

Highly Selective Detection of Cr(VI) in Water Matrix by a Simple 1,8-Naphthalimide-Based Turn-On Fluorescent Sensor

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Abstract 4-Bis-(2-hydroxyethyl)amino-*N-n*-butyl-1,8-naphthalimide was developed into a turn-on fluorescent sensor for Cr(VI). In the mixture of *N,N*-dimethylformamide and deionized water (1:9, v/v), the sensor could highly selectively distinguish CrO_4^{2-} from Cr^{3+} , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Mn^{2+} , Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , NO_3^- , SO_4^{2-} , PO_4^{3-} , IO_3^- , ClO^- and Cl^- by a 6-fold fluorescence enhancement. The working concentration of CrO_4^{2-} was from 0 to 90 μM with a detection limit of 3.6×10^{-7} mol/L. The detection could be carried out in water matrix and within a wide pH range (approximately from 2 to 12), and a large number of environmentally and biologically relevant ions including Cr^{3+} showed no significant interferences with the detection. The sensing mechanism was explored by reversibility and LC/MS analysis, as well as Job's plot experiment, and the results suggested that the recognition was based on the oxidation of the primary alcohol in the structure of the sensor by the Cr(VI) sources.

Keywords 1,8-Naphthalimide · Fluorescent · Probe · Sensor · Cr(VI)

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Introduction

Chromium is considered as one of the most dangerous heavy metals to environment and organism. Although trivalent Cr^{3+} is an essential trace element for human and of low toxicity, hexavalent Cr(VI) is about 100~1000 times more toxic than Cr^{3+} . Cr(VI) is highly soluble and biologically available in the ecosystems. It can easily enter human cells, make great damage on the liver, kidneys and other organs, and has high carcinogenicity [1–3]. Therefore, accurate and convenient detection methods for Cr(VI) is very necessary and meaningful. Many detection methods for Cr(VI) is reported, such as atomic absorption spectroscopy, inductively coupled plasma spectrometry, ion chromatography, spectrophotometry and so on [3–7]. However, some of these methods cannot distinguish Cr(VI) from Cr^{3+} and only provide the total amount of chromium, while others require complicated sample-pretreatment procedures.

In recent years, fluorescent sensors attract great attention in detection of various analytes because of their high sensitivity, rapid response, easy operation, low cost, in situ and real time monitoring, and noninvasiveness [8, 9]. Some nanoparticles were used as sensors to fluorimetrically measure Cr(VI) [1, 2]. Nevertheless, as compared to those for heavy metal ions like Cu^{2+} , Fe^{3+} , Hg^{2+} , Pb^{2+} and Cr^{3+} [9–16], fluorescent sensors for Cr(VI) were rare [17–20]. Hence, development of Cr(VI) fluorescent sensors are highly desirable.

1,8-Naphthalimide is a favorite reporter for fluorescent sensors [21, 22] owing to its high absorption coefficient, high fluorescence and quantum yield, large Stokes shift, good photostability and ease to modification. 1,8-Naphthalimide-based fluorescent sensors with new complicated structures are attractive. However, the research on new sensing performance of 1,8-naphthalimide derivatives with simple structures is also very important due to the better availability of the sensors. Amino and hydroxyl groups are potential Cr(VI)

receptor because they may be oxidized by Cr(VI), or form hydrogen bonds with $\text{Cr}_2\text{O}_7^{2-}$ or CrO_4^{2-} . Therefore, in this study, 4-bis-(2-hydroxyethyl)amino-*N-n*-butyl-1,8-naphthalimide (BHABN) was used as a fluorescent sensor for Cr(VI). BHABN has a simple structure and can completely distinguish Cr(VI) from Cr(III) and a large number of other cations and anions. The detection can be carried out in water matrix and within a wide pH range with high selectivity and sensitivity. $\text{K}_2\text{Cr}_2\text{O}_7$ was used as the Cr(VI) source, which could produce $\text{Cr}_2\text{O}_7^{2-}$ and CrO_4^{2-} in aqueous media at different pH. $\text{Cr}_2\text{O}_7^{2-}$ is stable in acidic condition, while CrO_4^{2-} in neutral and alkaline environments. Furthermore, CrO_4^{2-} can convert into $\text{Cr}_2\text{O}_7^{2-}$ under acidic environment, and vice versa [7, 17]. The detection of Cr(VI) was run in DMF/ H_2O (1/9, v/v), so CrO_4^{2-} was adopted to represent the Cr(VI) source hereinafter.

Experimental

Materials and Instruments

Materials

4-Bromo-1,8-naphthalic anhydride (BNA) (98 %) were supplied by Anshan HIFI Chemical Co., Ltd.. *n*-Butylamine (*n*-BA), diethanolamine (DEA) were bought from Sinopharm Chemical Reagent Co., Ltd.. The metal ions sources, NaCl, KCl, MgCl_2 , CaCl_2 , $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{FeCl}_2 \cdot 7\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$, HgCl_2 and $\text{Pb}(\text{NO}_3)_2$, and the anions sources, KIO_3 , $\text{K}_2\text{Cr}_2\text{O}_7$, Na_3PO_4 , NaNO_3 , NaClO and Na_2SO_4 , were provided by Sinopharm Chemical Reagent Co., Ltd.. All the reagents were used as received. The solvents used in synthesis were of analytical grade, others were spectroscopic grade. They were used without special treatment.

Instruments

LC-MS was collected on an Agilent 1200/6220 spectrometer (Agilent Co., USA). ^1H NMR spectra were carried out on a 400 MHz Varian Unity Inova spectrometer (Varian Co., USA). UV-vis spectra were obtained on a U-3900 spectrophotometer (Perkin-Elmer Co., USA). Fluorescence spectra were taken on a Fluoromax-4 spectrofluorometer (HORIBA Jobin Yvon Co., France). Melting point was determined on an X-6 Microscopic melting point tester (Beijing Tech Instrument Co., Ltd., China). pH values were measured on a Mettler-Toledo FE20 pH meter (Mettler-Toledo Co., USA). The pH values and the spectral measurements were performed at 25 °C unless otherwise specified.

Synthetic Procedures and Characterization Data

BHABN was synthesized by two-step reactions between 4-bromo-1,8-naphthalic anhydride (BNA), *n*-butylamine (*n*-BA) and diethanolamine (DEA), as shown in Scheme 1.

First, *N-n*-butyl-4-bromo-1,8-naphthalimide (BBN) was synthesized by the reaction between BNA and *n*-BA according to the literature [25]. Its melting point was 104–105 °C, which was in accordance with that reported in literature. Then, BBN reacted with DEA to afford BHABN, as described in the reference [26]. δ_{H} (400 MHz; CDCl_3 ; Me_4Si) 0.95 (3H, t, $J=8.0$ Hz), 1.41 (2H, m), 1.66 (2H, m), 3.60 (4H, t, $J=5.0$ Hz), 3.86 (4H, t, $J=5.0$ Hz), 4.08 (2H, t, $J=8.0$ Hz), 7.33 (1H, d, $J=8.0$ Hz), 7.58 (1H, t, $J=8.0$ Hz), 8.38 (1H, d, $J=8.0$ Hz), 8.41 (1H, d, $J=8.0$ Hz), 8.84 (1H, d, $J=8.0$ Hz) (Figure S1). LC-MS $[\text{M}+\text{H}]^+$: m/z , found. 357.1804 (Figure S2). Calcd. 357.1800.

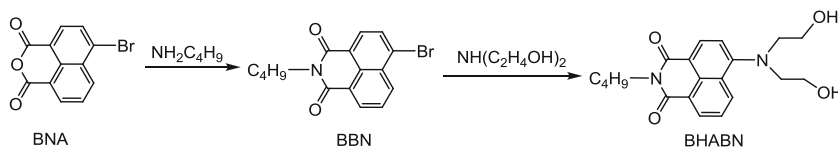
Methods

Sample Preparation

BHABN was dissolved in DMF to form 2 mM stock solutions. Anions and metal ions were dissolved in deionized water to get 10 mM stock solutions. When studying the fluorescence sensing behaviors of BHABN to metal ions and anions, 50 μL stock solution of BHABN was put into a 10 mL volumetric flask, mixed with 50 μL of each anion or metal ion stock solution, and diluted with DMF/ H_2O (1/9, v/v) in sequence. The concentration of BHABN and ions was 10 and 50 μM , respectively. In the UV-vis absorption and fluorescence titration, the mixed stock solutions of CrO_4^{2-} varied from 0–100 μL and the concentration of CrO_4^{2-} was 0–100 μM . When examining the fluorescence response of BHABN and BHABN/ CrO_4^{2-} system upon pH, a series of DMF/ H_2O (1/9, v/v) solutions of BHABN (10 μM) and BHABN (10 μM)/ CrO_4^{2-} (50 μM) with different pH values were prepared. The pH value was adjusted by 0.1 M sodium hydroxide and 0.1 M hydrochloric acid aqueous solutions. The used excitation and emission wavelengths of measurement were 440 and 542 nm, and slit width was 5 nm. The measurement was performed after 1.5 h.

Fluorescent Quantum Yield

When the fluorescent quantum yield (Φ_s) of BHABN was evaluated, the absorbance of the solutions was kept under 0.05 in order to make the testing results reliable. Φ_s was estimated from the absorption and fluorescence spectra of BHABN according to Eq. 1, where the subscript s and r stand for the sample and the reference (rhodamine B, $\Phi_r=0.97$ in ethanol), respectively. Φ is the quantum yield, A represents the absorbance at the excitation wavelength, S refers to the

Scheme 1 Synthetic route of BHABN

integrated emission band areas and n_D is the solvent refractive index.

$$\Phi_s = \Phi_r \frac{S_s A_r n_{Ds}^2}{S_r A_s n_{Dr}^2} \quad (1)$$

Calculation of the Detection Limit

The detection limit ($3\sigma/k$) was calculated based on the fluorescence titration [23, 24], where σ is the standard deviation of blank measurement, k is the slope of the linear fit lines in fluorescence titration. To determine σ , the emission intensity of BHABN in DMF/H₂O (1/9, v/v) without any metal ions was measured 5 times independently.

Analysis of Tap Water Spiked with Cr(VI)

In the analysis of tap water spiked with Cr(VI), 1 mL tap water from our lab in Dushu Lake Campus of Soochow University was added in 10 mL volumetric flask, then 50 μ L stock solution of BHABN (10 μ M), 60 μ L stock solution of Cr(VI) (60 μ M), and 67.8 μ L DMF were introduced. The mixed solution was diluted to volume with DMF/H₂O (1/9, v/v) and fluorescence spectra were recorded. The concentration of Cr(VI) in the samples was obtained from the linear relationship between the maximal fluorescence intensity of BHABN and the concentration of Cr(VI). Three parallel samples were tested.

Results and Discussion*Selectivity and Sensitivity of BHABN to Various Ions*

The UV–vis absorption response of BHABN (10 μ M) in DMF/H₂O (1/9, v/v) to multifarious metal ions and anions such as Na⁺, K⁺, Mg²⁺, Ca²⁺, Cr³⁺, Mn²⁺, Fe²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Pb²⁺, Cd²⁺, Hg²⁺, CrO₄²⁻, ClO⁻, NO₃⁻, SO₄²⁻, IO₃⁻, PO₄³⁻ and Cl⁻ (100 μ M) was firstly investigated. The results were showed in Fig. 1. Among different ions, only CrO₄²⁻ induced a small enhancement of the absorption peak at 440 nm, other cations and anions had no obvious influence in the UV–vis absorption spectra of BHABN. The peak around 370 nm belonged to the absorption of CrO₄²⁻.

Next the fluorescence response of BHABN to different cations and anions was studied. Upon addition of Na⁺, K⁺,

Mg²⁺, Ca²⁺, Cr³⁺, Mn²⁺, Fe²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Cd²⁺, Hg²⁺, Pb²⁺, CrO₄²⁻, ClO⁻, NO₃⁻, SO₄²⁻, IO₃⁻, PO₄³⁻ and Cl⁻ (100 μ M) to the solution of BHABN (10 μ M), only CrO₄²⁻ caused a significant fluorescence enhancement, as shown in Fig. 2. The maximum fluorescence intensity enhanced about 6 folds and the fluorescent quantum yield increased from 0.079 to 0.326. Other cations and anions, including Cr³⁺, showed almost negligible effect on the fluorescence profiles of BHABN. Accordingly, BHABN had high selectivity and sensitivity to CrO₄²⁻, and possibly can be used as a “turn-on” fluorescent sensor for Cr(VI) in DMF/H₂O (1/9, v/v).

Dependence of the Sensor’s Fluorescence on the Cr(VI) Concentration

In order to investigate the sensing behaviors of BHABN for Cr(VI) further, fluorescence titrations were conducted. The maximum fluorescence intensity (I) of BHABN was gradually enhanced with the addition of CrO₄²⁻ from 0 to 100 μ M (Fig. 3). Moreover, the fluorescence maxima increased linearly with the concentration of CrO₄²⁻ from 0 to 90 μ M (insets in Fig. 3). The linear regression equation was that $I = 39776.11164 + 23663.37278 [\text{CrO}_4^{2-}]$, with a correlation coefficient of 0.99686 ($n = 15$). The detection limit ($3\sigma/k$) based on the linear regression equation was 3.6×10^{-7} mol/L.

Effects of Competition Ions

To investigate the disturbance of the coexistent ions, 60 μ M of Na⁺, K⁺, Mg²⁺, Ca²⁺, Cr³⁺, Mn²⁺, Fe²⁺, Fe³⁺, Co²⁺, Ni²⁺,

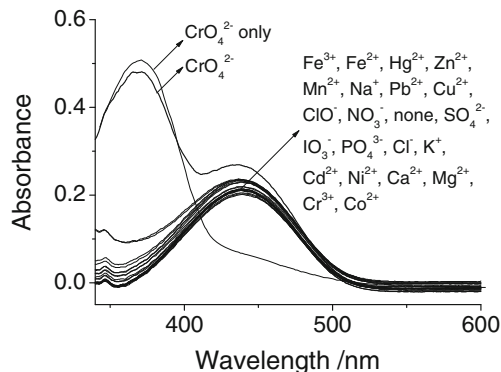


Fig. 1 UV–vis absorption spectra of CrO₄²⁻, BHABN and BHABN with various ions. Solvent: DMF/H₂O (1/9, v/v); c: 10 μ M for BHABN, 100 μ M for various ions

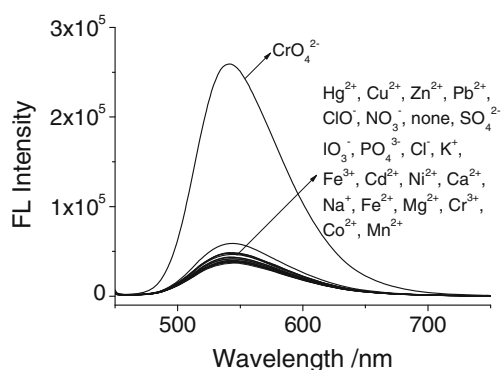


Fig. 2 Fluorescence spectra of BHABN and BHABN with various ions. Solvent: DMF/H₂O (1/9, v/v); c: 10 μM for BHABN, 100 μM for various ions; λ_{ex}: 440 nm; slit width: 5 nm

Cu²⁺, Zn²⁺, Cd²⁺, Hg²⁺, Pb²⁺, ClO⁻, NO₃⁻, SO₄²⁻, IO₃⁻, PO₄³⁻ and Cl⁻ was added into the solution of BTABN (10 μM) and CrO₄²⁻ (60 μM). Upon interaction with these ions, no pronounced changes were observed in the emission spectra except Fe³⁺ and Cr³⁺ enhanced the fluorescence of the solutions a little because of their higher acidity (Fig. 4). It is clear that BHABN has excellent anti-interference ability to miscellaneous background ions.

Effects of pH on the Detection of Cr(VI)

In order to examine the effect of proton to the detection of CrO₄²⁻, the fluorescence spectra of BHABN and BHABN/CrO₄²⁻ in DMF/H₂O (1/9, v/v) under different pH values were determined (Fig. 5). The maximum fluorescence intensity of BHABN and BHABN/CrO₄²⁻ both slowly decreased when the pH value increased from 2 to 12, but the maximal fluorescence intensity of BHABN/CrO₄²⁻ was remarkably stronger

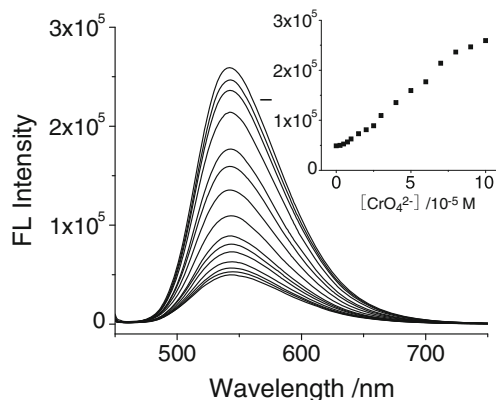


Fig. 3 Fluorescence spectra of BHABN (10 μM) with different concentrations of CrO₄²⁻. Solvent: DMF/H₂O (1/9, v/v), λ_{ex}: 440 nm, λ_F: 542 nm, slit width: 5 nm. From bottom to top, the equiv. of CrO₄²⁻: 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10. The insets showed the fluorescence maxima (*I*) vs the concentration of CrO₄²⁻

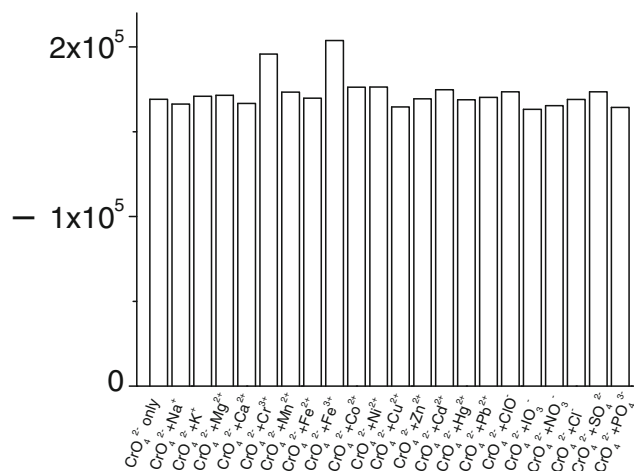


Fig. 4 Effects of coexisting ions on the fluorescence maxima of BHABN/CrO₄²⁻. Solvent: DMF/H₂O (1/9, v/v); c: 10 μM for BHABN, 60 μM for various ions; λ_{ex}: 440 nm, λ_F: 542 nm, slit width: 5 nm

than that of BHABN at the same pH value. Hence, BHABN can detect CrO₄²⁻ in a wide pH range (pH 2.56–12.16).

Time Response of BHABN to Cr(VI)

The time response of BHABN to Cr(VI) was illustrated in Fig. 6. The maximum fluorescence intensity of BHABN/CrO₄²⁻ gradually increased then plateaued after 1.5 h while the maximum fluorescence intensity of BHABN almost kept stable at a low level with time going. This indicated that the change of fluorescence intensity was caused by the interaction between BHABN and CrO₄²⁻ and BHABN can be used for the detection of Cr(VI) reliably.

Sensing Mechanism

The sensing mechanism was tentatively explored. Every one of Mn²⁺, Co²⁺, Ni²⁺, Cd²⁺, Hg²⁺, and Pb²⁺ could form a precipitation with CrO₄²⁻. However, the six cations induced no remarkable changes in the emission spectra of the BHABN/

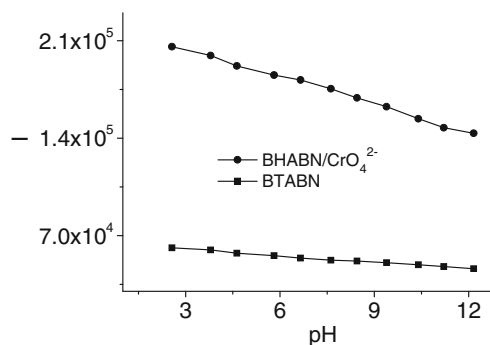


Fig. 5 Fluorescence response of BHABN and BHABN/CrO₄²⁻ to different pH values. Solvent: DMF/H₂O (1/9, v/v); c: 10 μM for BHABN, 60 μM for CrO₄²⁻; λ_{ex}: 440 nm, λ_F: 542 nm, slit width: 5 nm

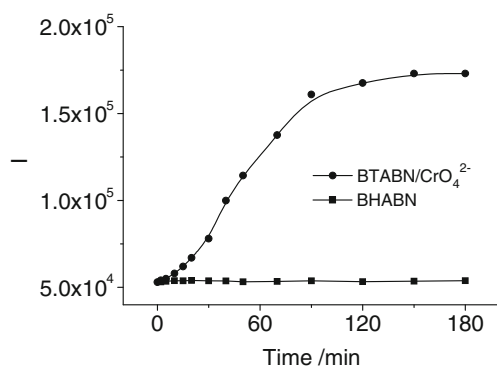


Fig. 6 Time response of BHABN to CrO_4^{2-} . Solvent: DMF/ H_2O (1/9, v/v); c: 10 μM for BHABN, 60 μM for CrO_4^{2-} ; λ_{ex} : 440 nm, λ_{F} : 542 nm, slit width: 5 nm

CrO_4^{2-} solution in the competition experiments (Fig. 4). This result implied that the recognition process of BHABN to CrO_4^{2-} was irreversible and chemical reactions between BHABN and CrO_4^{2-} possibly happened. The fact that the maximal fluorescence intensity of the BHABN/ CrO_4^{2-} solution almost linearly increased with the decrease of the pH value from 12 to 2 in accordance with the chemical-reaction mechanism because the Cr(VI) sources had stronger oxidation ability in acidic condition. The relatively slow time response also matched the chemical-reaction mechanism.

To verify the thoughts on the sensing mechanism further, LC-MS of the BHABN/ CrO_4^{2-} in DMF/ H_2O (1/9, v/v) was tested (Fig. 7). The peaks at m/z 100.9364 and 200.8515 were corresponding to $[\text{Cr}(\text{OH})_3\text{-}2\text{H}]^-$ and $[\text{Cr}(\text{OH})_3\text{+HCOOH+}3\text{H}_2\text{O-}2\text{H}]^-$. The peaks at m/z 311.1444, 398.1673, and 401.1764 were assigned to $[\text{BHABN-CH}_2\text{CH}_2\text{OH}]^-$, $[\text{BHABN+HCOOH-}4\text{H}]^-$, and $[\text{BHABN+HCOOH-H}]^-$ respectively. These results indicated the oxidation of BHABN by Cr(VI) and supported the chemical reaction-based sensing mechanism.

Fig. 7 LC-MS spectrum of BHABN (10 μM)/ CrO_4^{2-} (60 μM) in DMF/ H_2O (1/9, v/v)

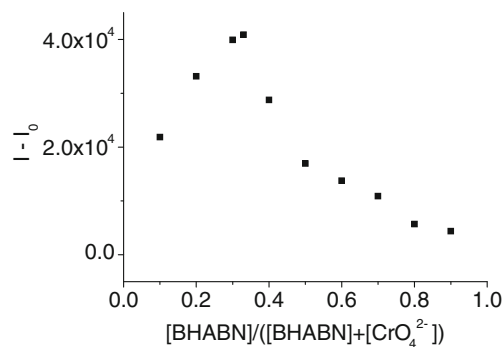
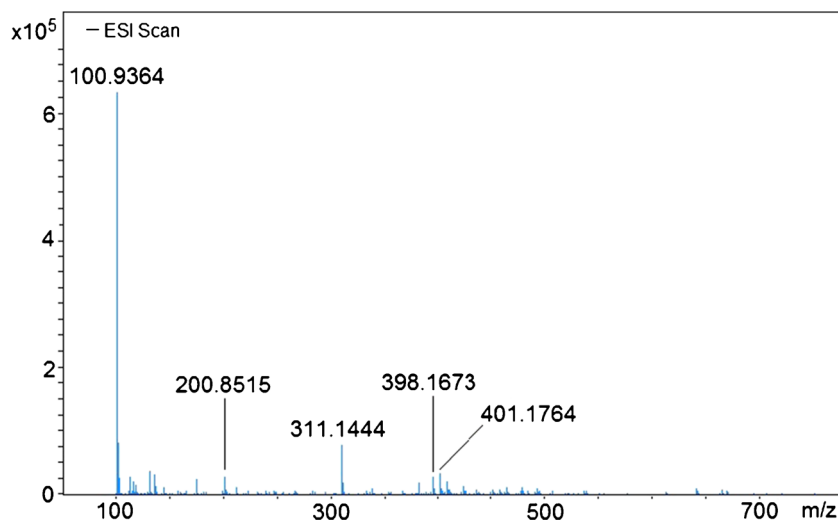


Fig. 8 Job's plot for BHABN versus CrO_4^{2-} with the total concentration ($[\text{BHABN}] + [\text{CrO}_4^{2-}]$) of 50 μM in DMF/ H_2O (1/9, v/v). λ_{ex} : 440 nm, λ_{F} : 542 nm, slit width: 5 nm

We also carried out Job's plot experiment and the result was exhibited in Fig. 8. The Job's plot did not show a satisfactory shape in which two lines with positive and negative slope usually crossed each other at the complex stoichiometry. This result may be ascribed to the complicated oxidation reactions. Therefore, we speculated that the turn-on fluorescent response of BHABN to Cr(VI) was based on the oxidation of the primary alcohol in the structure of BHABN by Cr(VI) sources. As a result, the electron-donating hydroxyls were turned into electron-withdrawing groups and the PET effect from the hydroxyls to the 1,8-naphthalimide fluorophore was suppressed, leading to the fluorescence enhancement.

Real Sample Analysis

An application of BHABN as a fluorescent sensor in analysis of tap water spiked with Cr(VI) was done. The detected concentration of Cr(VI) was 58.8 μM , close to that of the added Cr(VI) (60 μM). The recovery was 98.0 %. The relative standard deviation (RSD) of three parallel measurements was

2.5 %. These results indicate that BHABN has good accuracy in monitor Cr(VI) in environmental water.

Conclusions

In summary, a 1,8-naphthalimide derivative with a simple structure has successfully been developed into a turn-on fluorescent sensor for Cr(VI). The sensor can completely differentiate Cr(VI) from Cr³⁺ and various other cations and anions in DMF/H₂O (1/9, v/v), and has strong anti-interference ability to a large number of coexisting anions and cations including Cr³⁺. The sensor shows potential use in monitoring Cr(VI) in environmental and biological system with a detection limit of 3.6×10^{-7} mol/L. The response of the sensor to Cr(VI) may be resulted from the oxidation of the primary alcohol by Cr(VI) sources which weakens the PET effect from the hydroxyl to the fluorophore. This work will provide inspiration for the design of new Cr(VI) sensors.

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