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A Turn-On Fluorescence Chemosensor for Cyanide in Aqueous Media Based on a Nucleophilic Addition Reaction

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We synthesized a new cyanide (CN^-) chemosensor **CX** based on a nucleophilic addition reaction prompted by cyanide ion, which could be used for highly selective and sensitive fluorescence turn-on detection of cyanide in aqueous media. The **CX** showed selective fluorescence recognition for CN^-, the miscellaneous competitive anions (F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, S^{2-}, PO_4^{3-}, CO_3^{2-} and SCN^-) did not lead to any significant interference. The detection limit of the sensor towards CN^- is 1.15×10^{-7} mol•L^{-1}. The sensor has been successfully applied to estimate the cyanide ion in seeds of cherries. Test strips based on **CX** were fabricated, which could be used as a convenient and efficient CN^- test kit to detect CN^- in aqueous solution for "in-the-field" measurement.

Keywords cyanide, fluorescent, chemosensor, test kits, seeds of cherries

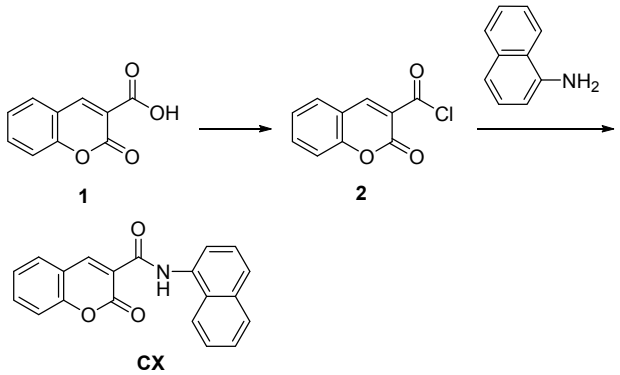
Introduction

The selective sensing of important anions is highly significant because anions are widely distributed and play important roles in both environmental and life sciences.[1-7] Many efforts have been made to design chemosensors for anions such as F^-, CN^-, Cl^-, $H_2PO_4^-$, SO_4^-, AcO^-, *etc*.[8-19] However, most of the synthetic receptors operate in nonprotonic organic solvents while few in aqueous solution, and they often require convoluted and time-consuming syntheses.[20-24] This is because it is particularly difficult to develop anion recognition systems that form hydrogen bonded complexes in aqueous solvent, for the obvious reason that the water competes strongly for the hydrogen bonding sites.

Cyanide is one among the toxic anions, cyanide ions could be absorbed through the lungs, skin, and gastrointestinal tract leading to vomiting, convulsions, loss of consciousness, and eventually death.[25] Despite their toxicity, their outstanding applications in various industrial processes including electroplating, metallurgy, heap leaching of gold ore, polymer synthesis, steel manufacturing and as raw materials for synthetic fibers, resins and herbicides are inevitable, which raise the risk of accidental or intentional release of cyanide ions into the environment as a toxic contaminant.[26] Therefore, a large number of good sensors for cyanide have been invented.[27-29] Sensitive, selective, simple, low-cost, and easy-to-prepare CN^- fluorometric chemosensors have become good choices for various applications.[30-32]

In view of this requirement and as a part of our research interest in ion recognition,[33-38] we attempt to develop an efficient optical chemosensor which can sense CN^- with specific selectivity and high sensitivity. Herein, we report a chemosensor **CX** based on a nucleophilic addition reaction prompted by cyanide ion, which could be used for highly selective and sensitive fluorescence turn-on detection of cyanide in aqueous media (Scheme 1).

Scheme 1 Synthesis of sensor molecule **CX**



Experimental

General information and materials

Fresh double-distilled water was used throughout the

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experiments, all other reagents and solvents for synthesis were commercially available at analytical grade and were used without further purification. All the anions were added in the form of tetrabutylammonium (TBA), which were purchased from Sigma-Aldrich Chemicals, and stored in a vacuum desiccator. ^{1}H NMR spectra were recorded on a Mercury-400BB spectrometer at 400 MHz. Chemical shifts are reported downfield from tetramethylsilane (TMS, δ scale with solvent resonances as internal standards). Electrospray ionization mass spectra (ESI-MS) were measured on an Agilent 1100 LC-MSD-Trap-VL system. UV-vis spectra were recorded on a Shimadzu UV-2550 spectrometer. Photoluminescence spectra were performed on a Shimadzu RF-5301 fluorescence spectrophotometer. Melting points were measured on an X-4 digital melting point apparatus (uncorrected). Infrared spectra were performed on a Digilab FTS-3000 FT-IR spectrophotometer.

Synthesis of sensor molecule CX

Coumarin-3-carboxylic acid (3 mmol) and bis(trichlormethyl) carbonate (BTC, 1.5 mmol) were added into dry dichloromethane (15 mL). Then the reaction mixture was stirred at 40 ℃ for 3 h under reflux. In this process, the coumarin-3-carboxylic acid has been converted to the coumarin-3-carbonyl chloride. The compound **2** (1 mmol), β-naphthylamine (1.2 mmol) and catalytic amount of acetic acid (AcOH) and triethylamine (20 mmL 2.0 g) were combined in hot absolute ethanol and tetrahydrofuran (25 mL). The solution was stirred under reflux for 4 h. After cooling to room temperature, the yellow precipitate was filtered, washed three times with hot absolute ethanol, then recrystallized with EtOH/H_2O to give a yellow powder product **CX** in 85% yield (m.p.＞300 ℃). ^{1}H NMR (DMSO-d_6) δ: 10.88 (s, 1H, NH), 8.97 (s, 1H, ArH), 8.43 (s, 1H, ArH), 8.05 (d, J=7.2 Hz, 1H, ArH), 8.00－7.64 (m, 5H, ArH), 7.64－7.22 (m, 4H, ArH); ^{13}C NMR (DMSO-d_6) δ: 160.94 (s), 160.47 (s), 154.36 (s), 147.87 (s), 135.98 (s), 134.79 (s), 133.80 (s), 130.74 (d), 129.19 (s), 127.96 (d), 127.08 (s), 125.77 (s), 125.55 (s), 120.72 (s), 120.35 (m), 118.96 (s), 116.60 (s); IR (KBr) v: 3437 (N－H), 1706 (C＝O) cm^{-1}. HR-MS calcd for $C_{20}H_{13}NO_3$: 315.09, found $[C_{20}H_{13}NO_3+H]^+$: 316.09.

General procedure for spectroscopy

All the fluorescence spectroscopy was carried out in the DMSO/H_2O (8∶2, *V*/*V*, containing 0.01 mol•L^{-1} 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.20) on a Shimadzu RF-5301 spectrometer. Any changes in the fluorescence spectra of the synthesized compound were recorded on addition of tetrabutylammonium salts while keeping the ligand concentration constant (2.0×10^{-5} mol•L^{-1}) in all experiments. Tetrabutylammonium salt of anions (F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, SCN^-, S^{2-}, PO_4^{3-}, CO_3^{2-} and CN^-) were used for the fluorescence experiments.

For ^{1}H NMR titrations, the solution of **CX** was prepared in DMSO-d_6 and the appropriate concentrated solution of the guest was prepared in D_2O. Aliquots of the two solutions were mixed directly in NMR tubes.

Results and Discussion

The sensing abilities of **CX** towards various anions (CN^-, F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, SCN^-, S^{2-}, PO_4^{3-}, and CO_3^{2-}) were investigated by fluorescence spectroscopy. We carried out experiments in the DMSO/H_2O (8∶2, *V*/*V*, containing 0.01 mol•L^{-1} HEPES, pH 7.20). Free sensor **CX** exhibited an emission maximum at 398 nm upon excitation at 335 nm. After addition of the DMSO solution of these different anions (1×10^{-3} mol•L^{-1}) to the DMSO/H_2O (8∶2, *V*/*V*, containing 0.01 mol•L^{-1} HEPES, pH 7.20) solution of sensor **CX** (2×10^{-5} mol•L^{-1}), respectively, a significant fluorescence enhancement was only seen in the case of CN^- and none of other anions induced any significant changes in the fluorescent spectrum of the sensor (Figure 1). Figure S1 demonstrated the absorption spectral changes of **CX** upon the addition of PO_4^{3-} and CO_3^{2-}.

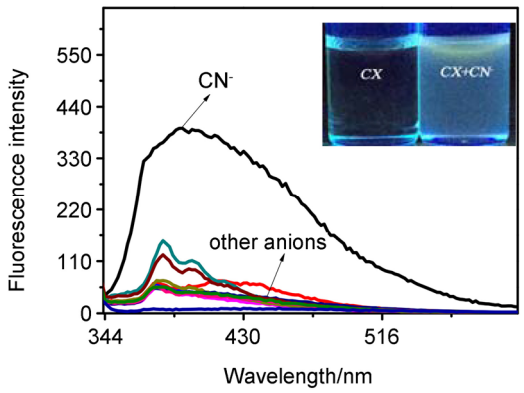

Figure 1 Fluorescence emission data for the sensor of **CX** (2×10^{-5} mol•L^{-1}) with various anions as the tetrabutylammonium salts, respectively, in the DMSO/H_2O (8∶2, *V*/*V*, containing 0.01 mol•L^{-1} HEPES, pH 7.20) solution (excitation wavelength＝335 nm). Inset: photograph of **CX** (2×10^{-5} mol•L^{-1}) upon addition of 50 equiv. of CN^-, which was taken under a UV-lamp (365 nm).

To further explore the utility of sensor **CX** as an ion-selective chemosensor for CN^-, competitive experiments were carried out in the presence of 50 equiv. of CN^- and 50 equiv. of various other anions (F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, SCN^-, S^{2-}, PO_4^{3-}, and CO_3^{2-}) in DMSO/H_2O (8∶2, *V*/*V*, containing 0.01 mol•L^{-1} HEPES, pH 7.20) for sensor **CX**. The other anions did not show any noticeable changes in the emission maximum band under the same condition. The plot of changes in the fluorescence intensity at 398 nm upon addition of various anions clearly showed excellent selectivity of sensor **CX** towards the CN^- (Figure 2).

To get further insight into the sensing behavior of **CX** to CN^-, fluorescence titration experiment was per-

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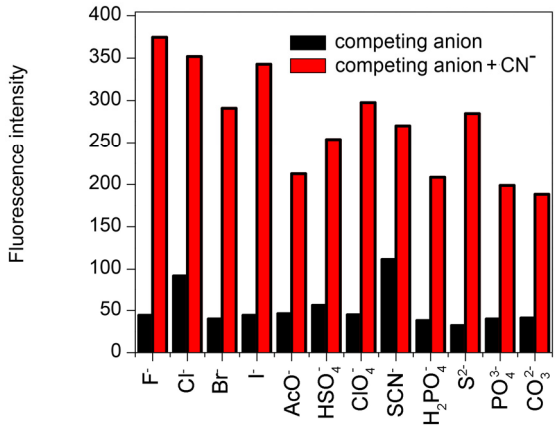


Figure 2 Fluorescence spectra response of **CX** (2×10^{-5} mol•L^{-1}) in the presence of various anions (50 equiv.) in DMSO/H_2O (8∶2, *V*/*V*, containing 0.01 mol•L^{-1} HEPES, pH 7.20) in response to CN^- (50 equiv.)

formed. With the gradual addition of pure water solution of CN^- to sensor **CX**, the emission peak at 398 nm gradually enhanced intensity, and the fluorescence of **CX** essentially reached saturation by 14 equivalents of CN^- (Figure 3). In other words, the free sensor **CX** was non-emissive, when it encountered CN^-, it resulted in a dramatic enhancement of the fluorescence intensity. Meanwhile, the fluorescence quantum yields increased from 0.047 to 0.22 (in ESI). On the basis of fluorescence titration data, the binding constant of **CX** for CN^- was calculated to be $K=1.25\times10^5$ mol•L^{-1} (in ESI). In the meantime, the detection limit of the fluorescence spectra changes calculated on the basis of $3\sigma B/S$ was 1.15×10^{-7} mol•L^{-1} for CN^- (in ESI).[39]

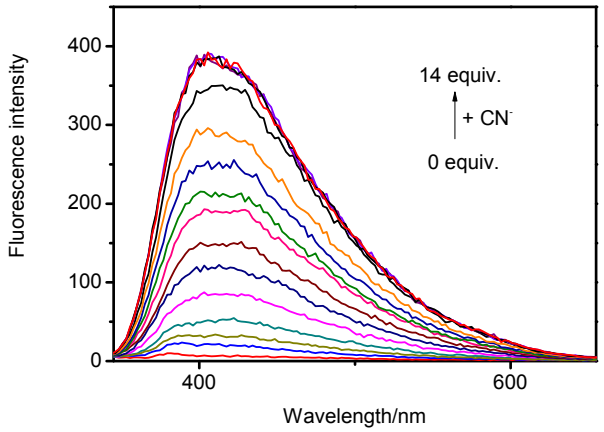


Figure 3 Fluorescence spectra of **CX** (2×10^{-5} mol•L^{-1}) in DMSO/H_2O solution (8∶2, *V*/*V*, containing 0.01 mol•L^{-1} HEPES, pH=7.20) upon addition of increasing CN^-.

As known, chemosensors always have a problem of long response time. In our case, the binding process of CN^- to **CX** was found to be very fast (Figure 4). After adding CN^- (1×10^{-3} mol•L^{-1}), the fluorescence emission intensity of **CX** was increased at 398 nm and reached the plateau region in less than 10 s, and remained quite stable, suggesting that the binding process might be completed instantly and the chemosensor has rapid detection ability for cyanide ions.

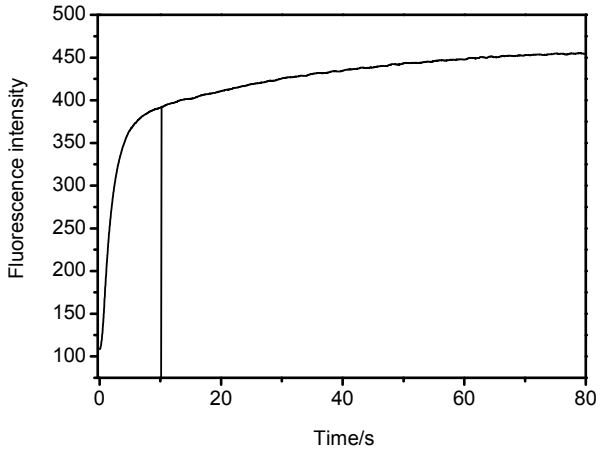
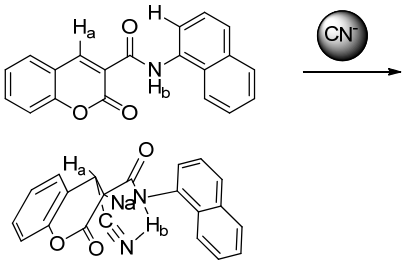


Figure 4 Fluorescence intensity at 398 nm for **CX** (2×10^{-5} mol•L^{-1}) in DMSO/H_2O (8∶2, *V*/*V*, containing 0.01 mol•L^{-1} HEPES, pH 7.20) after addition of CN^- (1×10^{-3} mol•L^{-1}).

To explore the sensing mechanism of sensor **CX** to CN^-, the ^1H NMR titration and MS were investigated, which illustrated the characteristic structural changes occurring upon interaction with CN^-. As shown in Figure 5, before the addition of cyanide anions, the ^1HNMR chemical shifts of H_a and H_b (N—H) appeared at δ 8.97 and 10.88, respectively. With the gradual addition of CN^-, protons H_b at **CX** disappeared and a new signal at δ 11.43 of H_b appeared. At the same time, the peak (H_a) at δ 8.97 disappeared, whereas new signals at δ 5.10 evolved. These results implied the nucleophilic attack of CN^- was added to coumarin group in **CX**, simultaneously, the —CN formed $H_b\cdots$CN hydrogen bond (Scheme 2). Further evidence obtained by MS experiments also supports this proposed mechanism. In the HR-MS spectrum of **CX** (Figure S5, in ESI), probe **CX** displays a peak at *m*/*z* 316.09 assigned to **CX** (*m*/*z* calcd 315.09). However, when 1 equivalent of CN^- was added to the solution of **CX**, a peak at *m*/*z* 365.12 ([CX+NaCN+H]$^+$) (Figure S6, in ESI) appears along with the disappearance of the peak at *m*/*z* 316.09. It is proved to be the ratio of 1∶1 between **CX** and CN^-.

Scheme 2 Proposed sensing mechanism of sensor **CX** for the detection of CN^-



To investigate the practical application of sensor **CX**,

Table 1 Determination of cyanide content in **CNCS** samples (n=3)

Sample	Determind CN^-/(μmol•L^{-1})	Adding CN^-/(μmol•L^{-1})	Found/(μmol•L^{-1})	Recovery/%	RSD/% (n=3)
1	2.27	0.5	2.75	96%	0.6
2	2.27	1.0	3.29	102	1.0
3	2.27	1.5	3.88	107	0.9
4	2.27	2.0	4.25	99	1.2

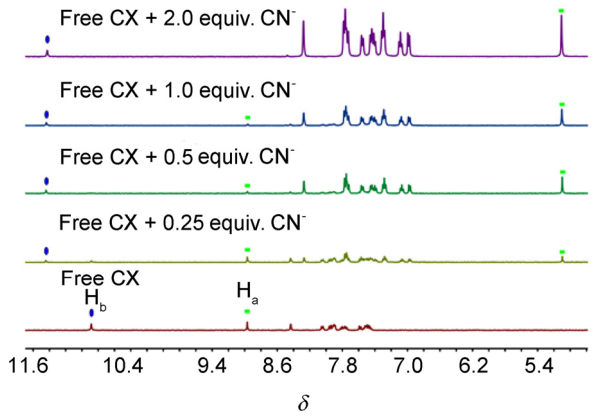


Figure 5 Partial ^{1}H NMR spectra of **CX** (0.01 mol•L^{-1}) in DMSO upon addition of CN^-.

we prepared test strips by immersing filter papers into the DMSO/H_2O (8∶2, *V*/*V*, containing 0.01 mol•L^{-1} HEPES, pH 7.20) of sensor **CX** (1×10^{-3} mol•L^{-1}), and then drying them in air. As shown in Figure 6, when the test strips coated with **CX** (1×10^{-3} mol•L^{-1}) were immersed into the pure water solutions of CN^-, the obvious fluorescence turn-on response can also be observed upon irradiation at 365 nm by a UV lamp. The development of such a 'dip-stick' approach is extremely attractive for 'in-the-field' measurements that do not require any additional equipment. Therefore, the test strips of **CX** have excellent application value in the detection of CN^-.

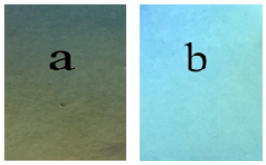


Figure 6 Fluorescence change of the test strips of **CX** (1×10^{-3} mol•L^{-1}). (a) only **CX**, (b) **CX** with CN^- in H_2O under irradiation at 365 nm.

To further demonstrate the potential of **CX** sensor for practical applications, 100 g of cherry nut were crushed and pulverized. The mixture was introduced into a flask, followed by addition of 300 mL of water and 0.5 g of NaOH. The obtained mixture was vigorously stirred for 15 min, then filtered through a 0.22 μm membrane to obtain the cyanide-containing solution (**CNCS**). **CNCS** samples were analyzed by using the standard addition method. The results show an average recovery of 101% with Relative Standard Deviation (RSD) of 0.92% for **CNCS** samples (Table 1). The experimental results indicate that the sensor possesses excellent applicability for real sample analysis.

Conclusions

In summary, we have presented a facile, low-cost and efficient example of a highly selective chemosensor for CN^-. The sensor gives an immediate response to the cyanide ion by fluorescence turn-on response. The detection of cyanide ion in solution using **CX** was found to be free of interference from any other anions. Moreover, the sensor demonstrates that the detection limit on fluorescence response of it to CN^- is down to 1.15×10^{-7} mol•L^{-1}, which is far lower than the maximum level of 2.7×10^{-6} mol•L^{-1} for cyanide in drinking water according to WHO guidelines. In addition, test strips based on **CX** were fabricated, which also exhibited a good selectivity to CN^- as in solution. Notably, the chemosensor was successfully applied to the detection of cyanide in cherry nut. So this recognition behaviour makes **CX** a potential probe to detect CN^- in our lives and environment.

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