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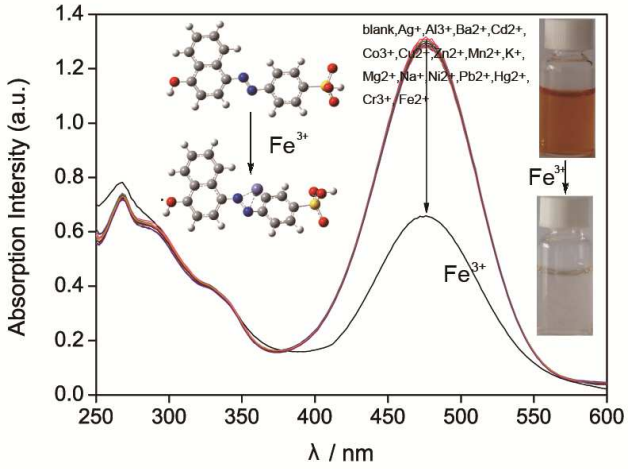
A multifunctional dye, 4-(4-hydroxy-1-naphthylazo)benzenesulfonic acid (**HNABA**) was identified and synthesized. The dye, combining hydroxyl group, azo group and carboxyl group, possessed excellent optical absorption properties changing with pH, solvents and coexisted metal ions. Especially, its spectral property kept tremendously stable under acid or neural conditions and was effectively applied for colorimetric determination of Fe^{3+} from brick-red to light red in aqueous under the physiological pH condition (pH 7.0). Under the optimum conditions, the detection possessed a linear range of 9.5~400×10^{-8} mol·L^{-1} with a correlation coefficient (R) of 0.9938 and a limit detection (3σ, n=20) of 4.2 ×10^{-9} mol·L^{-1}. The relative standard deviation (R.S.D.) was lower than 3.5% (n=5). The proposed method was successfully to determinate trace Fe^{3+} in three real environmental water samples. The action mechanism between **HNABA** and Fe^{3+} ion was discussed in detail.



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A multifunctional dye, 4-(4-hydroxy-1-naphthylazo)benzenesulfonic acid (**HNABA**) was identified and synthesized. The dye, combining hydroxyl group, azo group and carboxyl group, possessed excellent optical absorption properties changing with pH, solvents and coexisted metal ions. Especially, its spectral property kept tremendously stable under acid or neural conditions and was effectively applied for colorimetric determination of Fe^{3+} from brick-red to light red in aqueous under the physiological pH condition (pH 7.0). Under the optimum conditions, the detection possessed a linear range of 9.5~400×10^{-8} mol·L^{-1} with a correlation coefficient (R) of 0.9938 and a limit detection (3σ, n=20) of 4.2 ×10^{-9} mol·L^{-1}. The relative standard deviation (R.S.D.) was lower than 3.5% (n=5). The proposed method was successfully to determinate trace Fe^{3+} in three real environmental water samples. The action mechanism between **HNABA** and Fe^{3+} ion was discussed in detail.

1. Introduction

To detect metal ions has continually attracted tremendous attention in recent years due to its importance in environment, biology and chemistry domains. Iron, the third most abundant element in the earth's crust, is not only a very important macro-element in the environment, but also an essential mineral nutrient for human health.[1, 2] People who lack of iron will be suffering from iron deficiency anemia and other serious impact on human health, while excess iron would cause an increased risk of cancer, heart disease and other illnesses such as haemochromatosis.[3-8] Therefore, there is an urgent need to develop chemical sensors that are capable of detecting and monitoring iron levels in environmental samples. Considerable efforts have been made to develop various detection methods, such as fluorescent spectrometry,[9-14] electro-chromic techniques[15, 16] flow injection spectro-photometry,[17, 18] chromatography,[19, 20] mass spectrometry,[21] nuclear magnetic resonance,[22] and inductively coupled plasma mass spectrometry.[23] However, these instrumentally intensive methods often require utilizing sophisticated instrumentation or complicated pretreatment procedures, and are not suitable for on-line or in-field monitoring. By virtue of its simplicity, rapidity, non-destructive characteristics and the distinguishable ability by naked eyes instead of the complex instruments especially,[24-29] colorimetric sensors for Fe^{3+} have attracted considerable attention in recent years.[30-33] Typically, Yun, *et al*.[31] presented an easy naked-eye detection method of Fe^{3+} with a detection limit of 0.024 μg·mL^{-1}, based on 1-nitroso-2-naphthol, an excellent color-forming chelating agent. Adebayo, *et al*.[34] found a novel 8-hydroxyquinoline-based colorimetric sensor for the simple and rapid determination of Fe^{3+} using the reaction of Fe^{3+} with the sensor to form a metaloxine complex in chloroform solution. Wallace, *et al*.[30] developed a system that was able to detect low levels of Fe^{3+} using a squaraine dye to model on siderophores. All these confirm that organic colorimetric sensors are a promising, easy and practical strategy for detecting Fe^{3+}. However, the sensitivity of common colorimetric sensors is still lower than that of instrumentally intensive methods mentioned above. To develop novel and efficient colorimetric sensing materials remains a big challenge for a long time in the future.

Herein, to improve the sensitivity and selectivity of Fe^{3+} detection in aqueous, a multifunctional dye, 4-(4-hydroxy-1-naphthylazo)benzenesulfonic acid (**HNABA**), was identified and applied for Fe^{3+} detection practically after its optical properties were studied in detail. The multifunctional dye was expected to possess high selectivity and sensitivity to Fe^{3+} using both hydroxyquinoline group[31] and –N=N– group[35, 36] as chelating groups and to further increase the solubility in aqueous solution combining $–SO_3H$, –OH and –N=N– group. The action mechanism between **HNABSC** and Fe^{3+} was discussed by means of Job's plots and theoretical calculation.

2. Experiments

2.1 Reagents and apparatus

All the chemicals in the experiment were of AR grade and used as received from Sinopharm Chemical Reagent Co. Ltd. Water used throughout was doubly deionized.

A 1.0 mmol·L^{-1} Fe^{3+} standard solution for testing was prepared in doubly deionized water at room temperature and diluted to appropriate concentration daily. **HNABA** was synthesized according to our previous work[37, 38] and a 5.0×10^{-3} mol·L^{-1} **HNABA** stock solution was prepared in doubly deionized water at room temperature and stored at 4°C. Phosphate buffers (PB) were prepared by mixing a 0.01 mol·L^{-1} H_3PO_4 solution, a 0.01

mol·L^{-1} K_2HPO_4 solution, a 0.01 mol·L^{-1} KH_2PO_4 solution or a 0.01 mol·L^{-1} KOH solution in a proper ratio to acquire the desired pH (pH = 3.0, 4.0, 5.0, 6.0, 7.0, 7.5, 8.0, 9.0, 10.0).

FTIR spectra of **HNABA** with KBr disc were acquired using a Nicolet NEXUS 870 FTIR spectrophotometer at room temperature from 4000-500 cm^{-1}. ^{1}H NMR spectra were recorded using a Bruker AMX-500 spectrometer operating at 400 MHz, with tetramethyl-silane (TMS) used as the reference and D_2O as solvent. Elemental analysis was conducted using an Elemental Vario EL-III apparatus. UV-vis spectra were recorded on a Lambda 35 UV/Vis spectrometer using a 1-cm square quartz cell. pH was measured by a PHS-25pH meter.

2.2 Preparations of HNABA

According to the literatures,[37, 38] *p*-amino benzenesulfonic acid (0.87 g, 5 mmol) was dissolved in an ice-water solution of 15% sodium nitrite (0.38 g, 5.5 mmol). After cooling to 0 °C, the solution was added to concentrated hydrochloric acid (1.2 mL) and stirred for 30 min. The excess nitrous acid was destroyed with about 5 mg urea. The mixture was then added dropwise to 10 mL buffered aqueous solution (KH_2PO_4/ Na_2HPO_4, pH=6) containing naphthol (0.73 g, 5 mmol) and stirred for another 2 h at 0~5 °C. The resultant precipitate was filtered and purified by recrystallizing three times from ethanol to provide dark red crystal **HNABA** in the yield of 92.1 %.

IR (KBr), υ (cm^{-1}): 3433~2500 (OH, SO_3H), 1594, 1518 (Ar), 1376 (Ar-N(R_1R)), 1169 (S-O). ^{1}H-NMR (D_2O, 400 Hz) δ (ppm): 8.74 (d, J=8.5 Hz, 1 H, Ar-H), 8.61 (d, J=8.4 Hz, 1 H, Ar-H), 8.40 (d, J=8.4 Hz, 1 H, Ar-H), 8.12 (d, J=7.8 Hz, 1 H, Ar-H), 8.06 (d, J=7.8 Hz, 1 H, Ar-H), 7.48 (t, J=6.4 Hz, 1 H, Ar-H), 7.51 (t, J=6.7 Hz, 1 H, Ar-H), 7.15 (t, J=6.5 Hz, 1 H, Ar-H), 5.15 (s, 1 H, -OH). Anal. Calcd for $C_{16}H_{12}N_2SO_4$: H, 3.68; C, 58.53; N, 8.51; S, 9.77. Found: H, 3.73; C, 58.49; N, 8.50; S, 9.79.

2.3 Fe^{3+} detection procedure

For the Fe^{3+} determination, 1.0 mL PB (pH 7.0), 1.0 mL 5.0×10^{-4} mol·L^{-1} of **HNABA** and 1.0 mL of the appropriate Fe^{3+} solution or sample were transferred into a 10 mL volumetric flask. The mixture was stirred thoroughly and finally diluted to 10 mL with doubly deionized water. After 20 min, the absorption spectra were measured from 200 nm to 600 nm and the band-slit was set as 2.0 nm. The absorption intensity difference at 478 nm was used for quantitative analysis. The decreased absorption intensity of **HNABA** was represented as $\Delta A = A_0 - A$, where A_0 and A were the absorption intensities of the systems in the absence and presence of Fe^{3+}, respectively.

3. Results and discussion

3.1 The UV–vis absorption spectrum of HNABA

It is well known that **HNABA** is a strong polar compound, whose solubility is quite low in nonpolar solvents. To illustrate the effect of solvents on the absorption spectrum of **HNABA**, the UV-vis spectra in different polar solvents, *i.e.*, N,N-dimethyl formamide (DMF), tetrahydrofuran (THF), acetone, ethanol and water were recorded as shown in **Fig. 1**.

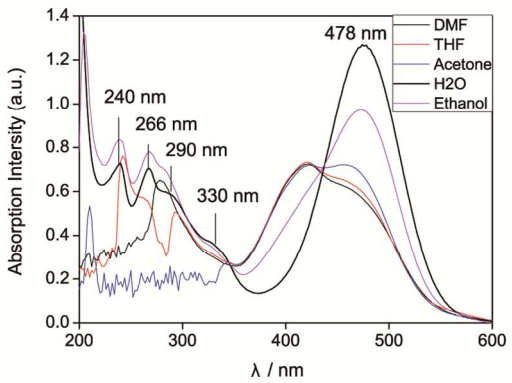

Fig. 1 The UV–Vis spectra of **HNABA** in nonpolar solvents, DMF, THF, Acetone, water and Ethanol

In Fig. 1, we can find that **HNABA** has a strong and sharp absorption peak at *ca*. 478 nm in polar protic water and ethanol, attributed to the whole molecular π-conjugated system, with a molar absorptivity (ε) of 2.54 ×10^4 L·mol^{-1}·cm^{-1} and 1.95 ×10^4 L·mol^{-1}·cm^{-1}, respectively, meaning that **HNABA** exists in the form of monomolecule, *i.e.*, polar protic water and ethanol are both good solvents for **HNABA**. While in polar aprotic acetone, THF and DMF, the absorption intensity at *ca*. 478 nm decreases and a new blue-shift absorption peak emerges at *ca*. 420 nm. The absorption gets wide and blue-shift might be attributed to the H-aggregation of **HNABA** in the poor solvents as acetone, THF and DMF.[39, 40] The conclusion could be further confirmed by the fact that the absorptions at *ca*. 240, 266, 290, 330 nm appear clearly in polar protic water and ethanol while decrease or disappear almost in polar aprotic acetone, THF and DMF, attributing to π→π*, n→π* transitions of C=C, N=N and O=C bonds in non-conjugated benzenesulfonic acid and naphthol moieties.

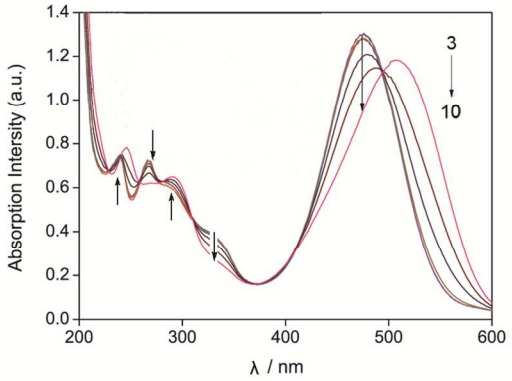

Fig. 2 Effect of pH on the UV–Vis spectra of **HNABA** (From up to down: 3.0, 4.0, 5.0, 6.0, 7.0, 7.5, 8.0, 9.0, 10.0)

For an acidic **HNABA** molecule, pH of the system will have a more significant influence on its existing formation and so make its absorption spectrum changed. Here the effect of pH on the absorption spectrum was investigated in polar protic water with a pH range of 3.0~10.0. As shown in **Fig. 2**, it is interesting to find that the absorption at 478 nm keeps quite stable as long as pH is

less than 7.0. When pH is more than 8.0, the absorption intensity at 478 nm decreases gradually and red shifts. The reason may be that SO_3H and OH groups in **HNABA** molecules all change into SO_3^- and O^- ions under basic conditions, which would enlarge the whole molecular π-conjugated system, but decrease the molecular dipole moment. The same phenomenon is also found in the absorbance at *ca*. 240, 266, 290, 330 nm as shown in Fig. 2, meaning that **HNABA** could possess the best optical absorption property under a wide pH range, *i.e.*, physiological conditions (pH *ca*. 7.0) will be selected in the next experiments.

3.2 Special response to Fe^{3+}

To demonstrate the selectivity of **HNABA** sensing to Fe^{3+}, we had investigated its colorimetric response to some other environmentally relevant metal ions, *i.e.*, Ag^+, Al^{3+}, Ba^{2+}, Cd^{2+}, Co^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, K^+, Mg^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Hg^{2+}, Cr^{3+} and Fe^{2+} at high concentrations in aqueous solutions at pH 7.0. After the addition of 100 equiv (4.0×10^{-4} mol·L^{-1}) different metal ions above and Fe^{3+} (4.0×10^{-6} mol·L^{-1}), the relative changes in absorption intensities of the sensing system were recorded as shown in **Fig. 3**, respectively. It can be seen from Fig. 3 that the absorption intensities of the **HNABA** sensing system in the presence of Ag^+, Al^{3+}, Ba^{2+}, Cd^{2+}, Co^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, K^+, Mg^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Hg^{2+}, Cr^{3+} and Fe^{2+} show negligible change even with 100-fold higher concentrations than that of Fe^{3+} and the alterations of ΔA are all less than 5% (detection error). The results indicate that **HNABA** possesses excellent selectivity to Fe^{3+} even in the presence of other coexisting metal ions under very high concentrations.

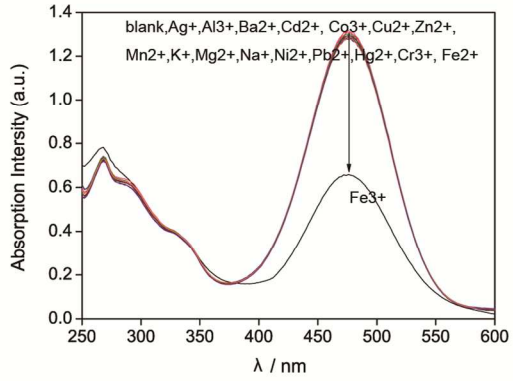

Fig. 3 Effect of different metal ions on the UV–Vis spectra of **HNABA**

To illustrate the response speed of **HNABA** to Fe^{3+} and the stability of the proposed system, the effect of incubation time on the absorption intensity was also investigated. The results show that a maximum and constant ΔA is reached after all reagents are added and incubated for *ca*. 20 min at room temperature. ΔA remains constant for more than 1 h, implying that the **HNABA** sensor to Fe^{3+} is stable and reliable.

The influence of ionic strength on ΔA of the system at 478 nm was also investigated by varying NaCl concentrations from 1.0×10^{-2} mol·L^{-1} to 1.0×10^{-6} mol·L^{-1}. It is worthwhile noting that all the ion strengths tested have no obvious effect on ΔA, hinting that the target sensing system is quite stable and may be applied in various kinds of surroundings.

3.3 Analytical parameters and samples detection

Fig. 4 shows the colour change and the absorption spectra of **HNABA** at different concentrations of Fe^{3+} between 9.5~400 $\times10^{-8}$ mol·L^{-1}. From Fig. 4a, it is easy to find that the colour of the sensing system changes from brick-red to light red, which can be detected by naked eye. Also, the calibration graph, the detection limit and precision for Fe^{3+} detection are obtained under the optimal conditions from Fig. 4b and 4c. A linear relationship between ΔA and Fe^{3+} concentration is exhibited in the range of 9.5~400 $\times10^{-8}$ mol·L^{-1} with a correlation coefficient of 0.9938. The regression equation is $A = -0.04834\times10^{-4} + 1.37\times10^{-3}$ c. Based on the definition of detection limit, three times of average deviation of UV absorption at 478 nm in 20 blank samples without Fe^{3+} divided by the slope absolute value of the standard curve in Fig. 4c here, the limit of detection (LOD) for Fe^{3+} is up to 4.2×10^{-9} mol·L^{-1}.

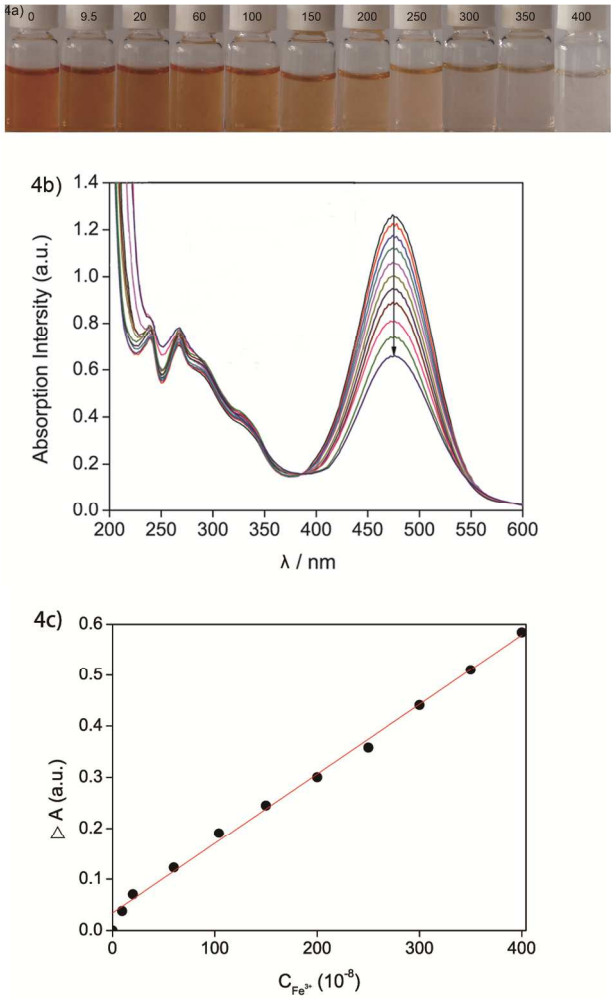

Fig. 4 The colour change (4a) and the absorption spectra (4b) of **HNABA** in different Fe^{3+} concentrations (From up to down: 0, 9.5, 20, 60, 100, 150, 200, 250, 300, 350, 400 $\times10^{-8}$ mol·L^{-1}); and the linear relationship between the ΔA of **HNABA** at 478 nm and $c_{Fe^{3+}}$ (4c)

To confirm its feasibility, the proposed method was applied to determine Fe^{3+} in 3 environmental water samples from the Pi River, underground water and tap water in campus, respectively (**Table 1**). All the samples were obtained by filtering several

times and concentrated by 100 times before testing. For recovery studies, some known concentrations of Fe^{3+} were added to the environmental water samples and the total Fe^{3+} concentrations were determined following the method proposed above. The recoveries of different known amounts of Fe^{3+} spiked were obtained from 97.8 % to 102.2% with a satisfying analytical precision (R.S.D. ≤ 3.5 %).

Table 1 Determination results for environmental water samples (n=5) [a]

Samples[b]	C_{Fe}^{3+} in sample[b] (nM)	Spiked (nM)	Found (nM)	Recovery (%)	R.S.D. (%)
1 (the Pi River)	788.5	500.0	1277.7	97.8	2.2
2 (underground)	985.2	500.0	1496.0	102.2	3.5
3 (pure water)	0.00	500.0	492.8	98.6	1.3

a. PB, pH 7.0.

b. The environmental water Fe^{3+} concentration determined using **HNABA** with the proposed method. The real values are the table values $\times 10^{-2}$ nmol·L^{-1} for the detected water samples were concentrated 100 times.

3.4 Action mechanism between HNABA and Fe^{3+}

To investigate the nature of the bonding between **HNABA** and Fe^{3+}, the binding stoichiometry of **HNABA** with Fe^{3+} was determined by using Job's plot. For the Job plot analyses, a series of solutions with varying mole fraction of Fe^{3+} were prepared by maintaining the total **HNABA** and Fe^{3+} concentration constant (6.0×10^{-5} mol·L^{-1}). The absorption intensity at 478 nm was measured for each solution. As shown in **Fig. 5**, a 1:1 stoichiometry for the complex between **HNABA** and Fe^{3+} can be drawn from Job's plots,[41] which confirms that Fe^{3+} might coordinate with nitrogen atoms in –N=N bonds or with oxygen atoms in naphthol rings.

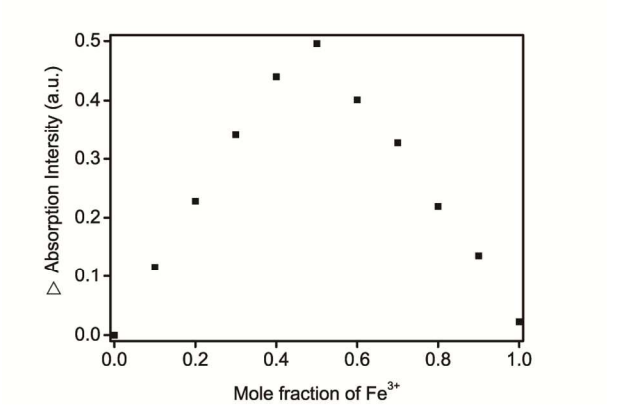

Fig. 5 Job's plot of **HNABA**-Fe^{3+} system. [**HNABA**] + [Fe^{3+}] = 8.0×10^{-5} mol·L^{-1} in aqueous at pH 7.0

Theoretical calculations have been carried out to further understand the nature of the bonding between **HNABA** and Fe^{3+}. The structures of **HNABA** before and after coordinating with Fe^{3+} were shown in **Fig. 6**, which were optimized using the the B3LYP/6-31G level of theory and method implemented in the Gaussian 03 suite of program.[42] From the results, we can find easily that the terminal phenyl rings distorted greatly, once the binding of Fe^{3+} with nitrogen atoms in –N=N bonds in **HNABA**, which resulted in the original conjugated system destroyed and so the absorbance at 478 nm deduced and even quenched.

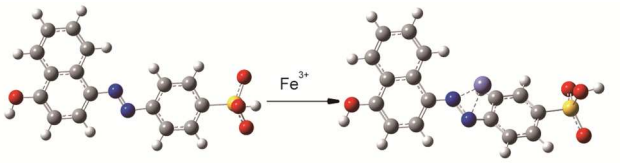

Fig. 6 Optimized geometries of **HNABA** before and after acted with Fe^{3+} ion. O, N, C, H, S and Fe atom are represented as red, blue, gray, white-gray, yellow and blue-purple, respectively

4. Conclusions

In conclusion, a multifunctional dye, 4-(4-hydroxy-1-naphthylazo) benzenesulfonic acid (**HNABA**) possessed a strong absorption (ε = 2.54×10^{4} L·mol^{-1}·cm^{-1}) at *ca*. 478 nm in polar protic water and kept tremendously stable under acid or neural conditions. Based on the results, the dye was successfully developed for trace Fe^{3+} detection with high selectivity and sensitivity under the physiological pH condition (pH 7.0). The optimal test conditions were obtained (After 20 min incubation time at room temperature under pH =7.0, $c_{\mathbf{HNABA}}$ 5.0×10^{-5} mol·L^{-1} in water) by investigating the influences of solvent, pH, ion intensity and incubation time on detection sensitivity. The linear range to detect Fe^{3+} in aqueous environment was 9.5~400 $\times10^{-8}$ mol·L^{-1} with a detection limit of 4.2 $\times10^{-9}$ mol·L^{-1} and a correlation coefficient of 0.9938. The action mechanism of **HNABA** and Fe^{3+} ion was confirmed by means of Job's plots, experimental and theoretical deduction as well.

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Notes and references

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