Synthesis and Study of a Novel Visualized Iron Ion Probe

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Summary: In this study, we synthesized probe R using Rhodamine 6G as the chromophore. The structure of probe R was characterized, and its probe properties and reaction mechanism were studied. The experimental results could be observed with the naked eye, and probe R could detect iron ions effectively. The LOD of UV-vis absorption determination was 6.4 µM, which was significantly lower than the international sanitation standard for drinking water. Therefore, R could be used as a new visual metal ion probe to determine Fe^{3+} content. Job's plot experiment demonstrated that the response of probe R to Fe^{3+} was based on complex formation with a ratio between R and Fe^{3+} of 2:1.

Keywords: Iron; Probe; Rhodamine 6G; Properties; Reaction mechanism; Visual.

Introduction

It is crucial to quickly and accurately measure iron levels in both food products and the human body [1, 2]. Compared with traditional detection methods such as Atomic Absorption Spectrum (AAS) and Cyclic Voltammetry (CV), Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [3-7]，fluorescent probes have become a rapid and efficient method for detecting metal ions due to their simple operation, excellent response performance and high sensitivity [8-9].

Rhodamine fluorescent dye molecules with modified amide groups are capable of carrying out an "open-close" function of helix. In its closed loop state, the molecule is colorless and non-fluorescent. However when encountering certain metal ions,the helix will open showing coloration fluorescence making them excellent candidates for use as fluorescent probes for metal ions [10-11]. Currently many new Rhodamine-like probe molecules have been studied mostly used for recognition Cu^{2+}, Zn^{2+}, Hg^{2+} etc., however they exhibit low sensitivity and weak anti-interference ability [12-13].

In order to obtain probe molecules with excellent performance, this study designed and synthesized a novel open-closed-loop metal ion probe using Rhodamine 6G, ethylenediamine, 2-hydroxynaphthalene formaldehyde, and other experimental materials through condensation reactions. The performance of the probe was tested, and the reaction mechanism was explored based on these tests.

Experimental

Chemicals and instruments

Rhodamine 6G, ethylenediamine, and 2-hydroxynaphthalene formaldehyde were purchased from Aladdin Chemical Co., Ltd. (Shanghai, China). Other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the chemicals used were of analytical grade. Most metal ions were used as their chloride salts, except for silver ions used as nitrate salts. Nuclear magnetic resonance (NMR) spectra were recorded on Brucker AM-400 MHz instruments with tetramethylsilane as internal standard. Mass spectrometry was performed using a Waters LCT Premier XE spectrometer. UV-vis absorption spectra were carried out on a SHIMADZU UV-2450 spectrophotometer with quartz cuvette (optical path length 10mm).

Synthesis of N-aminoethyl-Rhodamine 6G imide

Rhodamine 6G (2.0016 g) was dissolved in anhydrous ethanol (30 ml) until completely dissolved. Ethylenediamine (2 ml) was added and the mixture was refluxed at 110 °C for 6 hours. Thin-layer chromatography (TLC) was used to track the reaction in real time. After the reaction was complete, the

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mixture was cooled to room temperature. The solid obtained after suction filtration was washed several times with anhydrous ethanol and finally dried to yield a light pink solid. The yield: 1.2250 g (60%).1H NMR (400 MHz, CDCl3, TMS) 7.92(1H, m), 7.41 (2H, m), 7.03 (1H, m), 6.34 (2H, s), 6.22 (2H, d), 3.47 (4H, t), 3.11 (4H, m), 2.34 (2H, t), 1.89 (6H, s), 1.30 (6H, t), 0.95 (2H, s).

Synthesis of probe R

N-aminoethyl-rhodamine 6G imide (1.0080 g) was reacted with 2-hydroxynaphthalene formaldehyde (0.3965 g) at 110 °C for 10 h, and the reaction was tracked in real time by TLC. Probe R was washed with anhydrous ethanol and then recrystallized from anhydrous ethanol. The yield: 0.4301 g (32%).1H NMR (400 MHz, $CDCl_3$, TMS) δ 9.36 (s, 1H), 8.86 (dd, 1H), 8.67 (d, J = 8.3 Hz, 1H), 8.52 (d, J = 9.3 Hz, 1H), 8.46 (d, J = 6.6 Hz, 1H), 8.40 – 8.33 (m, 2H), 8.33 – 8.28 (m, 1H), 8.16 (s, 1H), 8.15 – 8.06 (m, 1H), 7.96 (dd, 1H), 7.74 (d, J = 9.3 Hz, 1H), 7.11 (s, 2H), 4.62 (q, J = 7.0 Hz, 2H), 4.34 – 4.26 (m, 4H), 4.08 (q, J = 7.1 Hz, 4H), 2.71 (s, 6H), 2.20 (t, J = 7.1 Hz, 6H), 2.13 (t, J = 7.0 Hz, 2H). LC/MS, m/z: $[M+H]^+$: found, 611.3.calcd,611.29.

Metal ion selectivity measurements

Fifteen clean centrifuge tubes were taken and the prepared probe R solution (0.3 mM) 1000 μL and acetonitrile aqueous solution [V(water)：V(acetonitrile)=1：1] 1.96 mL were measured into each centrifuge tube. 40 μL ultrapure water was added to one centrifuge tube as a blank control, and 40 μL of different metal ion solutions (3mM) were added to the remaining 14 centrifuge tubes respectively (Fe^{3+}, Ag^+, Zn^{2+}, Ni^{2+}, Cr^{3+}, Pt^{2+}, Co^{2+}, Cu^{2+}, Ca^{2+}, Ba^{2+}, Mg^{2+}, Na^+, Al^{3+} and Hg^{2+} in sequence).

Metal ion interference measurements

Based on the results of the metal ion selectivity measurements, the centrifuge tubes that had been added with Fe^{3+} were taken out, and 40 μL of Fe^{3+} solution (3mM) was added to each of the remaining 14 centrifuge tubes.

Metal ion sensing measurements

1000 μL of the probe solution (0.3 mM) was added to 15 centrifuge tubes, and then 0 ~ 140 μL Fe^{3+} solution was added successively at a gradient of 10 μL, and acetonitrile aqueous solution [V(water)：V(acetonitrile)=1：1] was added into the above centrifuge tubes to ensure that the total volume of solution in each centrifuge tube was 3 mL. The UV–vis absorption spectra were measured successively. The standard deviation σ is measured with the blank sample, and the limit of detection（LOD） was calculated.

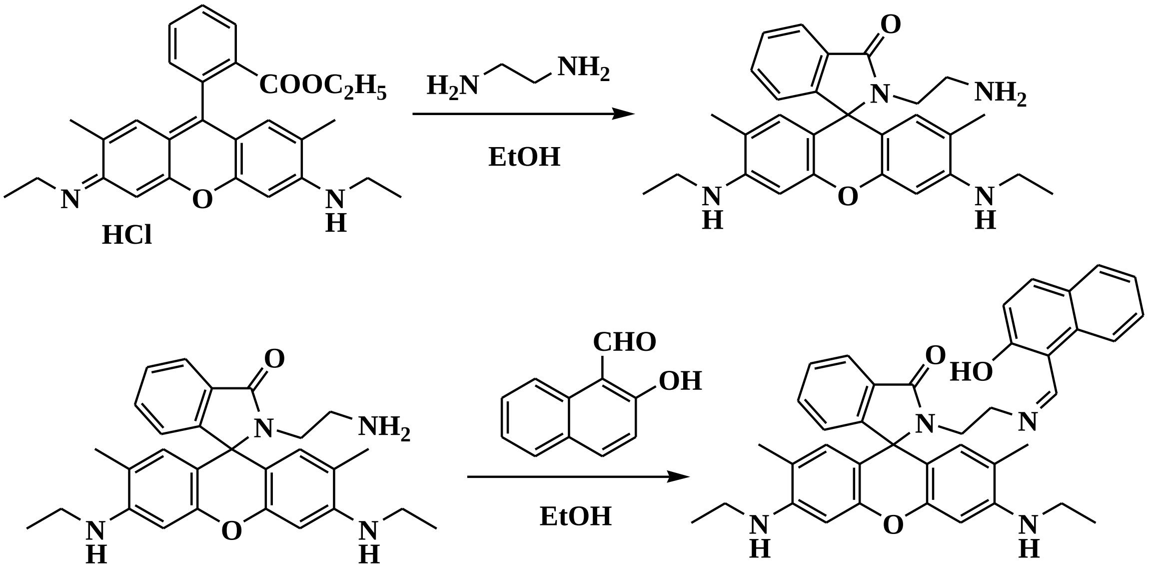
Job's plot measurements

Excessive EDTA solution was added into the centrifuge tube with Fe^{3+} solution in metal ion selectivity measurements, and the change in color of the solution was observed after standing for a period of time, and then the absorbance of the mixed solution at the maximum absorption peak of 530 nm was measured. Then 2.6 mL acetonitrile aqueous solution was removed by pipette and added into 11 centrifuge tubes. The probe R solution and Fe^{3+} solution were added in a gradient of 40 μL, that is, the volume of Fe^{3+} solution changes from 0μL to 400μL, and the volume of probe R solution pair changes from 400 μL to 0 μL. After the solution has been left to stand for 5 minutes, the absorbance at the maximum absorption peak of 530 nm was measured respectively.

Results and discussion

Design and synthesis of probe R

As shown in Scheme 1, N-aminoethyl-Rhodamine 6G-imide was firstly synthesized according to the literature and characterized by 1H NMR (Fig. S1), then 2-hydroxynaphthalene formaldehyde was connected to N-aminoethyl-Rhodamine 6G-imide by condensation reaction under certain conditions [14] . The probe R was successfully synthesized and characterized by 1H NMR and LC–MS, as can be seen in Fig. S2 and Fig.S3, respectively.



Scheme-1: Continuous two-step synthesis of R.

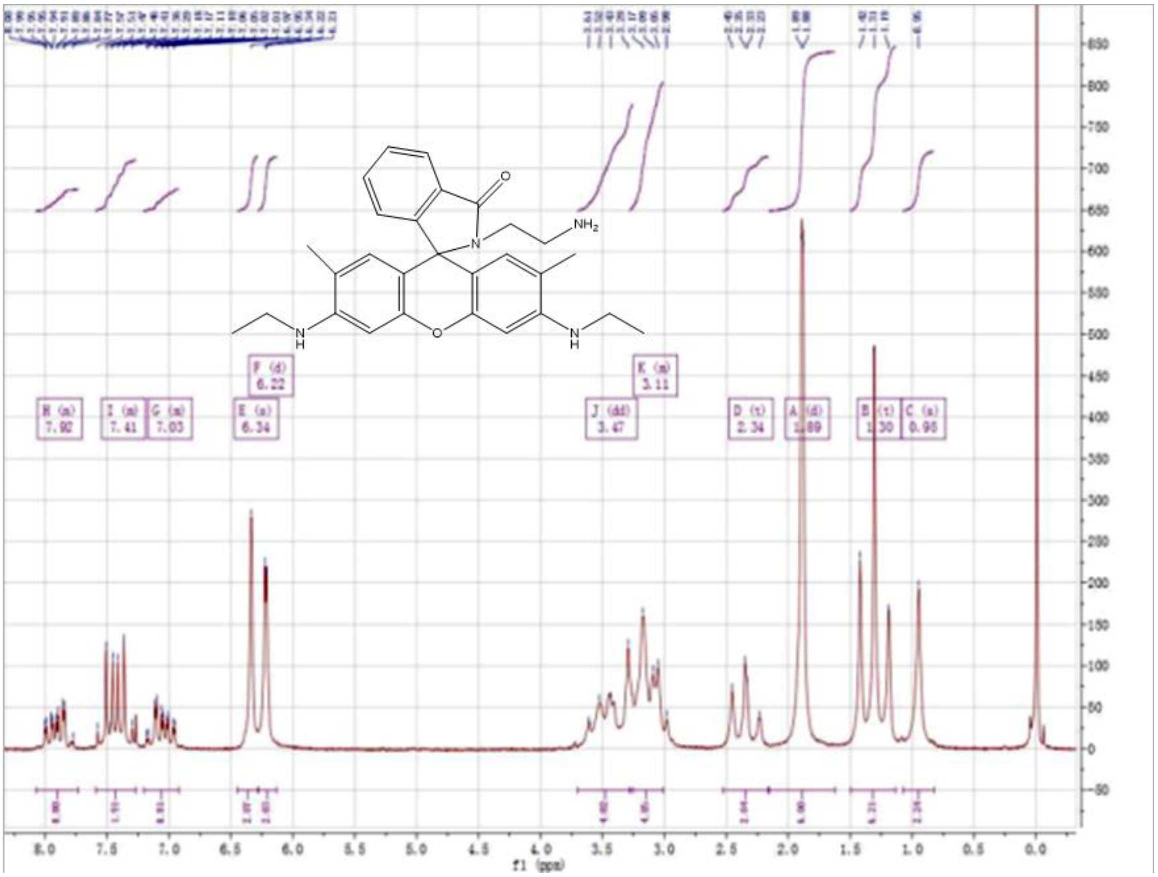


Fig. S1: ^{1}H NMR of N-aminoethyl-Rhodamine 6G imide (400 MHz, CDCl$_3$,TMS).

Fig. S2: ^{1}H NMR of probe R (400 MHz, CDCl$_3$,TMS).

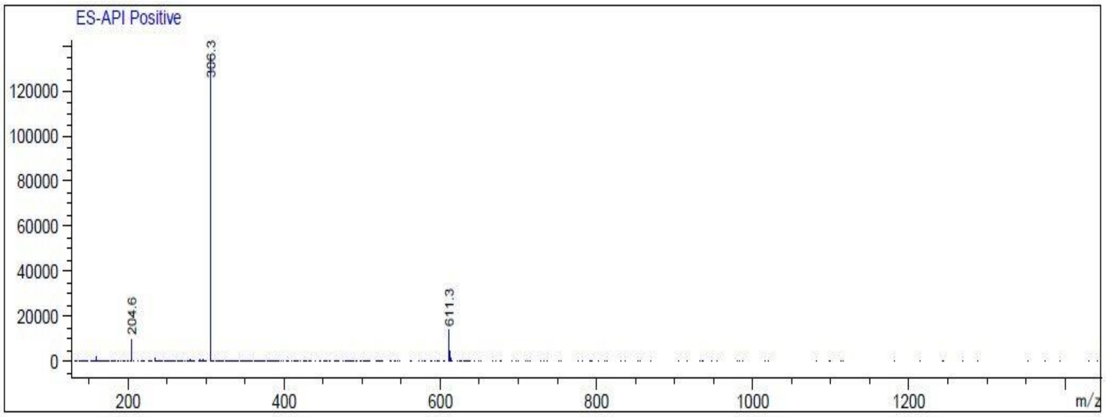


Fig. S3: LC-MS of probe R(LC condition: C18 column, ACN:H$_2$O=70:30, 1 ml/min, 254nm; MS condition: ESI-API, positive).

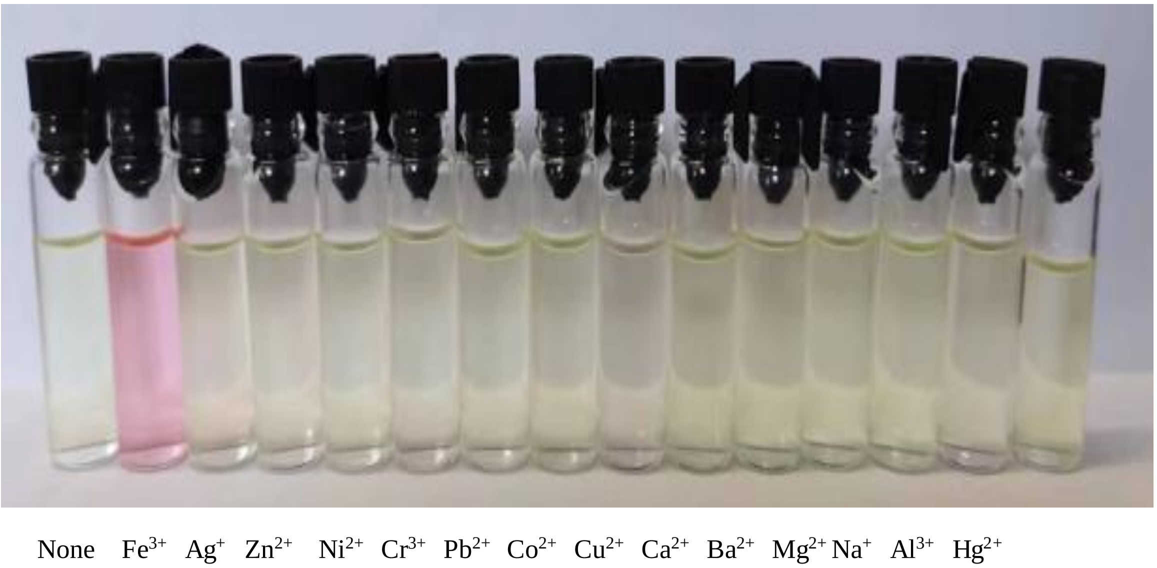


Fig. 1: Photographs of the corresponding R solutions after adding different metal ions under visible light.

Spectroscopic studies of Fe^{3+} on probe R

As shown in Fig 1, after the addition of various metal ions, the probe R solution showed a certain color change. The solution with Fe^{3+} added showed a light pink color. Besides, no obvious color change was visible to the naked eye in other solutions.

As shown in Fig 2, according to the UV-vis absorption spectra, it could be seen that the R solution with Fe^{3+} added had the maximum absorption peak at 530 nm, and a few other metal ions had the UV absorption peak at 530 nm, but the UV peak was lower than that of the probe R solution with Fe^{3+} added , which was Al^{3+}, Cr^{3+} and Cu^{2+} in order. Therefore, the probe R has the best selectivity for $Fe^{3+}$with a significant color change effect.

On the basis of the above ion selectivity experiments, in order to explore the interference of Fe^{3+} on the probe R, 3 mM Fe^{3+} solution of 40μl was added to all centrifuge tubes except that with Fe^{3+} added. After standing for a period of time, significant pink color changes were observed in all systems.This indicated that probe R still had a strong response to Fe in the presence of interference from other ions. According to Fig 3, similar peak height values appeared in each mixed solution at 530 nm of UV-vis absorption spectra. Although a few metal ions had a slight response to R and Fe^{3+}, the effect was not significant, indicating that probe R has a certain anti-interference ability.

As shown in Fig 4(a), when the concentration of Fe^{3+} increased from 0 μM to 140 μM with a gradient of 10 μM, the peak value at 530 nm of the strongest UV-vis absorption peak gradually increased. As shown in Fig 4(b), the UV-vis absorption intensity at 530 nm showed a good linear relationship with the concentration of Fe^{3+} when the concentration of Fe^{3+} varied within the range of 30 μM to 140 μM. Using this interval value for linear fitting, the linear regression equation can be obtained as:

$$y=0.00954x-0.03693，R^2 = 0.099131$$

The LOD can be calculated using the following formula:

$$LOD=3\sigma/k$$

σ：Standard deviation of blank sample；

k：The slope of absorbance with respect to Fe^{3+} concentration

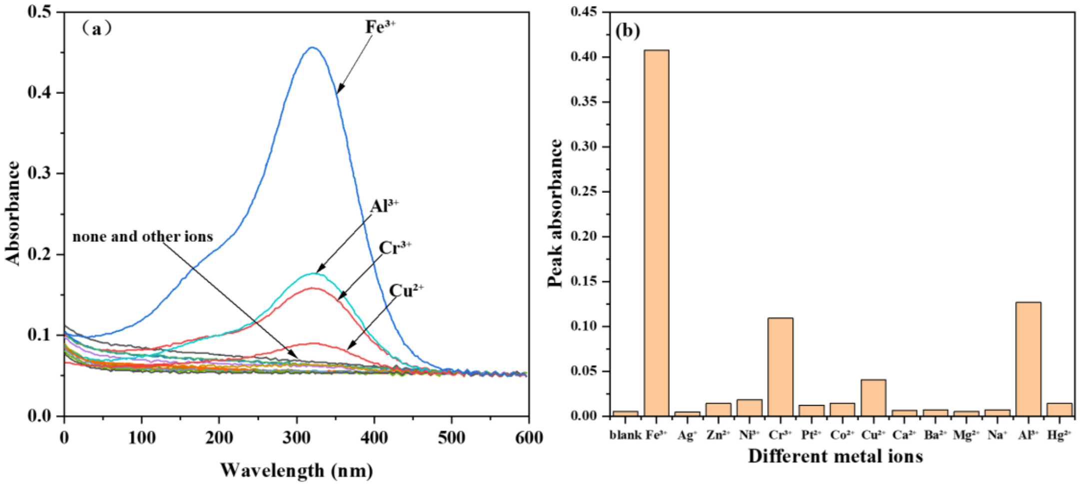


Fig. 2: (a) UV-vis absorption spectra of R solutions in the presence of different metals ions including Fe^{3+}, Ag^{+}, Zn^{2+}, Ni^{2+}, Cr^{3+}, Pt^{2+}, Co^{2+}, Cu^{2+}, Ca^{2+}, Ba^{2+}, Mg^{2+}, Na^{+}, Al^{3+} and Hg^{2+} (optical path length:10mm)

(b) The highest UV-vis absorption spectra of R solution in the presence of different metals.

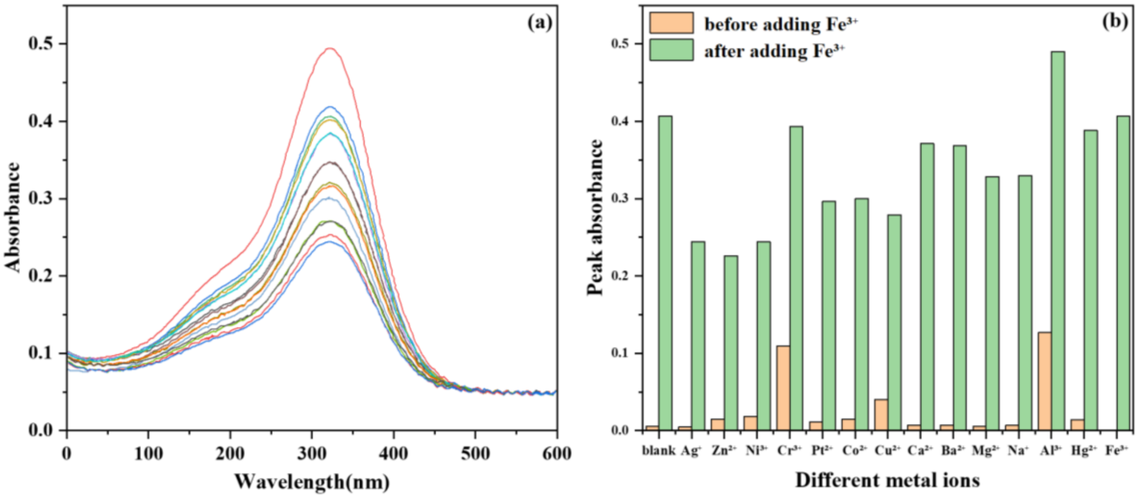


Fig. 3: (a) UV-vis absorption spectrum of R solutions with coexistence of Fe^{3+} and other metal ions including Ag^{+}, Zn^{2+}, Ni^{2+}, Cr^{3+}, Pt^{2+}, Co^{2+}, Cu^{2+}, Ca^{2+}, Ba^{2+}, Mg^{2+}, Na^{+}, Al^{3+} and Hg^{2+} (optical path length:10mm)

(b)The highest UV-vis absorption spectrum of R solutions with the coexistence of iron and other metal ions

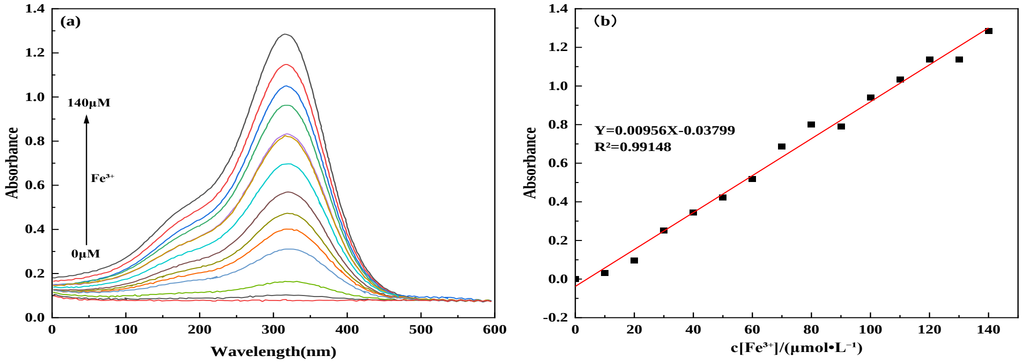


Fig. 4: (a) UV–vis spectra of R under different Fe^{3+} concentration increased from 0 μM to 140 μM with a gradient of 10 μM (optical path length:10mm)
(b) linear fitting curve of Fe^{3+} concentration and UV–vis absorption of R at 530 nm

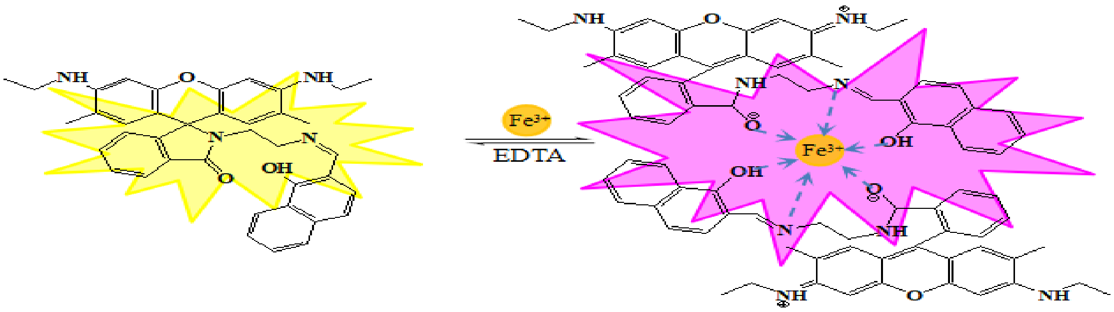
The results show that the LOD of probe R for Fe^{3+} UV-vis spectra in solution with acetonitrile aqueous solution [V(water)/V (acetonitrile) =1:1] as diluent is 6.4 μM, which is far lower than the Fe^{3+} content of 0.3 mg/L in drinking water sanitation standard.

Studies of interaction mechanism

To explore whether the reaction mechanism between probe R and Fe^{3+} was a complexation reaction, EDTA was selected as the complexing agent for the study.

When the probe R mixed with the solution containing Fe^{3+}, color reaction appeared without exception. The UV-vis absorption spectrum showed a maximum absorption peak at a wavelength of 530 nm. When EDTA was added to this mixture, the color of the solution became lighter with EDTA increasing until the color completely faded. After detection, the UV absorption peak also decreased significantly, and the whole process was reversible. It is not difficult to infer that the color reaction between probe R and Fe^{3+} is a complex phenomenon, which is closely related to the imide ring in the parent structure of Rhodamine. When the structure of probe R is in a spiral ring, the solution is light yellow, and when it is complexed with Fe^{3+}, the color reaction becomes pink. The color change visible to the naked eye can make the detection of metal ions simple and intuitive.

The total concentration of fixed Fe^{3+} and probe R remained unchanged, and the proportional relationship between metal ions and probe was mapped by absorbance. When ions and ligands are perfectly complexed, the peak value measured in the experiment is corresponding, so the complexation ratio of metal ions in the complex can be determined by Job's plot experiment. As shown in Fig 5, when the molar fraction of R was about 0.63, the maximum UV-visible absorption peak of the solution appeared at 530 nm, indicating that R and Fe^{3+} were combined in a complex ratio of 2:1. The possible reaction mechanism can be inferred as shown in Scheme 2.



Scheme-2: Reversibility of complexation of probe-Fe^{3+} with EDTA.

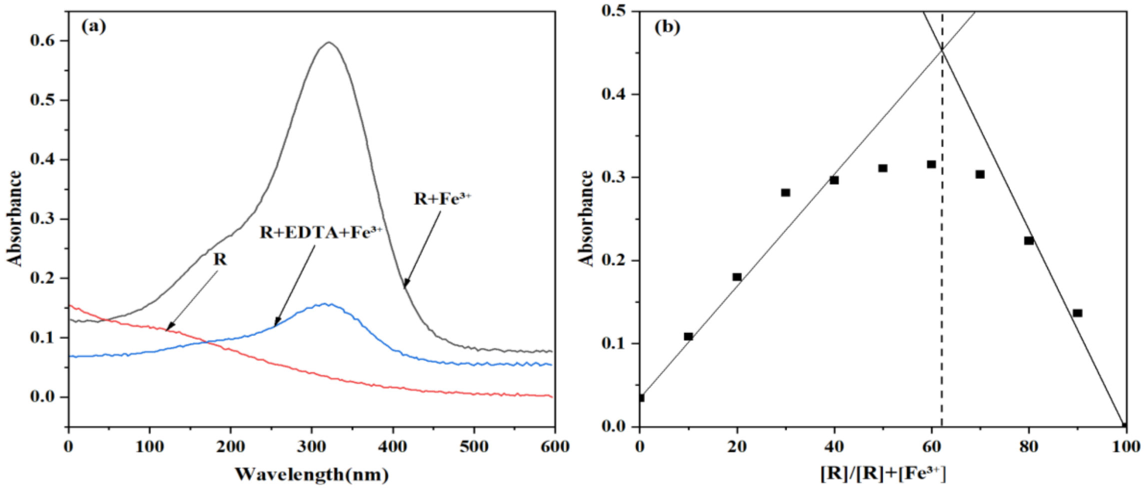


Fig. 5: （a） UV–vis spectra of probe R, probe R solution with Fe^{3+}, probe R solution with Fe^{3+}and EDTA
(b) the Job's plot

Conclusion

In this experiment, Rhodamine 6G, ethylenediamine, and 2-hydroxy-1-naphthalene formaldehyde were utilized as raw materials for the synthesis of probe R through condensation reactions. The structures were characterized using ^{1}H NMR and mass spectrometry. The ion selectivity, ion interference, probe sensitivity, and reaction mechanism of probe R with the metal ion Fe^{3+} were investigated using a UV-vis spectrophotometer. Probe R was a good iron ion chemosensor. It has high selectivity, sensitivity and anti-interference ability towards Fe^{3+}.With the increase of Fe^{3+} concentration, the signal was gradually enhanced. The LOD of probe R for Fe^{3+} (UV-vis spectra) was 6.4μM, which was significantly lower than the international sanitation standard for drinking water. Job's plot experiment demonstrated that the response of probe R to Fe^{3+} is based on complexation principles, with a complexation ratio between R and Fe^{3+} of 2:1. This result shows that the probe has a potential application prospect in the detection of Fe^{3+} content in food, environment, biology and other fields.

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