Using Heterocycle to Improve the Selectivity of Rhodamine-6G Dye: Synthesis of Pyrrole-Modified Rhodamine-6G and its Recognition to Zn^{2+}

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Summary: The fluorescent sensor XQN for Zn^{2+} based on rhodamine-6G have been designed and synthesized. XQN showed fluorescent specific selectivity and high sensitivity for Zn^{2+} against other metal ions such as Fe^{3+}, Cr^{3+}, Hg^{2+}, Ag^{+}, Ca^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+} and Mg^{2+} in CH_3CN-PBS(phosphate buffer saline) (10 mM, v/v=7:3, pH = 7.4) solution. The distinct color change and the rapid emergence of fluorescence emission provided naked-eyes detection for Zn^{2+}. The test strip results showed that these sensors could act as a convenient and efficient Zn^{2+} test kit. The recognition mechanism of the sensor toward Zn^{2+} was evaluated by IR and the Job's plots. The detection limits of XQN towards Zn^{2+} was calculated as 2.39 μM. In addition, XQN-Zn^{2+} fluorescence lifetime and fluorescence quantum yield were also measured.

Keywords: Rhodamine-6G; Pyrrole; The fluorescent sensor; Zn^{2+}; Test kit.

Introduction

In recent decades, the exploration of selective and sensitive fluorescent sensors for the detection of transition metal ions have been received great attention [1-6]. Amongst the important transition metal ions, Zn^{2+} is known for their ability in comprehensive applications. And Zn^{2+} plays a critical role in biological, environmental and chemical systems [7-10]. Nevertheless, the disruption of Zn^{2+} is very dangerous to human health. For example, the imbalance of Zn^{2+} has been strongly linked to hypoxia ischemia, epilepsy, amyotrophic lateral sclerosis, and several neurological disorders including Parkinson's disease and Alzheimer's disease [11-13]. So, it is highly desirable and absolutely necessary to develop the effective method to detect Zn^{2+} [14-16].

Fluorescent sensors, which are capable of recognizing guest species sensitively and sensitively, are of particular interest in supramolecular chemistry to check trace amount of transition metal ions in the last decades [17, 18]. The research efforts have been focused on the design and practical application of novel sensors because of their high selectivity and low cost.

It is well known for the favourable spectroscopic properties of rhodamine derivatives that include large absorption coefficient, high fluorescence quantum yield, long absorption and emission wavelength [19]. In particular, the modified spirolactam structure which enables colorless and nonfluorescent can be transformed into the colored and highly fluorescent ring-opened amide form in the presence of the corresponding metal ions [20]. Because of the particular "off-on" interaction mechanism, rhodamine derivatives have become the prior choice in constructing novel sensors, especially for the transition metal ions such as Zn^{2+}. In order to increase the sensitivity and selectivity to the metal ions, some heterocycles have been used to modify the rhodamine platform, such as furan, pyrrole and thiophene [21, 22]. However, most of them are obtained through C=N by aromatic aldehydes reacting with *N*-(rhodamine-B) lactam-ethylenediamine or *N*-(rhodamine-B) lactam-hydrazine hydrate [23-25]. But few compounds linked by amide between heterocycles and rhodamines have been reported. And it is clear that the derivatives modified by amide have more potential coordination sites to bind Zn^{2+} than by C=N unit, which may strengthen sensitivity and selectivity.

In this paper, the novel rhodamine-based fluorescent chemosensor **XQN** was designed and synthesized (Scheme-1), which was modified by pyrrole structure with amide to improve increase the selectivity of rhodamine-6G dye. To our surprised, the rhodamine derivative could "naked-eye"

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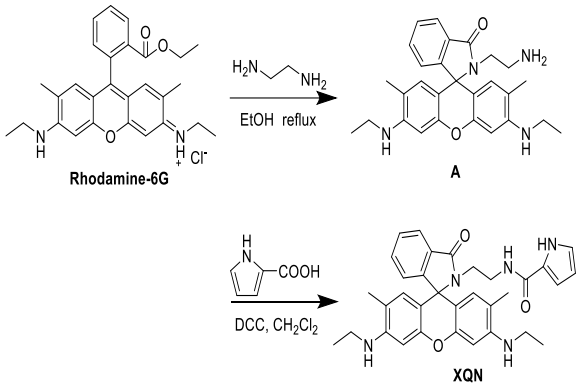
recognize Zn^{2+} with high selectivity and specific sensitivity in CH_3CN-PBS (phosphate buffer saline) (10 mM, v/v=7:3, pH=7.4). Meanwhile, other cations such as Fe^{3+}, Cr^{3+}, Hg^{2+}, Ag^{+}, Ca^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+} and Mg^{2+} could not cause any interference.



Scheme-1: Synthetic route to fluorescent sensor **XQN**.

Experimental

General spectroscopic methods

All UV-vis spectroscopy and fluorescent spectroscopy were carried out on a Shimadzu UV-1601 spectrometer and a HORIBA FLUOROMAX-4-NIR spectrometer. The concentration of **XQN** was kept constantly (3.3×10^{-4} M). The solution of metal ions were prepared from the nitrates salts of Fe^{3+}, Cr^{3+}, Hg^{2+}, Ag^{+}, Ca^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+} and Mg^{2+}.

^{1}H NMR and ^{13}C NMR spectra were performed on a Bruker at 400 MHz using TMS as an internal standard, DMSO-d_6 as the solvents. Melting points were measured on an X-4 digital melting-point apparatus (uncorrected). Infrared spectra were obtained on a Nicolete 5700 FT-IR spectrophotometer. Mass spectra were carried on with a Shimadzu LCMS-IT/TOF mass spectrometer. Solvents were purified and dried using standard protocol, the other chemical reagents were obtained commercially and used as received without further purification. All reagents were used of analytical grade.

*Synthesis of compound **XQN***

The synthesis of compound **XQN** was outlined in Scheme-1. According to the reported procedure, compound **A** was synthesized [17, 26]. Pyrrole-2-carboxylic acid (0.01 mol), DCC (0.02 mol) and **A** (0.01 mol) were dissolved in CH_2Cl_2 (10 mL) in a 25 mL round-bottom flask. The reaction mixture was stirred for 12 h at room temperature. When the solvent was evaporated under reduced pressure, the crude product was purified by silica gel chromatography (ethyl acetate: petroleum ether = 1: 2) to obtain the fluorescent sensor **XQN**. *N-(2-(3',6'-bis(ethylamino)-2',7'-dimethyl-3-oxospiro[isoindoline-1,9'-xanthen]-2-yl)ethyl)-1H-pyrrole-2-carboxamide* (**XQN**):Yield 83%, m.p. >300 °C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.34 (s, 1H), 7.92-7.72 (m, 2H), 7.56-7.38 (m, 2H), 7.03-6.90 (m, 1H), 6.79 (dd, J=3.9, 2.6 Hz, 1H), 6.61 (d, J=3.5 Hz, 1H), 6.27 (s, 2H), 6.13 (s, 2H), 6.01 (dd, J=5.9, 2.4 Hz, 1H), 5.06 (t, J=5.4 Hz, 2H), 3.28-3.04 (m, 6H), 2.92 (dt, J=11.7, 5.9 Hz, 2H), 1.86 (s, 6H), 1.22 (t, J=7.1 Hz, 6H). ^{13}C NMR (100 MHz, DMSO) δ 167.85, 160.80, 157.05, 154.36, 151.42, 148.11, 133.20, 130.51, 128.67, 127.85, 126.50, 124.05, 122.81, 121.64, 118.74, 110.05, 108.89, 104.88, 96.18, 64.81, 33.82, 17.48, 14.64. HRMS (ESI) m/z: $[M+H]^{+}$ Calcd for $C_{33}H_{35}N_5O_3$: 550.2813; Found 550.2816.

Results and Discussion

The optical responses of compound **XQN** to various metal cations including Fe^{3+}, Cr^{3+}, Hg^{2+}, Ag^{+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+} and Mg^{2+} was investigated in CH_3CN-PBS (10 mM, v/v=7:3, pH=7.4) through UV-vis and fluorescence spectroscopic methods. Firstly, free sensor **XQN** was colorless and no absorption was detected above 500 nm in UV-vis spectrum. When adding Zn^{2+} to the **XQN** solution, **XQN** immediately responded with dramatic color changes from colorless to yellow-green (Fig. 1). And a new absorption peak appeared at 525 nm. It might be due to the structure transformation from spirolactam to the ring-opened amide form with adding Zn^{2+}. To validate the selectivity to different metal ions, the same tests were investigated in use of Fe^{3+}, Cr^{3+}, Hg^{2+}, Ag^{+}, Ca^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+} and Mg^{2+} instead of Zn^{2+} by UV-vis spectroscopy. Although Fe^{3+} and Hg^{2+} also led to color changes, their changes are less pronounced than Zn^{2+}. The results exhibited that **XQN** could discriminate Zn^{2+} from other ions.

In addition, the fluorescent properties of XQN in the presence of various cations were studied. The sensor did not display any appreciable emission excited at 525 nm. However, the fluorescence spectra of sensor XQN dramatically changed along with the Zn^{2+} concentration increase. For sensor XQN, an intense emission peak arose at 550 nm (Fig. 2). As a result, the fluorescent intensity at 550 nm was enhanced gradually with the increase of Zn^{2+} concentration (Fig. 3). Although the emission peak of Fe^{3+} and Hg^{2+} also appeared at 550 nm the same as Zn^{2+}, the fluorescent peak of sensor XQN at 550 nm was strongest caused by Zn^{2+} (Fig. 2). It was considered that the nonfluorescent spirolactam was transformed into the high fluorescent ring-open amide form when Zn^{2+} was added.

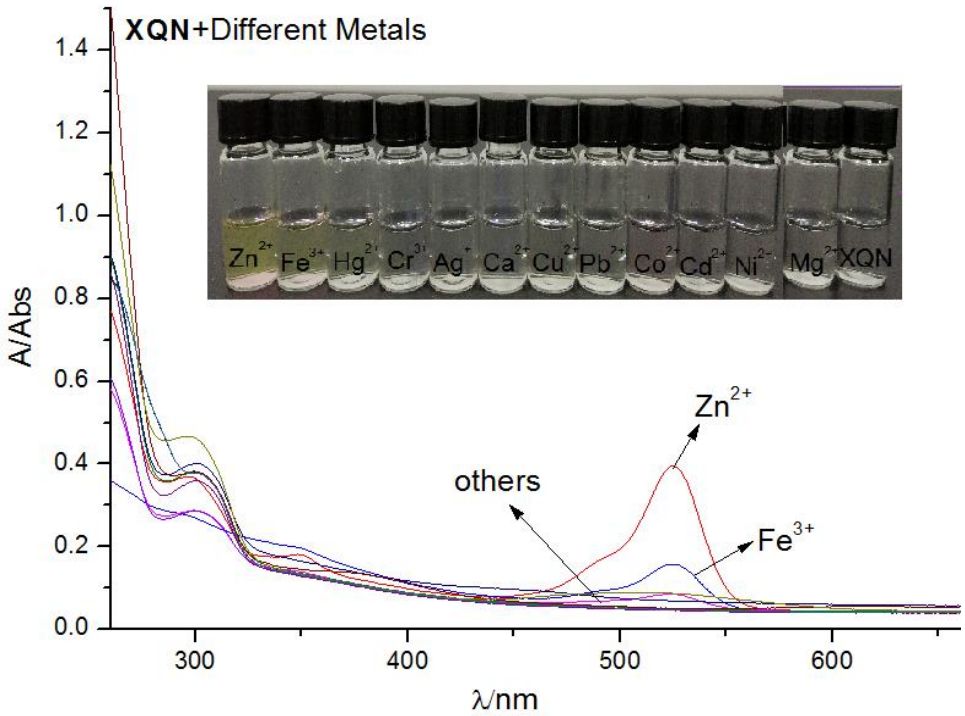


Fig. 1: Absorption spectra of the sensor XQN (3.3×10^{-4} M) in the presence of various metal ions in CH_3CN-PBS (10 mM, v/v=7:3, pH = 7.4). Inset: Photographs of chemosensor XQN (3.3×10^{-4} M) in the presence of various metal ions in CH_3CN-PBS (10 mM, v/v=7:3, pH = 7.4).

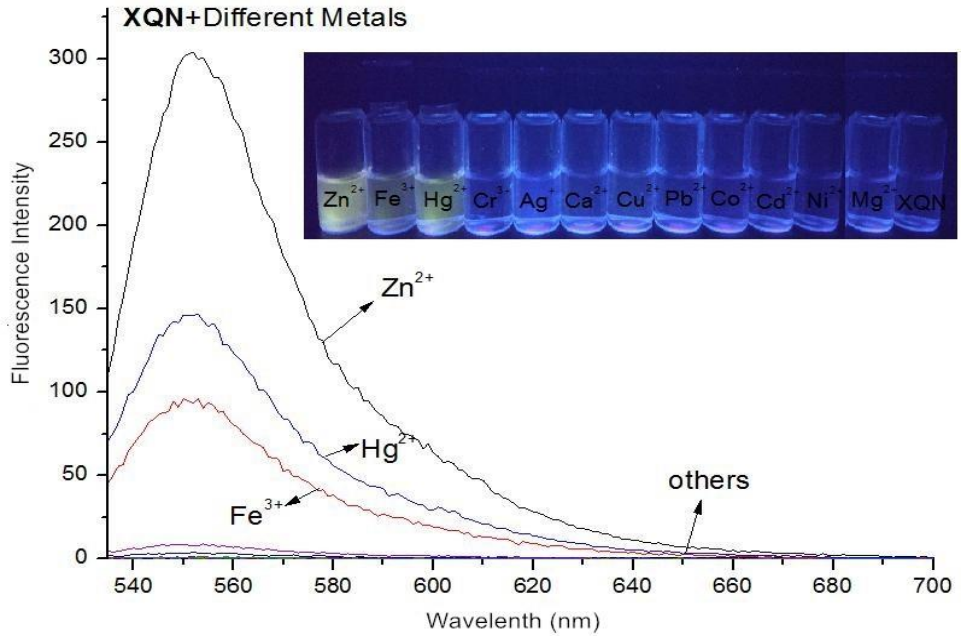


Fig. 2: Fluorescence spectra changes of the sensor XQN (3.3×10^{-4} M) in the presence of various metal ions in CH_3CN-PBS (10 mM, v/v=7:3, pH = 7.4), λ_{ex} =525 nm, detection from 540 to 720 nm. Inset: Photographs of chemosensor XQN (3.3×10^{-4} M) in the presence of various metal ions in CH_3CN-PBS (10 mM, v/v=7:3, pH = 7.4)

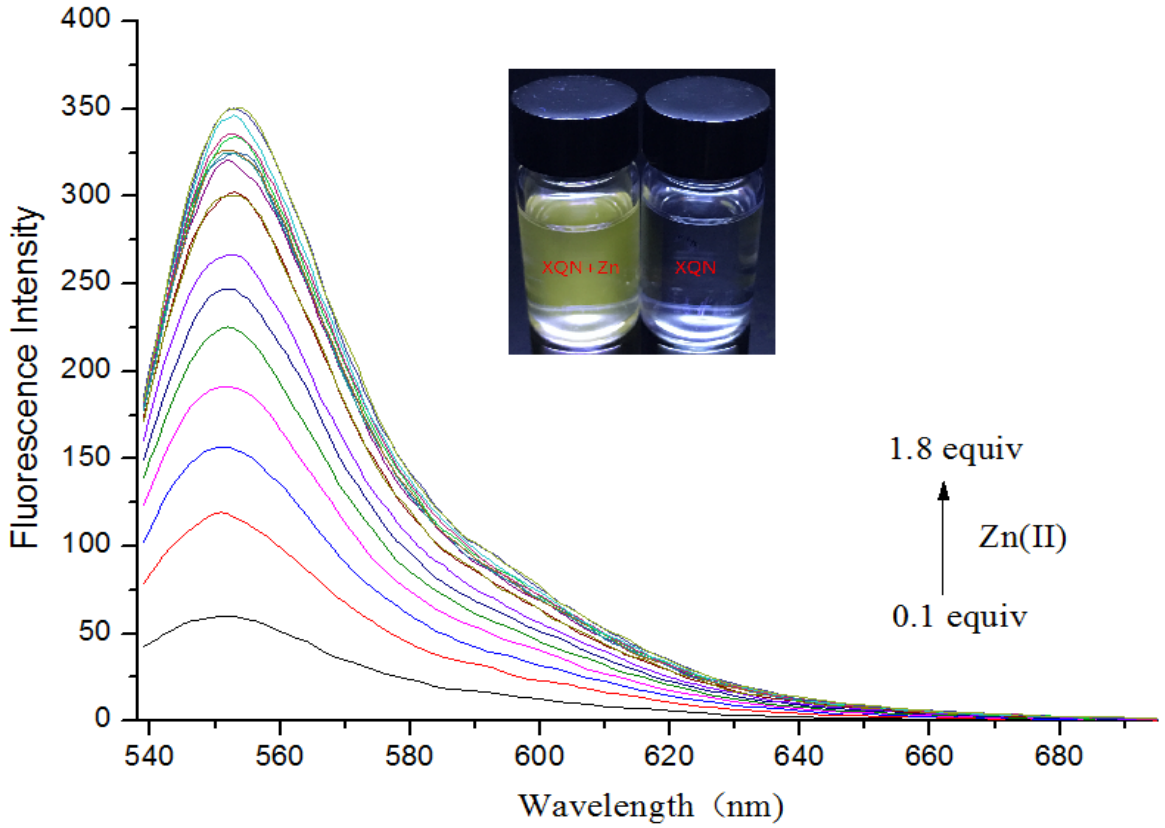


Fig. 3: Fluorescence spectra of XQN (3.3×10^{-4} M) in the presence of different concentration of Zn^{2+} (0-1.8 equiv.) in CH_3CN-PBS (10 mM, v/v=7:3, pH=7.4), λ_{ex} =525 nm, detection from 540 to 700 nm.

To further exploit the utility of sensor XQN, competitive experiments were carried out (Fig. 4). The results indicated that the recognition of sensor XQN towards Zn^{2+} was hardly affected by coexistent cations. And it also suggested that XQN could be used as a potential fluorescent sensor for Zn^{2+}. Moreover, the changes in the fluorescence intensity at 550 nm depending on the reaction time were recorded from 0 to 60 min (see SI Fig. S4). The experimental results confirmed that the fluorescence intensity significantly increased immediately when 20 equivalents of Zn^{2+} solution was added to the solution of sensor XQN (3.3×10^{-4} M). And it clearly showed that the reaction completed quickly after Zn^{2+} was added into the solution of sensor XQN.

To confirm the stoichiometry of the binding of sensor with Zn^{2+} as well as the mechanism of interaction between XQN and Zn^{2+}, the Job's plots analyses and IR analyses were conducted [26, 27]. As the main object, the Job's plots of sensors XQN clearly showed that the maximum fluorescence intensity at 550 nm with a mole fraction at 0.5 which indicated that the stoichiometric ratio of the interaction between them was 1: 1 (see SI Fig. S5 and Fig. 6).

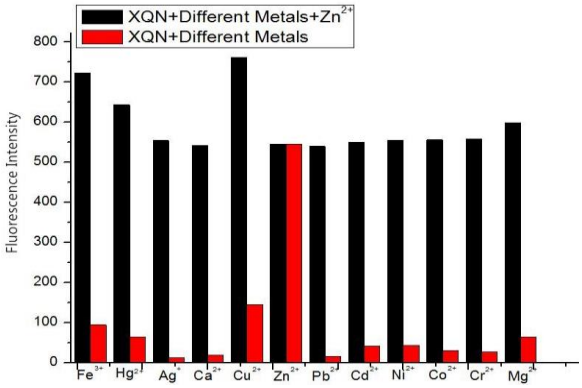


Fig. 4: Fluorescence response of XQN (3.3×10^{-4} M) in CH_3CN-PBS (10 mM, v/v=7:3, pH = 7.4) at 550 nm upon addition of respective metal ions, followed by addition of Zn^{2+} (1.5 equiv.), λ_{ex} =525 nm. The anti-interference of XQN (3.3×10^{-4} M) for Zn^{2+} (1.5 equiv.) detection was carried out by adding a mixture of other metal ions and Zn^{2+} to the sensor solution.

In the IR spectra of XQN, the stretching vibration absorption peaks of amide N-H appeared at 3473 cm^{-1} (Fig. 5). However, when XQN was coordinated with Zn^{2+}, the stretching vibration absorption peak disappeared, because the amide O=C-N-H deprotonated and tautomerized to C=N, and a new stretching vibration absorption peaks appeared at 1666 cm^{-1}. In accordance with the 1:1 stoichiometry, sensor XQN was the most likely to chelate with Zn^{2+} *via* N, O-donor atoms because of the delocalization of O=C-N group. And a new peak of C=N appeared at 1629 cm^{-1} because the spriolactam ring opened. For the complex [XQN-Zn^{2+}], the NO_3^- was incorporated and balanced the positive charges of Zn^{2+} ion, which was confirmed by the characteristic peak of the NO_3^- with the intense band at 1383 cm^{-1}. Therefore, IR and the Job's plots may suggest that XQN interact with Zn^{2+} *via* N, O-donor atom because of the delocalization of O=C-N group as shown in Scheme-2.

By the absorption spectrum and the emission spectrum, it showed that the obvious change in color could be watched by naked-eyes. When the spirolctam was closed, it looked colorless. But after Zn^{2+} was added in the solution of sensor XQN, the ring was opened and transformed into amide form. So the absorption peak at 525 nm appeared. And the luminescent peak at 550 nm was detected. With the concentration of Zn^{2+} rising, the absorption peak and emission peak of [XQN-Zn^{2+}] were enhanced. In addition, the detection limits of XQN towards Zn^{2+} was calculated as 2.39 μM (see SI Table S1) [28]. In addition, the comparative analyses of XQN with the previous reported probe were displayed in Table-1. In order to further study the fluorescent properties, the binding constant, the life time and fluorescence quantum yield of XQN-Zn^{2+} was also measured (see SI Table S1).

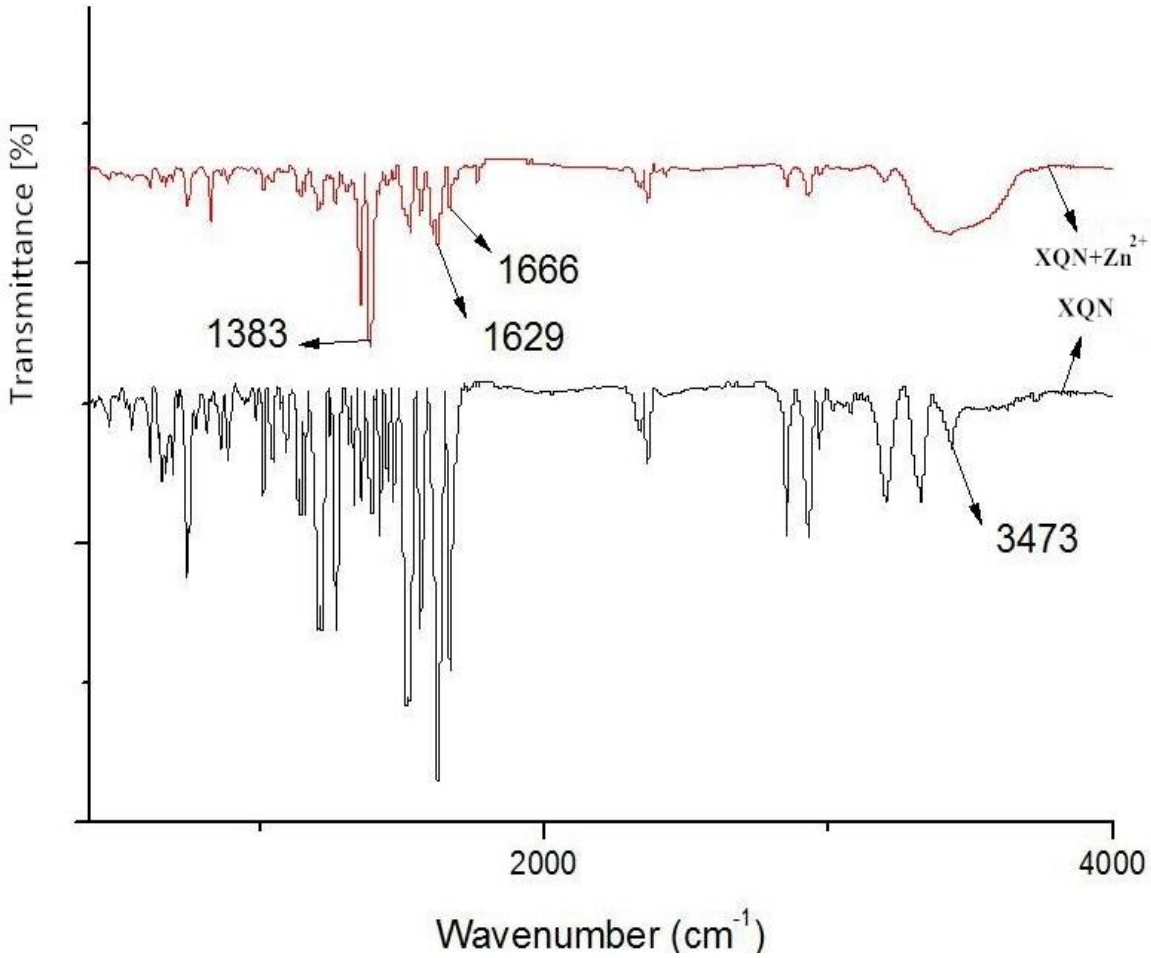
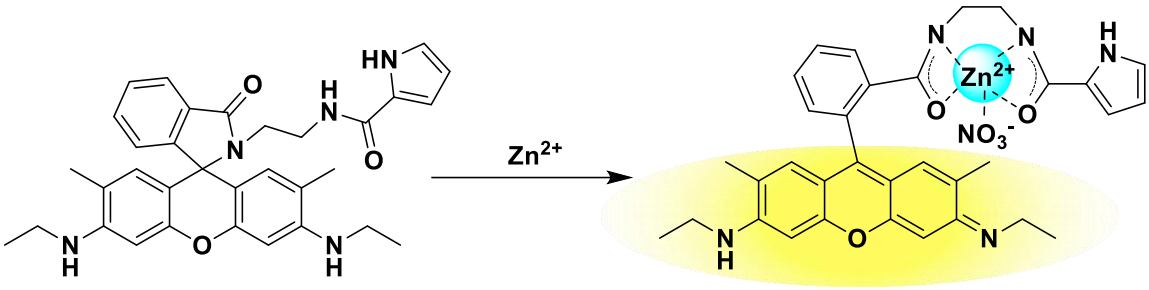


Fig. 5: Comparison between the FT-IR spectral data for XQN and its Zn^{2+} complex.



Scheme-2:The proposed mechanism of XQN for Zn^{2+} ion.

Table-1: Fluorescent sensors reported for the detection of Zn^{2+}.

Probe name	Solvent	Detection limit	References
CTS	EtOH/HEPES buffer	5.03×10^{-7} M	[15]
L2	CH_3CN	1.4×10^{-6} M	[16]
BAR	MeCN-H_2O	2.21 μM	[17]
This wok	CH_3CN-PBS buffer	2.39 μM	Present work

To investigate the practical application of sensor, test strips were prepared by immersing filter papers into a CH_3CN-PBS (10 mM, v/v=7:3, pH=7.4) solutions of sensor **XQN** (1×10^{-4} M) followed by air drying. Then these test strips were utilized to sense Zn^{2+} (1×10^{-4} M). As shown in Fig. 6, the obvious color change was observed under the 365 nm UV lamp in the presence of Zn^{2+}. So, this study indicated that test strips of **XQN** had the potential to detect Zn^{2+} colorimetrically and fluorimetrically in solid state as well.

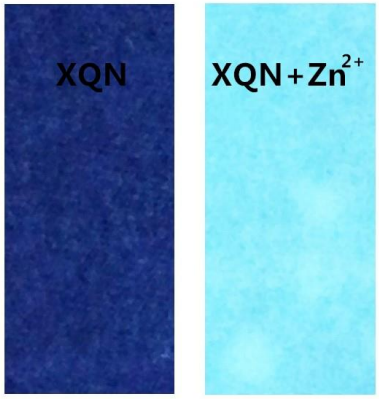


Fig. 6: Photographs of XQN (1×10^{-4} M) on test papers under irradiation at 365 nm.

Conclusions

In summary, the rhodamine-based sensor XQN was synthesized and characterized. The sensor XQN exhibited highly selective and sensitive recognition toward Zn^{2+} against other metal ions in CH_3CN-PBS (10 mM, v/v=7:3, pH=7.4) solution. The binding mode was proposed based on the the Job's plots analyses and IR analysis. Moreover, test strips were prepared by immersing filter papers into the solution of **XQN**, which exhibited good selectivity to Zn^{2+}. These findings indicated that sensor **XQN** modified by pyrrole unit had potential applications in physiological and environmental systems for Zn^{2+} detection.

Acknowledgement

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