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Acridinium Salt Based Fluorescent and Colorimetric Chemosensor for the Detection of Cyanide in Water

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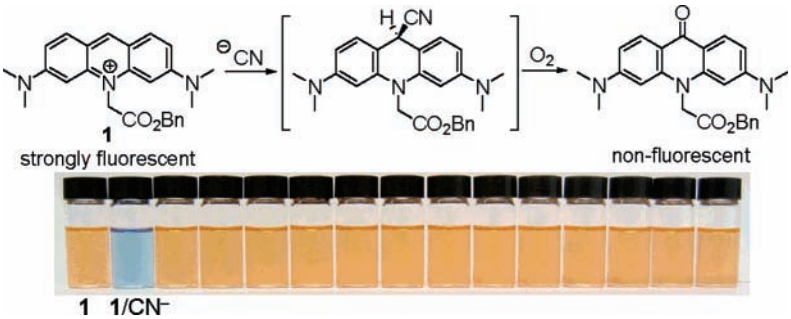
ABSTRACT



A new, selective chemosensor has been developed to detect cyanide in water at micromolar concentrations. The acridinium salt used in this sensor system is prepared in a single step from an acridine orange base. Detection is based on the irreversible, 1:1 stoichiometric, nucleophilic addition of cyanide to the 9-position of the acridinium ion. This process induces a large decrease in fluorescence intensity and a marked color change. The selectivity of the system in aqueous media for CN^- over other anions is remarkably high. Also, the sensitivity of both the fluorescence- and colorimetric-based assay is below the 1.9 μM suggested by the World Health Organization (WHO) as the maximum allowable cyanide concentration in drinking water. Thus, the chemodosimeter should be applicable as a practical system for the monitoring of CN^- concentrations in aqueous samples.

The cyanide ion is extremely toxic to mammals, leading to vomiting, convulsions, loss of consciousness, and eventual death.[1] In addition to being found in many foods and plants, cyanides are used industrially in gold mining, electroplating, and metallurgy.[2] According to the World Health Organization (WHO), cyanide concentrations lower than 1.9 μM are acceptable in drinking water.[3] Recent studies have shown that the lethal cyanide concentration in the blood of fire victims is ca. 20 μM.[4]

The cyanide anion is strongly nucleophilic and forms stable complexes with many transition metals. These properties of cyanide have been used advantageously in the design of fluorescent and colorimetric chemosensors. Chemosensors that rely on the formation of cyanide complexes with Zn(II)−porphyrin,[5] Ru(II)−pyridine,[6] boron derivatives,[7] and CdSe quantum dots have been developed.[8] Recently, nucleophilic addition reactions of cyanide to oxazine,[9] pyry-

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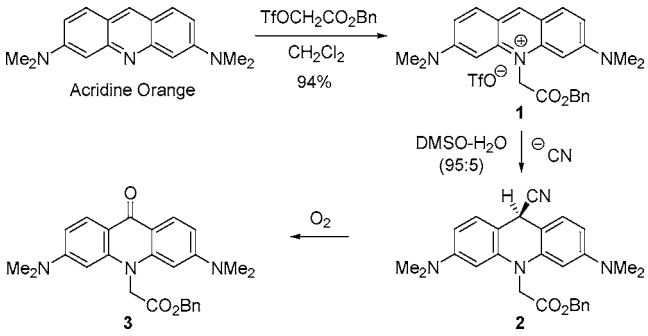
lium,[10] squarane,[11] and trifluoroacetophenone[12] derivatives that possess suitable signaling properties have been used for the design of cyanide chemosensors. In contrast to other anions, the recognition systems of cyanide through hydrogen bonding have been rarely considered.[13]

Some of the known fluorescent chemosensors of cyanide have low detection limits in the micromolar concentration region. Especially, the boronic acid based cyanide probes developed by the Geddes group have reached fluorescence and colorimetric sensitivity down to physiologically lethal levels (>20 μM) in aqueous media.[7a−c] In general, however, interference by other anions, such as fluoride and acetate, is a serious problem with the known cyanide selective sensors. Below, we describe the results of recent studies carried out in our laboratory which have led to the development of an acridinium salt based, highly selective and sensitive dual fluorescent and colorimetric chemosensor for cyanide.

The strategy used for the design of cyanide-selective chemosensors takes advantage of the nucleophilic addition reaction of cyanide at the 9-position of *N*-methylacridinium ions.[14] Exploratory efforts with several acridinium salts showed that the acridine orange derivative **1** has ideal properties (Scheme 1). The salt **1** was prepared from an

Scheme 1. Synthesis and Reaction of **1** with Cyanide



acridine orange base in a single step in 94% yield.[15] As expected, this substance has strong absorption at ca. 532 nm (log ϵ = 3.65) and a high fluorescence quantum yield (Φ_f = 0.30).[16] Addition of cyanide ions to an aqueous solution of **1** causes a color change from orange to pale blue accompanying a dramatic decrease in fluorescence intensity. The addition reaction which causes these changes is highly solvent sensitive, especially to the amount of water present in the medium. This phenomenon is probably due to the fact that the nucleophilicity of cyanide is significantly diminished in water. Consequently, a DMSO−water (95:5) solvent system was used for the titration experiments in which each reaction was run for 10 min at 50 °C.[17] ^{1}H NMR (DMSO-d_6/D_2O, 95:5) monitoring of the process showed that adduct **2** is formed initially and that it rapidly reacts with oxygen to produce the acridinone **3** (Scheme 1).[14] Acridinone **3** is produced exclusively when the initially formed product mixture, comprised of a 1:1 ratio of **2**:**3**, is exposed to air (Supporting Information).[18]

Fluorescence monitoring of the cyanide addition reaction was performed by using a 10 μM solution of **1** in DMSO−water (95:5 v/v) at 50 °C. Upon addition of 1 equiv of cyanide, the fluorescence intensity of the solution experiences an ca. 14-fold (F_0/F) decrease (Figure 1) in a manner that is

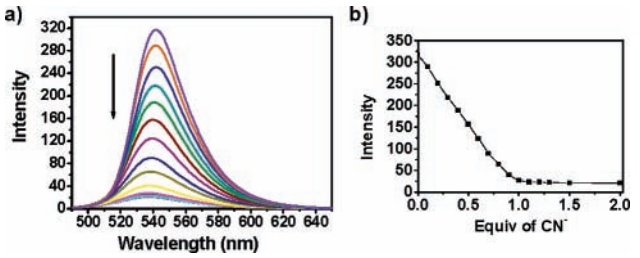


Figure 1. (a) Fluorescence emission of a solution of **1** (DMSO−water (95:5 v/v) 10 μM) upon addition of CN^- (excitation at 480 nm). (b) Plot of fluorescence intensity at 541 nm vs number of equivalents of CN^-.

inversely proportional and 1:1 stoichiometrically related to the cyanide concentration.

To evaluate the cyanide-selective nature of acridinium salt **1**, fluorescence changes caused by the addition of other anions, including CN^-, AcO^-, F^-, Cl^-, Br^-, I^-, $H_2PO_4^-$, HSO_4^-, SCN^-, NO_3^-, BzO^-, N_3^-, CH_3S^-, and ClO_4^-, were evaluated. Solutions of **1** (10 μM), containing 1 equiv of each of these anions, were maintained for 10 min at 50 °C and then subjected to fluorescence analysis. As the data in Figure 2a demonstrate, anions other than CN^- do not cause significant changes in the fluorescence intensity. Thus, the selectivity profile for CN^- over other anions is remarkably

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(16) Fluorescence quantum yields were calculated by using acridine orange (Φ_f = 0.46 in EtOH) as a reference (Soep, B.; Kellamnn, A.; Lindqvist, L. *Chem. Phys. Lett.* **1972**, *13*, 241−244).

(17) All the NMR, fluorescence, and colorimetric titration experiments were performed in commercial solvents without degassing. The reaction of CN^- (0.1 equiv) and **1** reaches completion within 10 min at 50 °C (see Supporting Information for time-dependent fluorescence changes).

(18) Attempts to isolate **2** were unsuccessful, producing only **3**. The related conversion of *N*-methyl-9-cyanoacridan to *N*-methylacridone by oxygen is known (ref 14b).

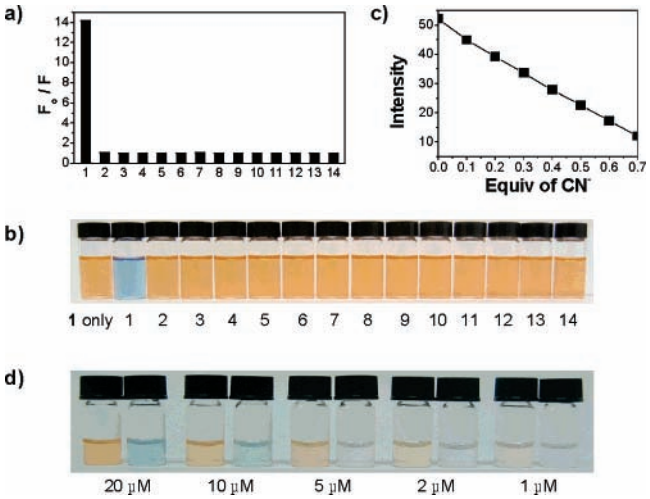

Figure 2. (a) Fluorescence intensities of **1** (10 μM) and (b) color changes of **1** (25 μM) in DMSO−water (95:5 v/v) in the presence of 1 equiv of the following anions: 1, CN^-; 2, AcO^-; 3, F^-; 4, Cl^-; 5, Br^-; 6, I^-; 7, $H_2PO_4^-$; 8, HSO_4^-; 9, SCN^-; 10, NO_3^-; 11, BzO^-; 12, N_3^-; 13, CH_3S^-; 14, ClO_4^-. (c) Fluorescence intensities at 539 nm of **1** (1 μM) upon additions of CN^- in DMSO−water (95:5 v/v). Each point (0.1 equiv of CN^-) corresponds to 2.0 μM of [CN^-] in water. (d) Color changes of **1** in 1:1 DMSO/water solution in the presence of 1 equiv of CN^- (20, 10, 5, 2, 1 μM) and drying reagents followed by filtration.

high. ^{1}H NMR analysis indicates that basic anions, such as AcO^- and F^-, function as bases to deprotonate the acidic α-carbonyl protons.[19]

The changes in fluorescence intensity caused by the addition of CN^- are not influenced by the presence of other cations or anions. Also, other nucleophilic reagents, such as Et_2NH, $BnNH_2$, PhSH, NaSMe, NaOPh, and *n*-Bu_4NOH, do not react with **1** (Supporting Information). As a result, this acridinium salt should be an ideal chemodosimeter for monitoring cyanide ions.

The selectivity observed by using fluorescence monitoring is matched when **1** is employed as a colorimetric detector for CN^-. In contrast to the visually observed orange to pale blue color change associated with the reaction of **1** (25 μM) with CN^-,[20] no significant color changes are promoted by addition of other anions (Figure 2b).

The limits for detecting cyanide in water by using chemodosimeter **1** as a colorimetric and fluorimetric sensor were determined. Fluorescence titration of **1** (1.0 μM) with CN^- in DMSO−water (95:5 v/v) showed that the fluorescence intensity of the solution is nearly inversely proportional to the amount of CN^- added (Figure 2c). Addition of 0.1 equiv of CN^-, which corresponds to 2.0 μM of [CN^-] in water,[21] promotes a significant fluorescence intensity change. Therefore, the fluorescent system based on **1** can be used to detect the WHO suggested maximum allowed cyanide concentration in drinking water (1.9 μM).

Colorimetric detection of micromolar concentrations of cyanide using **1** was then explored. By using the DMSO−water (95:5 v/v) solvent system, the colorimetric detection limit is restricted to >1 μM levels of **1**, which corresponds to >20 μM of [CN^-] in water (orange vials in Figure 2d). To lower this limit, 1:1 DMSO/water (v/v) solutions containing drying reagents were used. Equal volumes and concentrations of DMSO solutions of **1** and water solutions of CN^- were mixed and then treated with anhydrous $MgSO_4$.[22] Upon warming the mixtures at 50 °C for 10 min, the orange-colored solutions change to pale blue (almost colorless for 5∼1 μM concentrations). For 20−1 μM of cyanide concentrations in water, it is possible to discern this color change visually (Figure 2d). Thus, the colorimetric detection limit for cyanide by **1** is well within WHO guidelines of 1.9 μM.

In summary, this investigation has led to the development of a highly selective and sensitive chemodosimeter for detecting micromolar concentrations of CN^- ions in water. Acridinium salt **1** that serves as the basis of the detection system is easily prepared in a single step from acridine orange. This salt reacts irreversibly with CN^- in a 1:1 stoichiometric manner, a process which induces a large reduction in the fluorescence intensity and a marked color change. Importantly, the selectivity of this system for CN^- over other anions is extremely high. In addition, the detection limits for cyanide by both the fluorescence- and colorimetric-based sensor systems fall well below 1.9 μM of aqueous cyanide concentrations. Consequently, the chemodosimeter **1** appears to be a practical system for monitoring CN^- concentrations in aqueous samples.

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Supporting Information Available: Experimental procedures for the synthesis, spectral data, and copies of ^{1}H NMR and ^{13}C NMR of **1** and **3**; data for UV−vis, fluorescence, and ^{1}H NMR titrations of **1**; and other data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) The facts that this deprotonation process accompanies no significant fluorescence intensity changes and that the disappearance of α-carbonyl protons in ^{1}H NMR titration spectra suggest that the equilibrium between **1** and its ylide generated by the basic anions causes H/D exchange of α-carbonyl protons.

(20) Upon addition of cyanide to **1**, the solution turns colorless then pale blue. The intensity of the blue color becomes more intense with time. This may imply that the acridinone **3** is responsible for the blue color.

(21) See Supporting Information for the sample calculation.

(22) $MgSO_4$ did not induce color changes without CN^-.