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Artigo

Fe^{3+}-SELECTIVE ENHANCED FLUORESCENCE PROBE BASED ON A RHODAMINE DERIVATIVE

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A novel Fe^{3+}-selective and turn-on fluorescent probe **1** incorporating a rhodamine fluorophore and quinoline subunit was synthesized. Probe **1** displayed high selectivity for Fe^{3+} in $CH_3CN–H_2O$ (95:5 v/v) in the presence of other relevant metal cations. Interaction with Fe^{3+} in 1:1 stoichiometry could trigger a significant fluorescence enhancement due to the formation of the ring-open form. The fluorescent response images were investigated by a novel Euclidean distance method based on red, green, and blue values. A linear relationship was observed between fluorescence intensity changes and Fe^{3+} concentrations from 7.3×10^{-7} to 3.6×10^{-5} mol L^{-1}.

Keywords: fluorescence; iron probe; Euclidean distance.

INTRODUCTION

Iron is an essential metal that plays crucial roles from the core of the earth to animal living organisms.[1] Either its deficiency or excess closely coincides with human health such as the prevention of certain diseases, the disturbances of glucose levels and the promote oxidation of lipids and proteins.[2,3] In addition, iron deficiency chlorosis is a great matter of concern in agriculture.[4] Thus, numerous conventional methods have been developed for the determination of Fe^{3+}, such as voltammetry,[5] atomic absorption spectroscopy,[6] and flow injection spectroscopy.[7,8] These standard techniques usually require complicated pretreatment procedures and necessitate the destruction of the sample. Thus, in recent years, fluorescent Fe^{3+} probes have attracted burgeoning interest mainly due to their sensitivity and non-destructive property.[9-12] In general, the Fe^{3+} fluorescent probe displays fluorescence quenching owing to the paramagnetic nature of Fe^{3+}.[13] As an alternative, fluorescent "turn-on" probes have stimulated active research. Consequently, the rhodamine frame work is an ideal candidate because of their simplicity and excellent spectroscopic properties.[14] Particularly, it undergoes non-fluorescent spirocyclic ("off" signal) and strongly fluorescent ring-open amide form ("on" signal). Heretofore, numerous rhodamine-based probes for Fe^{3+} have been reported.[15-19] As an effort for achieving more excellent fluorescent probes for Fe^{3+}, we herein designed and synthesized a new rhodamine derivative **1**, with rhodamine B as the fluorophore and hydroxyquinoline as the ion acceptor. Apart from the typical fluorescence analysis, Euclidean Distance analytical method was also ingeniously introduced to study Fe^{3+} sensing properties in this paper. As results demonstrated, **1** suggested a selective enhanced fluorescent response to Fe^{3+} over other tested metal cations in CH_3CN-H_2O (95:5 v/v) solution.

EXPERIMENTAL

Materials

All reagents were purchased from commercial suppliers and used without further purification. Rhodamine B and 8-Hydroxyquinoline-2-carboxaldehyde were purchased from Alfa-Aesar. All metal salts were obtained from shanghai Chemical Reagent Corporation (Shanghai, China). Chromatographic CH_3CN and de-ionized H_2O were used throughout the experiments. The stock solutions (1×10^{-4} mol L^{-1}) of the perchlorate salts of Ba^{2+}, Cu^{2+}, Ni^{2+}, Mg^{2+}, Ca^{2+}, Na^{+}, the nitrate salts of Pb^{2+}, Ag^{+} and chloride salts of Fe^{3+},Co^{2+}, Zn^{2+}, Fe^{2+}, Hg^{2+} in CH_3CN were prepared, respectively. The solution (2×10^{-6} mol L^{-1}) of compound **1** was prepared in CH_3CN and then diluted by H_2O to obtain aqueous solution CH_3CN-H_2O (95:5 v/v) of **1** (1×10^{-6} mol L^{-1}). The fluorescence spectra were recorded on Perkin Elmer LS-55 spectrofluorometer. ^1H NMR and ^{13}C NMR spectra were determined on Varian INOVA spectrometer in $CDCl_3$. IR spectra were performed with a Bruker Tensor 27 FT-IR spectrometer. ESI-MS were obtained using a Waters Micromass ZQ-4000 spectrometer. C, H, N elemental analysis was made on a Vario-EL. Under the UV lamb at 365 nm lighting, the fluorescence images were acquired before and after exposure of Fe^{3+} at different concentrations. The digital red (*R*), green (*G*), and blue (*B*) values were then subtracted to calculate the Euclidean Distance (*ED*) by the formula (1):[20]

$$ED = \sqrt{(\Delta R)^2 + (\Delta G)^2 + (\Delta B)^2} \quad (1)$$

Synthesis of probe 1

Rhodamine hydrazide (0.46 g, 1 mmol)[21] was dissolved in ethanol (20 mL), 8-Hydroxyquinolin-2-carboxaldehyde (0.17 g, 1 mmol) in ethanol (10 mL) was added dropwise (Scheme 1). The mixture was refluxed in an oil bath overnight and then cooled to room temperature. The yellowish color precipitate obtained was filtered and washed by cold ethanol (3 × 10 mL). After drying under reduced pressure, the reaction afforded yellowish solid **1** 0.31 g. Yield: 50%; m.p. 290 °C. Its structure was characterized by IR, NMR, ESI-MS, and elemental analysis (Figure 1S - Figure 4S, supporting information). IR (KBr, cm^{-1}): 3413 cm^{-1} (-OH), 1724 cm^{-1} (-C=O), 1697 cm^{-1} (-C=N-), 1613 cm^{-1} and 1513 cm^{-1} (-C=C-); ^1H NMR (400 MHz, $CDCl_3$, δ / ppm): 1.15 (12H, *t*, *J* = 6.8 Hz, -CH_2-C$\underline{H}_3$), 3.35 (8H, *q*, *J* = 6.8 Hz, -C$\underline{H}_2$-CH_3), 6.25 (2H, *d*, *J* = 7.6 Hz), 6.53 (4H, *m*), 7.09 (2H, *d*, *J* = 7.6 Hz), 7.16 (2H, *d*, *J* = 7.2 Hz), 7.24 (2H, *d*, *J* = 8.4 Hz),7.37 (1H, *t*, -N=CH-), 7.51 (2H, *m*), 8.04 (4H, *m*), 8.63 (1H, *s*, -OH). ^{13}C NMR (100 MHz, $CDCl_3$, δ / ppm): 12.63, 44.35, 66.18, 98.09, 105.69, 108.12, 110.05, 117.72, 118.82, 123.66, 124.00, 127.89, 128.09, 128.41, 133.84, 135.86, 137.54, 149.10, 152.19, 153.17, 165.52. ESI-MS: 612.5 ($M+H^+$, 100). Anal. Calcd. For **1** ($C_{38}H_{37}N_5O_3$): C, 74.61; H, 6.10; N, 11.45. Found: C, 74.52; H, 6.15; N, 11.48.

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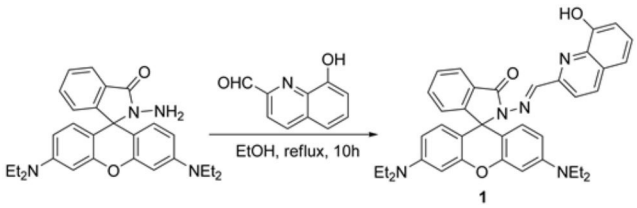

Scheme 1. *The synthetic procedure for probe* ***1***

RESULTS AND DISCUSSION

Due to its relative insolubility in aqueous media, the metal cation sensing system of probe **1** can only tolerate 5% H_2O in CH_3CN (v/v) (Figure 5S, supporting information). Figure 1 showed fluorescence spectra of **1** in the absence and the presence of tested metal ions (Fe^{3+}, Cu^{2+}, Co^{2+}, Zn^{2+}, Fe^{2+}, Hg^{2+}, Pb^{2+}, Ag^{+}, Ba^{2+}, Ni^{2+}, Mg^{2+}, Ca^{2+}, Na^{+}) in CH_3CN-H_2O (95:5 v/v). **1** itself was very weakly fluorescent, indicating the predominant ring-closed spirolactam. Upon addition of 36 *equiv.* of Fe^{3+}, 1 exhibited approxiamtely 250-fold fluorescence enhancement at 582 nm, refering to the delocalization in the ring-open amide form.[22] Correspondingly, pink colour and red fluorescence appeared. In contrast, other metal ions showed negligible change except that Cu^{2+} led to minor fluorescence response by 10-fold, suggesting the trace ring-open formation.

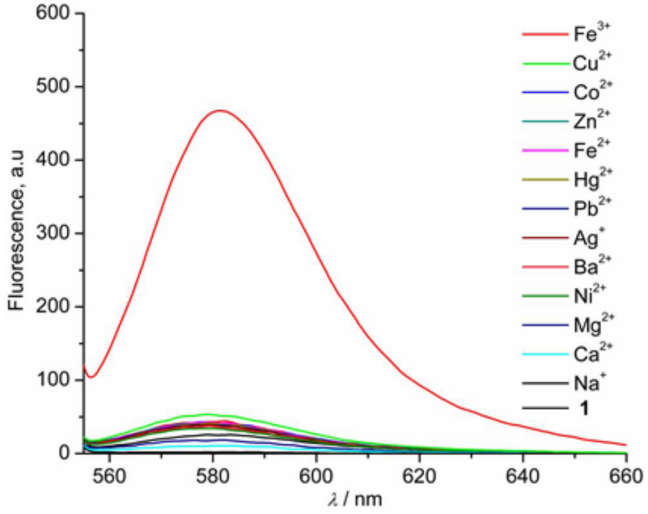

Figure 1. *Fluorescence spectra of* ***1*** *(1 × 10^{-6} mol L^{-1}, λ_{ex} = 540 nm) in CH_3CN-H_2O (95:5 v/v) in the presence of various metal ions (3.6 × 10^{-5} mol L^{-1})*

Furthermore, fluorescence titration of **1** (1 × 10^{-6} mol L^{-1}) with Fe^{3+} was performed in Figure 2. Upon adding Fe^{3+}, the maximum intensity at 582 nm gradually increased, which indicates the formation of ring-open amide form leading to turn-on fluorescence. Namely the formation of **1**-Fe^{3+} complex might contribute to obvious fluorescence enhancement with emission quantum yield of 0.41 using rhodamine B (Φ_f = 0.49 in EtOH) as a standard.[23] Besides, Job's plot demonstrates a 1:1 binding stoichiometry between **1** and Fe^{3+} (Figure 2 inset). According to this 1:1 model, the binding constant was calculated to be 8.20 × 10^4 L mol^{-1} indicating a strong binding ability of **1** to Fe^{3+}.[24]

Meanwhile we examined the relations between *ED* values of the fluorescent changes of probe **1** as a function of Fe^{3+} concentrations. As shown in Figure 3, the *ED* tracks linearly with increasing Fe^{3+} concentration from 7.3 × 10^{-7} to 3.6 × 10^{-5} mol L^{-1}, which generates a linear equation $Y = 4.082 \times 10^5 C - 0.131$ with high coefficient of 0.9994. This monotonic behaviour in the response probably reflects

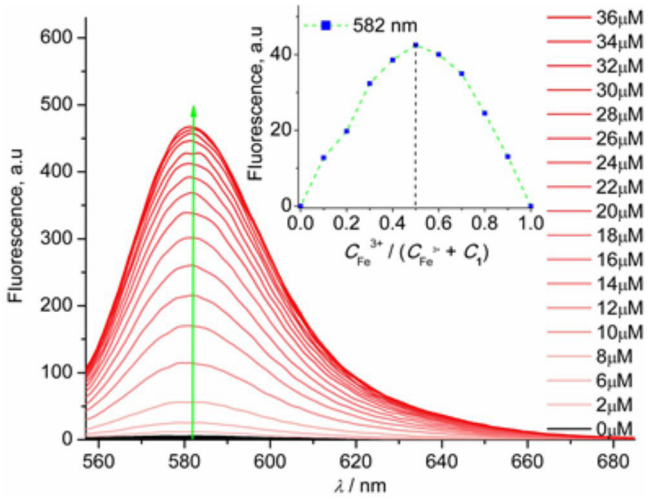

Figure 2. *Fluorescence titration of probe* ***1*** *(1 × 10^{-6} mol L^{-1}, λ_{ex} = 540 nm) in CH_3CN-H_2O (95:5 v/v) upon the addition of Fe^{3+} from 0 to 3.6 × 10^{-5} mol L^{-1}. Inset: Job plots of* ***1*** *with Fe^{3+} measured by fluorescence intensity at 582 nm (λ_{ex} = 540 nm). [**1**] + [Fe^{3+}] = 1 × 10^{-6} mol L^{-1}*

a 1:1 stoichiometry between probe **1** and Fe^{3+}.[25] The detection limit could reach the 2.4 × 10^{-7} mol L^{-1} from 3 times signal to noise, which exhibits relative moderate sensitivity compared to other repoeted probes.[26-28] This might be due to that *ED* data are acquired from simple fluorescence images rather than precise spectrofluorometer instrument.[29] Among three colour channels, *R* channel contributed mostly to the total fluorescent response, and the corresponding intensity also displayed a linear response to the Fe^{3+} solution with the correlation coefficient of 0.9955. Thus, this can be responsible for the observation of red fluorescence of **1**-Fe^{3+} complex by naked eyes. *G* and *B* channel did not show many changes relative to *R* channel. However, in these two channels the relative intensity illustrates a tolerable relationship to the concentration of Fe^{3+} with the correlation coefficient of 0.8988 and 0.9810, separately. The quantitative determination of Fe^{3+} is easily achieved by *ED* response and the concentration of Fe^{3+} ions. Base on *ED* data analysis, the control experiment was also carried out and showed in Figure 4. It can be found that the sensing of Fe^{3+} by probe **1** is hardly influenced by those co-existent metal ions.

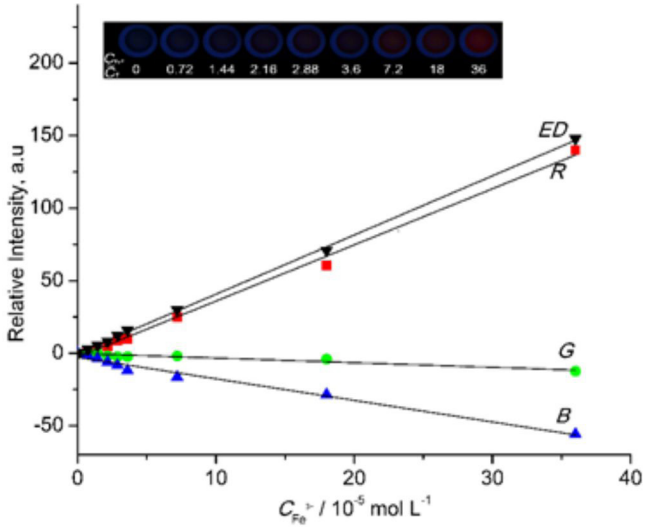

Figure 3. *The fluorescence intensity responses of* ***1*** *(1 × 10^{-6} mol L^{-1}) with the concentrations of Fe^{3+} from 0 to 3.6 × 10^{-5} mol L^{-1}. Inset: The fluorescence images of* ***1*** *with various concentrations of Fe^{3+}*

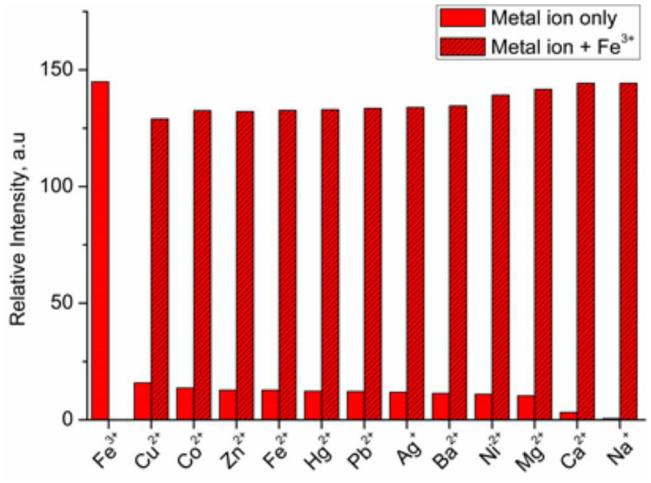

Figure 4. *Fluorescence response of* ***1*** *upon addition of various metal ions under the absence and presence of background Fe^{3+} (in the same equivalence of Fe^{3+}) at 582 nm (λ_{ex} = 540 nm)*

Based on above mentioned observations, we can safely propose the most possible binding mode of **1** and Fe^{3+} in Figure 5. The spirocyclic ring possibly opens to efficiently capture Fe^{3+}, a highly delocalized π-conjugated structure develops and a significant fluorescence enhancement occurs. With this special coordinate mode, involving two O atoms and two sp^2 N atoms, only Fe^{3+} can be allowed to enhance the fluorescence, though the other ions fail, indicating the coordinate moiety of **1** match perfect with Fe^{3+}. This binding behavior can be definitely confirmed by the new peak at *m/z* = 671.3 for $[1 + Fe^{3+} + 3H]^+$ instead of the original peak at 612.5 for $[1 + H]^+$ in ESI-MS spectra. By maximizing the binding from **1**, the 4-binding mode is consequently suggested.

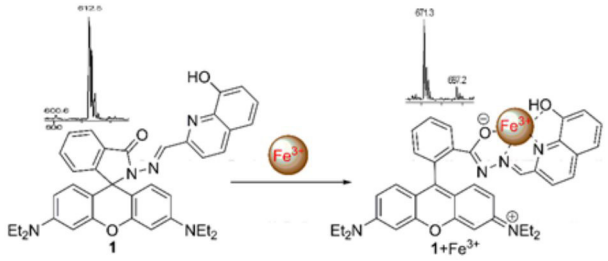

Figure 5. *Proposed binding mode and ESI-MS data of probe* ***1*** *and Fe^{3+}*

CONCLUSIONS

In summary, we have developed a novel rhodamine-based probe **1** for Fe^{3+} over other tested metal ions based on the enhanced fluorescence. **1** exhibits 250-fold fluorescence enhancement intensity in the presence of 36 *equiv.* of Fe^{3+}, indicating the mild fluorescence response. *ED* analytical method was successfully employed to perform the relationship fluorometric changes of **1** and the concentration of Fe^{3+}. The future efforts will be focused on the structural modification of rhodamine-based probe in order to elaborate special metal ion sensing applications in completely aqueous system.

SUPPLEMENTARY MATERIAL

IR, ^{1}H-NMR, ^{13}C-NMR spectra of probe 1, as well as the evaluation of its fluorescence intensity can be found at http://quimicanova.sbq.org.br, in pdf format with free access.

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