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Chenyan Lv

1873578630@qq.com

Hunan Institute of Science and Technology

Bowen Hu

Hunan Institute of Science and Technology

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A Novel AIE-active Salicylaldehyde-Schiff Base Probe with Carbazole group for Al³⁺ Detection in Aqueous Solution

Chenyan Lv, Bowen Hu *

College of Chemistry and Chemical Engineering, Hunan Institute of Science and Technology, Yueyang 414006, Hunan Province, PR China.

Abstract: A donor-acceptor Schiff-base fluorescent probe BKS with chelation enhanced fluorescence (CHEF) mechanism was designed and synthesized via benzophenone(Acceptor), salicylaldehyde and carbazole(Donor) for Al³⁺ detection, which exhibited typical aggregation-induced emission (AIE) characteristic. BKS probe could provide outstanding selectivity to Al³⁺ with a prominent fluorescence “turn-on” at 545nm in a wide pH range of 2-11. Which was significantly lower than the standard of WHO^[1-2] guidelines for drinking water. By the Job’s plot, the binding stoichiometry ratio of probe BKS to Al³⁺ was determined 1:1. The proposed strategy offered a very low limit of detection at 1.486μM in THF/H₂O(V/V=1:4 HEPBS=10mM, pH=7.40) and a wider linear range of 0-500μM. Furthermore, the probe could hardly be interfered by other examined metal ions. The analysis of Al³⁺ in real water samples with propriate recovery (100.72~102.85) with a relative standard deviation less than 2.82% indicated the accuracy and precision of BKS probe and the great potential in the environmental monitoring of Al³⁺.

Keywords: aggregation-induced emission (AIE), fluorescent probe; turn-on; aluminium ion; Schiff base

Introduction

It’s well known that Aluminum (Al) is the third most abundant metallic element in the earth's crust, and is commonly found in nature as oxides, fluoride, and silicide.^[3] Compounds of aluminum have been widely used in aeronautics and astronautics, manufacturing, food processing and other fields, such as in production of computers, making aircraft fuselage frames, foods and food ingredients, clinical medicine, which have brought great convenience to human society^[4-5]. Nevertheless, the trace level of Al³⁺ ions in the environment can affect the growth of roots, which can interact with cell wall, cytoplasm and plasma membrane in acid soil. Moreover, it is verified that

aluminum can also affect human growth and development, impair intelligence, and have impartible relationship with Parkinson's disease, gastrointestinal problems and osteal porosis^[6-7]. The limit of aluminum in drinking water is 0.2 ppm ($\sim 7.41 \mu\text{M}$) set by World Health Organization (WHO). Therefore, it is essential to develop a rapid and sensitive method for detecting aluminum.

To date, some instrumental methods have been developed to detect lower concentrations of Al^{3+} , such as atomic absorption (AAS)^[8], electrochemiluminescence and electrochemical^[9], inductively coupled plasma atomic emission spectroscopy technique (ICP-AES)^[10] and inductively coupled plasma mass spectrometry (ICP-MS)^[11-12], while they commonly require time-consuming sample preparation and tedious operations, which are unsuitable for real-time analysis.^[13] Therefore, in recent years, various fluorescent probes for Al^{3+} ion detection have been attracted considerable interests due to their advantages such as high selectivity, real-time detection, and versatility^[14]. Compared with the fluorescent chemosensor with the aggregation-caused quenching (ACQ) effect, the probes with aggregation-induced emission (AIE) activity have been reported for turn-on detection of Al^{3+} ion by incorporating several receptor ligands such as Schiff base, pyridines, hydrazines, carboxylic acids, and sulfonate salts in different media^[15-18], which is attributed to the effective suppression of the non-radiative transition^[19]. Meanwhile, as the preference of Al^{3+} ion coordinating a sphere containing O and N as hard-base sites, Schiff base compounds exhibit higher fluorescence performance than solid state in organic solution and AIE medium, so they are often used in the designing of fluorescent probes^[20].

In the present work, a novel D-A Schiff based fluorescent probe BKS with AIE effect was designed and synthesized with the reactants including 4-nitro-(4'-fluorine)-benzophenone, carbazole and salicylic aldehyde. The as-prepared fluorescent probe BKS exhibits fluorescence "turn-on" response to Al^{3+} in THF/ H_2O (V/V=1:4 HEPBS=10mM, pH=7.40) solution with high selectivity and sensitivity within a wide pH range of 2-11. In addition, the as-prepared probe was successfully employed in actual water samples for the detection of Al^{3+} with satisfactory recovery.

2 Experimental

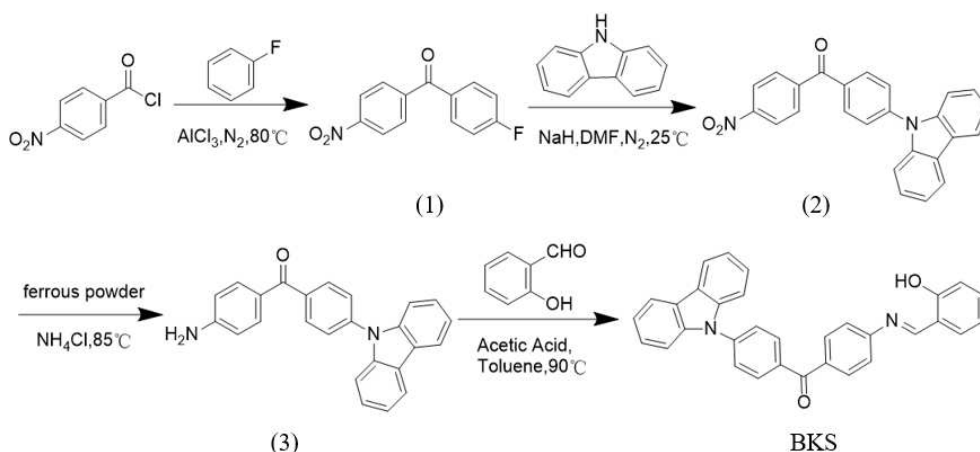
2.1 Materials and instruments

All chemicals and reagents were of analytical grade and used directly without further purification. ^1H NMR and ^{13}C NMR spectra were recorded on AVANCE III HD 400 NMR spectrometer (Brock, Switzerland). The infrared spectra were obtained through the American Nicolet AVATAR370 type infrared spectrometer. UV-Vis absorption was measured with a UV-2600 spectrophotometer. The fluorescence measurements were performed using a Hitachi F-700 fluorescence spectrometer (Japan). The pH values were measured with a PHS-3C (Shanghai Leici instruments Co., Ltd., China).

2.2 Synthesis and characterization of the probe (BKS)

The compound BKS was designed and synthesized as shown in Scheme 1. The compounds (1) ~ (3) were prepared according to the known method. The details were given in the Supplementary information (SI, Fig. S1).

Successively, a mixture of compound (3) (0.5 g, 1.38 mmol) and salicylic aldehyde (0.46 g, 4.14 mmol) was added in 150 mL single-port round-bottom flask with 20 mL toluene. When the mixture solution was heated to 50 °C, acetic acid (1 mL) was added dropwise, the solution was continuously stirred and refluxed for 24 hours at 110 °C. Then the residue was obtained after evaporation of solvents. Finally, the yellow solid product of 0.43 g (yield 68.5%) was obtained after recrystallization from anhydrous ethanol. ^1H NMR (400 MHz, Chloroform- d) δ 12.90 (s, 1H), 8.71 (s, 1H), 8.16 (d, J = 7.8 Hz, 2H), 8.09 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 7.9 Hz, 2H), 7.76 (d, J = 8.2 Hz, 2H), 7.54 (d, J = 8.2 Hz, 2H), 7.48 – 7.39 (m, 5H), 7.34 (t, J = 7.4 Hz, 2H), 7.05 (s, 3H). (SI, Fig. S2). ^{13}C NMR (101 MHz, Chloroform- d) δ 164.37, 161.32, 152.37, 141.71, 140.26, 136.05, 135.53, 134.00, 132.78, 131.82, 131.74, 126.36, 126.27, 123.87, 121.33, 120.67, 120.53, 119.42, 119.02, 117.48, 109.83. (SI, Fig. S3).



Scheme 1. The synthetic route of target compound BKS.

2.3 Fluorescence and UV-vis spectroscopic studies

A stock solution of the probe BKS (20 μM) was prepared in THF. 2 mM of different metal ions (Al^{3+} , Li^{+} , Na^{+} , K^{+} , Ag^{+} , Cu^{2+} , Fe^{3+} , Zn^{2+} , Mn^{2+} , Ca^{2+} , Mg^{2+} , Cd^{2+} , Cr^{2+} , Pb^{2+} , Ba^{2+} , Ga^{2+} , La^{3+} , Ni^{3+} , In^{3+} , Ce^{3+}) from their chloride salts were separately prepared in HEPBS buffer solution (THF/ H_2O (V/V=1:4 HEPBS=10mM, pH=7.40). In the UV-vis and fluorescence studies, the probe BKS and each metal ion used were 10 μM and 50 μM , respectively. The fluorescence titration experiments were conducted at 400 nm with emission slit of 10 nm, by adding the stock solutions of metal ions into the probe solution. Each measurement was performed in triplicate.

2.4 Analysis of Al^{3+} in real water samples.

All water samples were the surface water collected from a local river and lake belonging to the Dongting Lake area (located in Yueyang City, Hunan Province, China). Tap water sample was freshly collected from our laboratory. All water samples were tested after the addition of THF at the volume ratio of 1:4 and 10 μM BKS probe. Spiked samples were prepared as the same mention above, with the pH adjusted with 5 M NaOH. All results were expressed as mean values of at least three replicates in the experiments.

3. Results and discussion

3.1 Aggregation-induced emission (AIE) behaviors of BKS probe

Contrast to the phenomenon of aggregation-caused quenching (ACQ), the AIE activity shows no emission in dilute solutions but strongly emitted in the aggregate-state and solid^[21]. So for as-prepared BKS probe, the AIE property was firstly investigated. In our pre-experiments, fluorescence intensity of BKS probe was measured in six different pure organic solvents, respectively. The results revealed that the BKS probe was highly soluble and the strongest fluorescence emission intensity in THF (SI, Fig. S4). Therefore, the mixture of THF/H₂O with different content of water (% volume, f_w) was chosen as test solutions in the follow-up experiments. As shown in Fig. S5, the UV absorption of probe BKS gradually red shifted with the increase of water content and displayed the maximum shift of 30nm at 95% f_w , compared with that of the probe in THF. Then the typical test in mixed solution of THF/H₂O were executed and recorded the fluorescence emission spectra in Fig. 1a. BKS probe (2×10^{-5} M) initially exhibited quite weak luminescence. From $f_w = 70\%$, the fluorescence intensity rapidly increased and achieved the maximum at 95% f_w , as 6.4 times as that of the probe in THF. Moreover, the optical photo in Fig. 1b obviously illustrated BKS probe possessed AIE effect. The results above were attributed to the reduced solubility and the aggregation of the probe BKS with the increased water content, which led to the transformation of the D-A configuration of probe BKS from spatial torsion to planarized configuration, thus increasing the effective conjugate length. Moreover, in the aggregate state, due to the formation of intramolecular hydrogen bonds according to the hydroxyl group and N atoms, the rotation or vibration of the benzene ring was restricted (RIR)^[22-23], which blocks the non-radiative decay and favors the radiative transition, resulting in enhancing fluorescence.

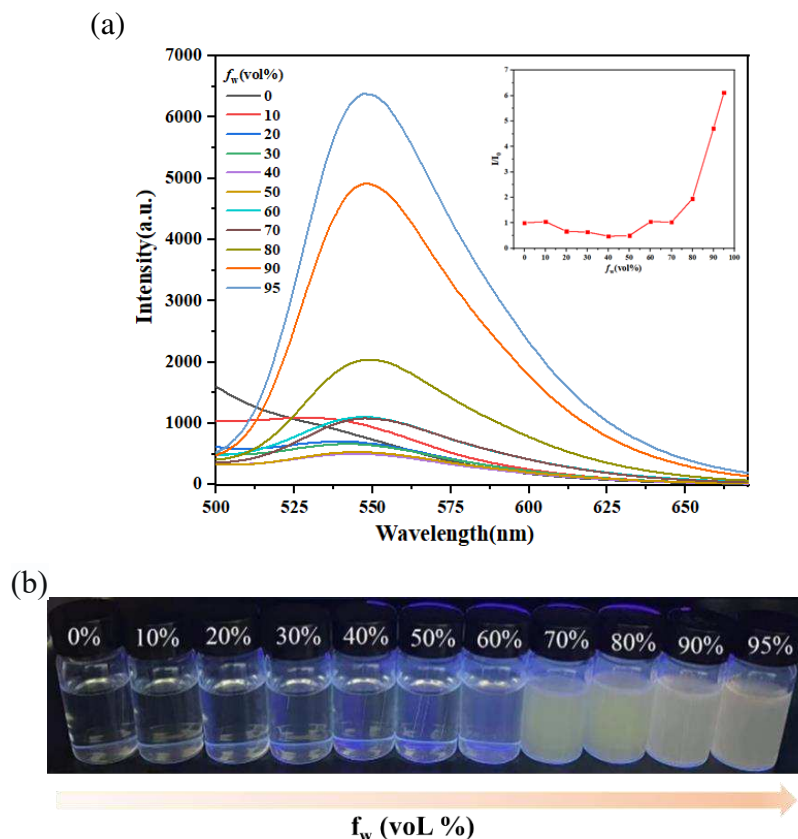


Fig.1 (a) Fluorescence emission spectra of BKS probe in THF/H₂O solutions ($f_w=0$ -95%) at 400 nm of excitation wavelength. Inset: Plots of FL intensity of BKS probe versus f_w in THF/H₂O mixtures; (b) the photos of BKS probe (10 μ M) under 365 nm UV light in different THF/H₂O mixed solution.

3.2 Fluorescent response of BKS to Al³⁺

The selectivity for AIE-active probes is very important because it is closely related to the efficiency of these chemical probe in itself. At first, the metal ion response performance of BKS (10 μ M) was investigated by UV-Vis absorbance and fluorescence spectra. As shown in Fig. S6, the UV absorption spectrum of the probe BKS had no obvious change with adding other metal ions, while a new absorption band was formed at 410 nm with addition of Al³⁺ ion. Herein, the red shift of absorption peak could be deducted that there might be the production of a new BKS-Al³⁺ complex. Moreover, the fluorescence intensities of all the tested cations other than Al³⁺ (in Fig.2a)

were found to be very weak at 545 nm, which was attributed to the isomerization of C=N bond by the excited state intramolecular proton transfer (ESIPT), the probe has weak fluorescence^[24]. As we all know, Al³⁺ is recognized as a hard acid and prefers to coordinate hard-base donors (N & O)^[25]. The O/ N-rich Schiff based fluorescent probes have rich hard alkaline centers and can provide binding sites for hard acid Al according to HSAB^[26]. The aluminum ion binding through O donor site and the C=N moiety of BKS restricted C=N isomerization^[27].

Therefore, chelated enhanced fluorescence (CHEF) can enhance the fluorescence of the probe. In the THF/H₂O solution of BKS, Al³⁺ had a strong fluorescence emission at 545 nm due to enhanced fluorescence by chelation, which may be the addition of Al³⁺ made the probe form a rigid structure, leading to the destruction of ESIPT characteristics^[28].

The addition of Al³⁺ to coordinate the non-bonded electrons in the C=N atom, thus inhibiting the isomerization of C=N and enhancing the fluorescence of the probe. The alignment of Al³⁺ and probe BKS inhibits the rotation of the C=N bond, thus inhibiting the ESIPT effect and enhancing the fluorescence of the probe, resulting in chelation-enhanced fluorescence effect (CHEF)^[29].

The probe selectively and sensitively recognizes Al³⁺ and inhibits the isomerization of the C=N bond by CHEF, enhancing the PL emission of the probe. The aluminum ion is a hard metal that can be coordinated with the C=N and O sites on the probe BKS, showing strong affinity. Therefore, BKS can selectively and sensitively recognize Al³⁺, which is a promising turn on-fluorescence sensor. (Scheme 2)

Meanwhile, the optical photo obtained under a 365 nm UV light directly certified the high fluorescence response of probe BKS to Al³⁺ in Fig.2c, only where Al³⁺ ion was presence, the dazzling white fluorescence appeared, while other ions produced no visible fluorescence, except Cu²⁺, Fe³⁺ solutions getting dark by completely quenching.

These results demonstrated that BKS has an excellent selectivity to Al^{3+} over other interested metal ions.

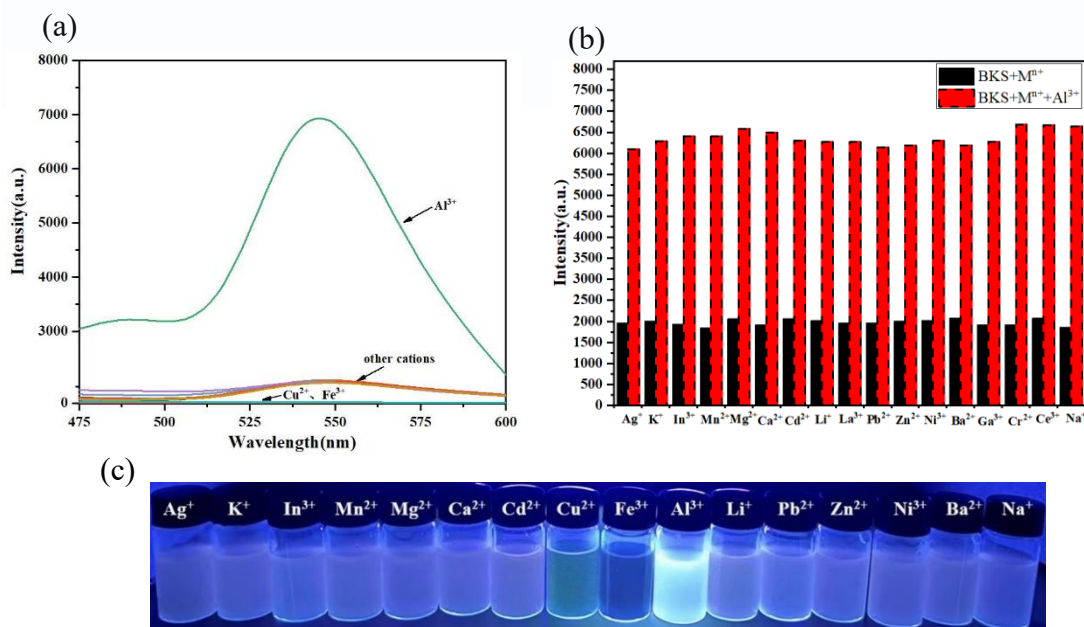
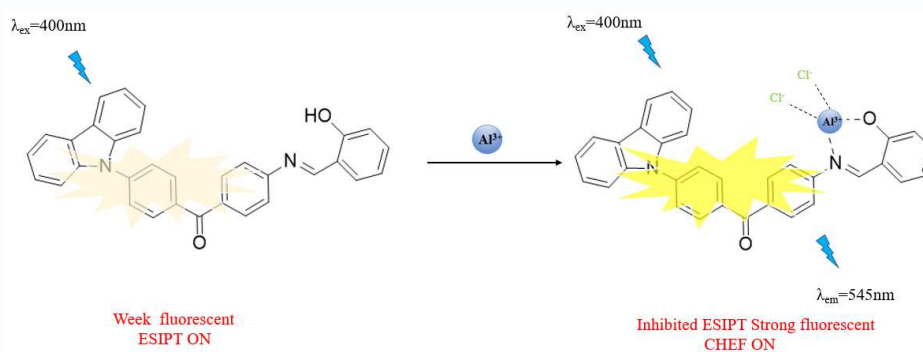


Fig. 2 (a) Fluorescence spectra of BKS probe (10 μM) upon addition of different metal ions (5.0 equiv.) in THF/H₂O (v/v=1:4 HEPBS=10 mM, pH=7.40); (b) Fluorescence response of adding Al^{3+} to the solution containing BKS and various ions (black bars are the solution of BKS With various cations, red bars are the solution after adding Al^{3+}); (c) visual fluorescence color changes of BKS probe (10 μM) in the presence of different metal ions under UV light at 365 nm.



Scheme 2 Mechanism of BKS response to Al^{3+} .

3.3 Competition studies

To further verify the anti-disturbance from other coexistent metal ions, the competition experiments for Al^{3+} were carried out by measuring the fluorescence intensity at 545nm after adding other competitive metal ions, respectively. The fluorescence emission spectrum was measured at an excitation wavelength of 400nm in the presence of aluminum ions and other ions. As depicted in Fig. 2b, the presence of other ions has no obvious influence on the determination of Al^{3+} . These results illustrated that the probe could detect Al^{3+} with high selectivity.

3.4 Fluorescence titration study and limit of detection (LOD) of aluminum ions

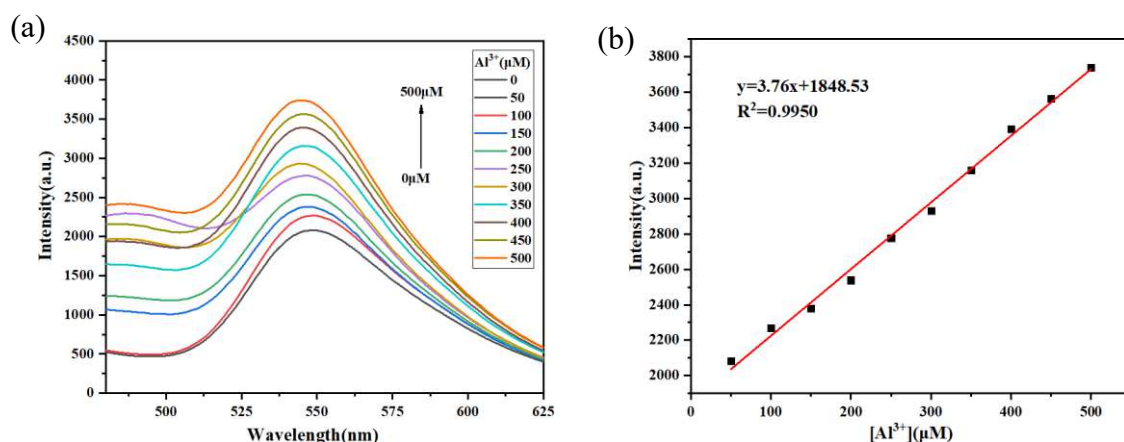


Fig. 3 (a) Fluorescence spectra of BKS at 545 nm with various concentrations of Al^{3+} (0-500 μM); (b) The linear relationship of the fluorescence intensity of BKS- Al^{3+} at 545 nm versus the concentration of Al^{3+} .

To check the sensitivity of sensor probe BKS for Al^{3+} ion, the fluorescence titration experiments were investigated in THF/ H_2O (v/v=1:4, HEPBS=10 mM, pH=7.40) solution. Probe BKS exhibited a strong emission peak at 545 nm with excitation at 400 nm. With the increase of Al^{3+} (0-500 μM) concentration, the fluorescence intensity of the probe gradually increased at 545 nm. When the amount of Al^{3+} was over 500 μM , the fluorescence intensity was not in the linear range. The fluorescence intensity of the probe BKS- Al^{3+} at 545 nm has a good linear relationship with the concentration of Al^{3+} ($R^2 > 0.99$, Fig.3b). The results indicate that detection of BKS- Al^{3+} can be used for quantitative analysis of Al^{3+} . With the increasing of Al^{3+} ion concentration, the fluorescence intensity gradually increased, with a slight blue shift from 545nm to 544nm. Based on the equation $\text{LOD} = 3\sigma/k^{[30-31]}$, the detection limit of

probe BKS- Al^{3+} for Al^{3+} was calculated to be 1.486 μM , indicating that probe BKS had a high sensitivity in identifying Al^{3+} .

3.5 pH effect on BKS with Al^{3+}

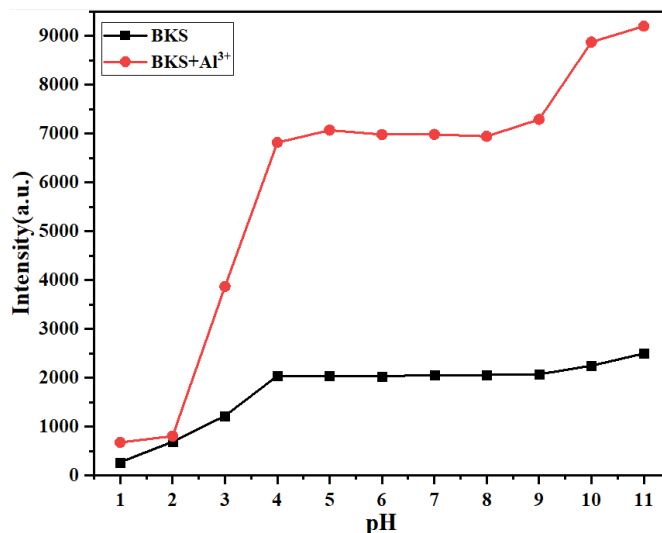


Fig.4 Scatter plot of fluorescence intensity at 545 nm with pH.

The influence of pH on the probe BKS and BKS- Al^{3+} systems was investigated. As shown in Fig. 4, the fluorescence spectrum of the probe BKS does not show any significant change in the pH range from 4.0 to 9.0. After the addition of Al^{3+} ions, a prominent fluorescence “turn-on” at 545nm in a wide pH range of 2-11, indicating that the change in pH has little effect on the probe and that the BKS can sensitively detect Al^{3+} ions over a wide range of pH.

3.6 Response time of probe BKS to Al^{3+}

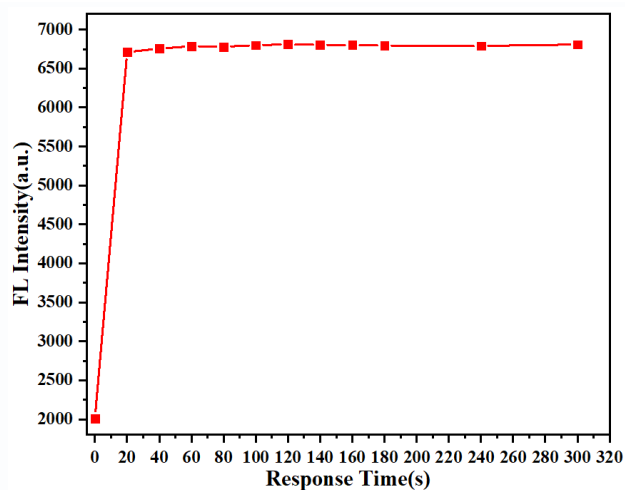


Fig.5 Response time spectra of fluorescent probe BKS to Al^{3+}

The interaction time between the probe BKS and Al^{3+} was investigated. As shown in Fig. 5, the fluorescence response strength of the probe BKS to the aluminum ion increases linearly with time within 0-20 s after the addition of the aluminum ion, and a stable fluorescence intensity can be reached within 20 s. After the addition of Al^{3+} , the fluorescence intensity of the probe BKS remained constant after 5 minutes of continuous irradiation, indicating that the BKS is stable enough to detect Al^{3+} .

3.7 Reversibility study

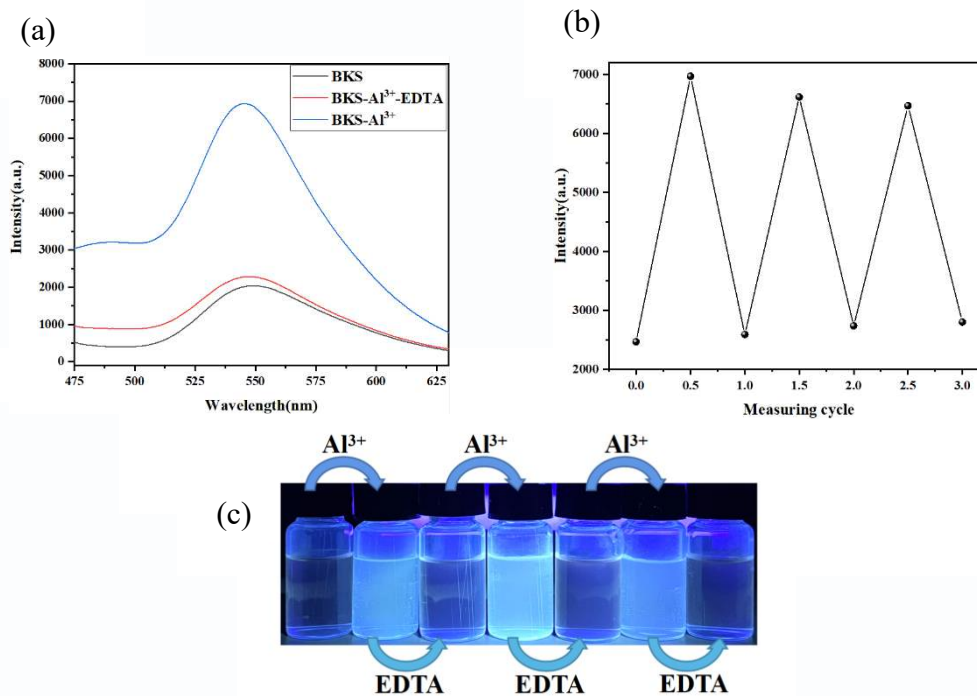


Fig.6 (a) Fluorescence spectra of reversibility study of probe BKS to Al^{3+} ; (b) Cyclic fluorescence diagram of fluorescence probe BKS after adding Al^{3+} ; (c) Fluorescence probe BKS was added to Al^{3+} under 365nm UV light

It is well known that the good reversibility is also a significant characterization for a fluorescent probe. The reversibility of BKS to Al^{3+} was determined by reversible experiment. As shown in Fig. 6, the fluorescence intensity is restored after EDTA is added to the BKS- Al^{3+} system, and it is enhanced again after Al^{3+} . When Al^{3+} and EDTA were added alternately, the fluorescence "on-off" response was clearly visible and repeated well (three times). This proves that the probe BKS has good invertibility.

3.8 Binding mechanism of probe BKS with Al^{3+}

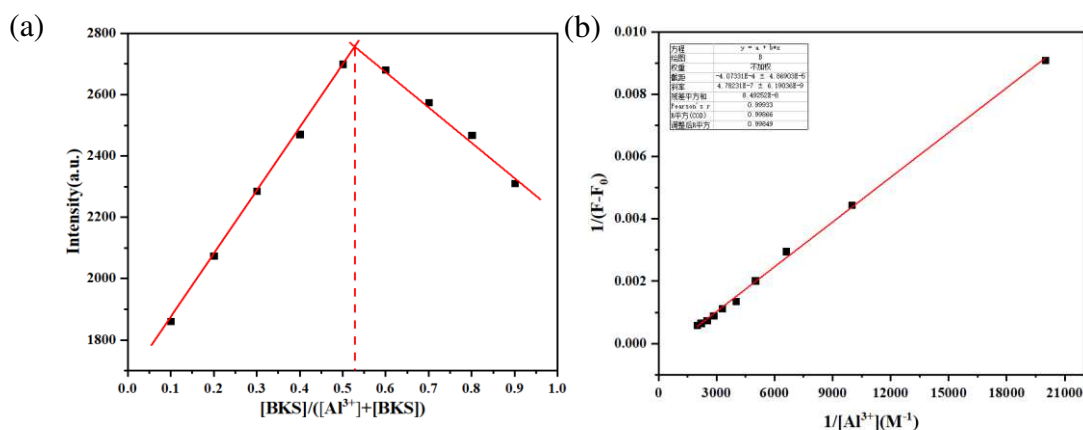


Fig.7 (a) Job's plot of probe BKS with Al^{3+} ; (b) Benesi-Hilbrand plot of probe BKS with Al^{3+} .

To determine the coordination stoichiometry between probe and Al^{3+} (BKS- Al^{3+}) a Job's plot analysis was carried out by continuous variation method^[32-33]. Finally, their absorption spectra were verified. The highest absorbance intensity was reached at a 0.52 mole fraction (Fig.7a) which designates that BKS- Al^{3+} complex formation happened with a 1:1 binding ratio. According to the modified Benesi-Hildebrand equation as followed (Fig.7b)^[34], the association constant (K_a) of probe BKS with Al^{3+} was calculated to be $0.477 \times 10^3 \text{M}^{-1}$.

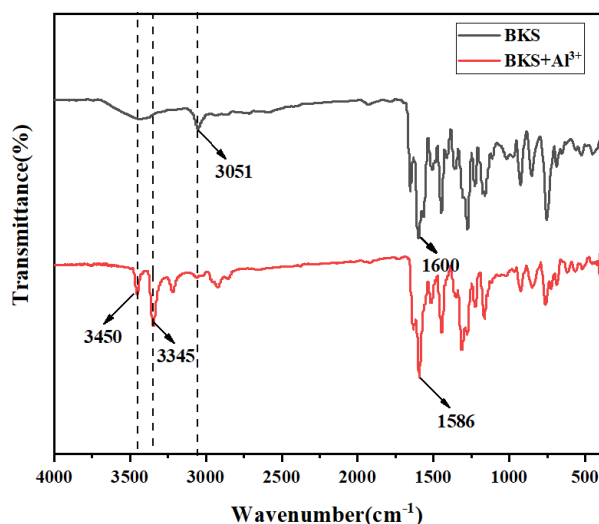


Fig.8 IR spectrum of probe BKS and probe BKS+Al³⁺.

To accurately master the combination detail of BKS with Al³⁺, FT-IR spectra were carried out in the absence and presence of Al³⁺ respectively^[35-36] (Fig. 8). It can be seen that -OH stretching vibration peak of the probe BKS appeared at 3051 cm⁻¹. However, BKS binding to Al³⁺ does not have a stretching vibration peak of -OH at 3051 cm⁻¹, but showed new stretching vibration peak at 3450 cm⁻¹ and 3345 cm⁻¹, which might be attributed to the disappearance of the -OH peak due to the combination of Al³⁺ with BKS. Meanwhile, it can be seen that compared with the infrared spectrum of BKS, the -N=CH- stretching vibration peak of the BKS binding to Al³⁺ were moved from 1600 cm⁻¹ to 1586 cm⁻¹, which might be attributed to the displacement of the -N=CH- peak due to the combination of Al³⁺ with BKS. The intensity were increased, and the intensity of the stretching vibration peak of -OH at 3450 cm⁻¹ and 3345 cm⁻¹ were increased.

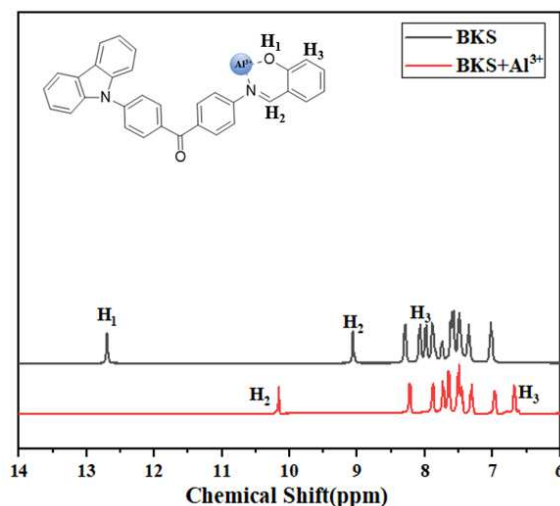


Fig. 9 ^1H NMR titration of BKS with Al^{3+} in DMSO-d_6 .

For further insight, an NMR study on BKS and its corresponding Al^{3+} complex was carried out in deuterated DMSO [37-38](Fig. 9). In the spectrum of BKS, the phenolic $-\text{OH}$ signal (H_1) is clearly visible at 12.70 ppm. The peak for the imine proton (H_2) appears at 9.07 ppm. In the spectra of the BKS- Al^{3+} complex, the $-\text{OH}$ (H_1) peak disappeared, and the aromatic protons (H_3) linked to hydroxyl group had a slight to higher field, indicating that phenol O atom coordinated with Al^{3+} ion through deprotonated oxygen atom. The movement of imine protons (H_2) towards the lower field indicates that the imine N participates in the coordination of Al^{3+} ions.

3.9 Applications

Water samples	Added (μM)	Detected ($\bar{x} \pm \text{SD}$)(μM)	Recovery (%)	RSD (%)
1	5	5.1 $\pm$ 0.12	101.08	2.455
	10	10.1 $\pm$ 0.14	100.89	1.405
2	5	5.0 $\pm$ 0.14	100.72	2.816
	10	10.1 $\pm$ 0.13	100.98	1.317
3	5	5.1 $\pm$ 0.06	102.85	1.206
	10	10.2 $\pm$ 0.11	101.25	1.051

Annotation: 1: Laboratory tap water;2: Dongting lake water;3: Drinking water

Table 1: Quantitative detection of Al^{3+} in real environmental samples by proposed sensor methods.

To understand and evaluate the application potential of probe BKS in the detection of Al^{3+} ions in ambient water, different water samples were collected from Dongting lake water, laboratory tap water and drinking water and were filtered for further testing. Fluorescence intensity of probe BKS was significantly enhanced at 545nm($\lambda_{\text{ex}}=400\text{nm}$). The recoveries of Al^{3+} in Dongting lake water are 100.72%~100.98%, 100.89%~101.08% in laboratory tap water and 101.25%~102.85% in laboratory drinking water. Therefore, probe BKS has high selectivity and specificity and can be used for monitoring Al^{3+} in all kinds of water environment.

4. Conclusion

A donor-acceptor Schiff base chemosensor comprising carbazole, benzophenone and salicylic aldehyde was developed for the selective recognition of aluminum ions in THF/H₂O system. Simple and inexpensive fluorescent probe exhibited a turn-on fluorescence response toward Al³⁺ at micromolar range over other metal ions. The Al³⁺ ion and probe coordination hindered the rotation of C=N isomerization, thus inhibiting the ESIPT effect. The CHEF effect predominated in the system, to enhance the fluorescence of the probe. The change in fluorescence intensity was such that the synthesized Schiff base exhibited “turn-on” mode of high sensitivity towards Al³⁺ ions. The limit of detection (LOD) of BKS and the binding constant (K_a) was found to be 1.486 μM and 0.477 × 10³ M⁻¹. The results of Job's plot and ¹H NMR confirmed that the complexation ratio of BKS to Al³⁺ was 1:1. Finally, the probe was used to detect Al³⁺ ions in environmental water samples, and it was found that it could be used to monitor Al³⁺ in various water environments.

Acknowledgements

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Conflict of Interest

There are no conflicts to declare.

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