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A turn-on fluorescent chemosensor selectively detects cyanide in pure water and food sample

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ARTICLE INFO	ABSTRACT
Article history: Received Revised Accepted Available online	A turn-on fluorescent chemosensor (H-1) for cyanide anions based on dihydroxy phenazine was designed and synthesised. The sensor H-1 exhibits high sensitivity and good selectivity for cyanide in pure water. The CN^- response mechanism involves a hydrogen bonding and deprotonation process in the sensor, which induced prominent fluorescence enhancement. The detection limit of the sensor towards CN^- is 5.65×10^{-7} M, and other anions had nearly no influence on the probing behavior. In addition, test strips based on the sensor were fabricated, which also exhibit a good selectivity to CN^- in water. Notably, this sensor was successfully applied to detect CN^- in food samples, which proves a very simple and selective platform for on-site monitoring of CN^- in agriculture samples.
<i>Keywords:</i> Dihydroxy phenazine Cyanide Test strips Food samples	

The cyanide (CN⁻) has been continuously of concern all over the world due to its extreme toxicity and widespread use in metallurgy, plastics production, and silver or gold extraction.¹ Cyanide-containing substance, found in foods, plants, water, soil, polluted air, vehicle exhaust, and even cigarette burning gas, are extremely detrimental to living organisms and most animals by causing lethal effect to their central nervous system.² Uptake of toxic cyanide could occur through absorption by lungs and skin, and also from contaminated food and drinking water.³ Therefore, much interest has been sparked in the design of new methods to monitor CN⁻ in biological and environmental samples.

Thanks to the enthusiastic efforts of scientists, a large number of good sensors for cyanide have been invented.⁴ Among the various sensors, fluorescent chemosensors present numerous advantages, including high sensitivity, low cost, and easy operation.⁵ Moreover, phenazine derivatives, which have dramatic fluorescent emissions, have been synthesized for a long time, but they were seldom been used in ions recognition field.⁶ On the other hand, in biological and environmental systems, CN⁻ sensors interactions commonly occur in aqueous solution, therefore, much attention has been paid to develop the cyanide sensors that work in water. However, few, if any, CN⁻ sensors are able to display high selectivity over other anions such as F⁻, AcO⁻, and H₂PO₄⁻ in pure water.⁷ Recently, we have also reported 4-amino-3-hydroxynaphthalene-1-sulfonic acid (Scheme S2) and 2, 4-dimethyl-7-amino-1, 8-naphthyridine as the fluorescent cyanide sensors in pure water.⁸

In view of this requirement and as a part of our research interest in ion recognition.⁹ Herein, we report a rationally designed, simple, and efficient fluorescent sensor **H-1** based on dihydroxy phenazine. The chemosensor **H-1** could act as a turn-on fluorescent sensor for cyanide anions in pure water. The recognition progress occurred via deprotonating between the hydroxyl of **H-1** and cyanide. When exposed **H-1** to the low concentration solution of cyanide anion there was a dramatically increase of the emission maximum of **H-1**, and the color of the solution changed from dim orange to bright yellow, which can be seen by naked-eyes under the UV lamp (365 nm). As practical applications, the chemosensor was successfully applied to the detection of CN^- in sprouting potatoes and bitter seeds, which prove a very simple and selective platform for on-site monitoring of CN^- in agriculture samples.

The 2, 5-dihydroxy-*p*-benzoquinone (2) was reacted with *o*-diaminobenzenes (1.1 equiv) in water to afford high yields of 2, 3-dihydroxyphenazines **H-1** (Scheme S1).¹⁰ It was characterized by ¹H NMR, IR and ESI-MS (Fig. S5, S8, and S9 in SI).

In order to investigate the CN⁻ recognition abilities of the sensor **H-1** in water, we carried out a series of host-guest recognition experiments. The recognition profiles of the chemosensor **H-1** toward various anions, including F⁻, Cl⁻, Br⁻, Γ , AcO⁻, H₂PO₄⁻, HSO₄⁻, ClO₄⁻, CN⁻ and SCN⁻, were primarily investigated using fluorescence and UV-vis spectroscopy in water. When 50 equiv. of CN⁻ were added to the sensor **H-1** (20 μ M), there was not a significant color change. Interaction of **H-1** with 50 equiv. of CN⁻ resulted in a negligible red shift in the absorption spectrum, which was not visible to the naked eye (Fig. S1 in SI).

As shown in Figure 1, in the fluorescence spectrum, the maximum emission of **H-1** appeared at 548 nm in water when excited at $\lambda_{ex} = 415$ nm. On the addition of 50 equivalents of CN⁻, the fluorescence intensity of sensor **H-1** (20 μ M) increased rapidly, and the maximum emission shifted to 568 nm. The color change from dim orange to bright yellow could be distinguished by the naked eye on UV lamp

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(365 nm). To validate the selectivity of sensor **H-1**, the same tests were also conducted using F^- , Cl^- , Br^- , Γ , AcO^- , $H_2PO_4^-$, HSO_4^- , ClO_4^- , and SCN⁻, and none of these anions induced any significant changes in the fluorescent spectrum of the sensor (Fig. S3 in SI). Therefore, in water solution, **H-1** showed specific fluorescent selectivity to CN⁻.



Figure 1. Fluorescence spectral response of **H-1** (20 μ M) in water upon addition of 50 equiv. of CN⁻ (λ_{ex} = 415 nm). Inset: photograph of **H-1** (20 μ M) upon addition of 50 equiv. of CN⁻, which was taken under a UV-lamp (365 nm).

To get further insight into the sensing behavior of **H-1** to CN⁻, fluorescence titration experiment was performed (Fig 2). Upon incremental addition of CN⁻ to solution **H-1**, the emission peak at 548 nm gradually enhanced intensity and blue-shifted maximum emission, as well as the fluorescence of **H-1** was essentially reached saturation by 10.16 equivalents of CN⁻. Meanwhile, the fluorescence quantum yields increased from 0.24 to 0.68 (in SI).¹¹ The binding constant K_a was determined to be 2.65 × 10⁹ M⁻² (in SI).¹² Furthermore, the detection limit of the fluorescent spectrum changes calculated on the basis of $3\sigma/m^{16}$ is 5.65 × 10⁻⁷ M for CN⁻, which is far lower than the WHO guideline of 1.9 µM cyanide (Fig. S4 in SI).¹³



Figure 2. Fluorescence spectra of **H-1** in the presence of different concentrations of CN^- in water ($\lambda_{ex} = 415$ nm). Inset: a plot of fluorescence intensity depending on the concentration of CN^- in the range from 0.00 to 10.16 equivalents ($\lambda_{ex} = 415$ nm), $\lambda_{em} = 568$ nm).

To further explore the utility of sensor **H-1** 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^- , CI^- , Br^- , Γ , AcO^- , $H_2PO_4^-$, HSO_4^- , CIO_4^- , and SCN^-) in a water solution of sensor **H-1**. The fluorescent emission spectrum

of sensor H-1 with CN^- was not influenced by the subsequent addition of competing anions, which indicated that H-1 has specific selectivity to CN^- (Fig. 3).



Figure 3. Fluorescence intensity changes of **H-1** (20 μ M) in the presence of CN⁻ (50 equiv.) and additional anions (50 equiv.) in water ($\lambda_{ex} = 415$ nm, $\lambda_{em} = 568$ nm).

In addition, the recognition mechanism was investigated by ¹H NMR titration of the sensor **H-1** with CN⁻. ¹H NMR titration displayed the chemical shift changes of **H-1** upon the addition of CN⁻, as shown in Fig. 4, sensor **H-1** showed a peak at 10.94 ppm in DMSO- d_6 , we confirmed that which correspond to the protons of \neg OH. Cyanide could take the protones away via deprotonating during the \neg OH moieties of **H-1**, in the corresponding ¹H NMR spectra, upon addition of 1.0 equiv. CN⁻ by deuterium water solution, the signal at 10.94 ppm (\neg OH) disappeared. At the same time, the phenazine signals from 7.20 to 8.10 ppm have a significant upfield shift, indicating that the density of intramolecular charge increased through deprotonating.



Figure 4. ¹H NMR spectra (400 MHz, DMSO- d_6) of free **H-1** and in the presence of CN⁻.

In order to quantify the reaction ratio between **H-1** and CN⁻ ion, besides the fluorescence Job's plot measurement (Fig. 5) was conducted by varying the concentration of both the receptor and the CN⁻ ion with a total concentration of 4×10^{-5} M. The maximum point appears at the mole fraction of 0.75, which indicates the reaction ratio of **H-1** and CN⁻ is 1:2. It was further confirmed by the appearance of a peak at m/z 257.00 assignable to [**H-1** + 2Na⁺] (m/z = 256.99) in the ESI-MS spectrum (Fig. S10 in





Figure 5. Job's plot examined between H-1 and CN⁻, indicating the 1:2 stoichiometry.

Based on the above findings, we propose that the mechanism is the deprotonation process between the probe **H-1** and cyanide in this system may proceed through the route depicted in Figure 6.



Figure 6. The proposed mechanism of H-1 for CN⁻.

The selectivity of **H-1** to CN^{-} was also examined over a wide range of pH values. The detection of CN^{-} can work well in the pH range of 3.0 ~ 8.0 in HEPES buffered solution in water (Fig. 7).



Figure 7. Influence of pH on the fluorescence of H-1 and H-1+CN⁻ in HEPES buffered solution in water ($\lambda_{ex} = 415 \text{ nm}, \lambda_{em} = 568 \text{ nm}$).

The reversibility of sensor **H-1** has been measured by alternating addition of hydrochloric acid to the solution of H-1+CN^- , which induced the opposite trend in the fluorescent spectra to that observed. Upon addition of 1.0 equivalent of hydrochloric acid, the optical fluorescence intensity returned to the levels observed for the free compound **H-1**, and the solution became orange under the UV lamp (365 nm) again. This cycle could be repeated more than several times without considerable loss of sensing ability of the sensor (Fig. 8).



Figure 8. Reversible switching cycles of fluorescence intensity ($\lambda_{ex} = 415 \text{ nm}$, $\lambda_{em} = 568 \text{ nm}$) by the alternating addition of CN⁻ and HCl in water. Above are the vials showing visual fluorescent color.

Generally, chemosensors suffer from a long response time. In our case, the response of **H-1** toward CN^- was found to be very fast (Fig. 9). After addition of a solution of CN^- (50 equiv.), the fluorescence emission intensity of **H-1** in water at 568 nm increased rapidly and reached a plateau in about 30 s at room temperature.



Figure 9. Fluorescence intensity ($\lambda_{ex} = 415 \text{ nm}$, $\lambda_{em} = 568 \text{ nm}$) for H-1 (20 μ M) in water after addition of pure water solution of 50 equiv. of CN⁻ (0.01 M).

To investigate the practical application of chemosensor **H-1**, test strips were prepared by immersing filter papers into a water solution of **H-1** (0.1 M) and then drying them in air. The test strips containing **H-1** were utilized to sense CN^- and other anions. As shown in Figure 10, when CN^- and the other anions were added on the test strips, the obvious color change was observed only with CN^- solution under irradiation at 365 nm by a UV lamp. And potentially competitive ions exerted no influence on the detection of CN^- by the test strips. Therefore, the test strips could conveniently detect CN^- in solutions.



Figure 10. Photographs of H-1 on test strips (A) only H-1, (B) after immersion into water solutions with CN^- , (C) after immersion into water solutions with other anions, (D) after immersion into water solutions with CN^- and other anions under irradiation at 365 nm.

In order to further investigate the practical utilities of probe in our lives, one hundred grams of bitter seeds must be crushed and pulverized. Then the mixture must be introduced into a flask. This is followed by addition of 300 mL of water and 0.5 g of NaOH. The obtained mixture must be vigorously stirred for 15 min. Then the mixture was filtered to obtain the cyanide-containing solution (BSCS). Next, we chose the sprouting potatoes to implement the following experiment. The sprouting potato (100 g) was first mashed before being soaked in water (200 mL) for two days until the extract became turbid. The mixture was filtered and the filtrate was eluted with 125 mM NaOH solution (100 mL) to obtain the cyanide-containing solution (SPCS). We diluted the BSCS and **SPCS** with fresh double water, which ensured the Ph < 8. As shown in Fig. 11, upon the addition of the two different cyanide solution into H-1 (20 μ M), the fluorescence intensity of sensor H-1 increased rapidly. The color change from dim orange to bright yellow could be distinguished by the naked eyes on UV lamp (365 nm).



Figure 11. Fluorescence spectral response of H-1 (20 $\mu M)$ in diluted bitter seeds and sprouting potatoes filter.

In summary, a simple sensor (H-1) for highly selective and sensitive detection of cyanide anions based on dihydroxy phenazine was designed and synthesized, which can be used as a turn-on fluorescent sensor for cyanide in pure water. The detection limit of the sensor towards CN^- is 5.65×10^{-7} M, and other anions, including F⁻, Cl⁻, Br⁻, I⁻, AcO⁻, H₂PO₄⁻, HSO₄⁻, ClO₄⁻, and SCN⁻ had nearly no influence on the probing behavior. In addition, test strips based on H-1 were fabricated, which also exhibited a good selectivity to CN^- in water. Notably, the chemosensor was successfully applied to the detection of cyanide in sprouting potatoes and bitter seeds. Thus, it will be regarded as an environmental friendly material which can sense cyanide anion in water and make great contribution to the development of cyanide anion sensors.

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Graphical abstract: In this work, a turn-on fluorescent chemosensor (**H-1**) for cyanide anions based on dihydroxy phenazine was designed and synthesised. The sensor **H-1** exhibits high sensitivity and good selectivity for cyanide in pure water.



Highlights

- 1. A structurally simple anion sensor for highly selective and sensitive detection of CN⁻ anions via fluorescent OFF-ON mechanism.
- 2. Taking advantage of a simple mechanism of deprotonation.
- 3. This sensor was successfully applied to detect CN⁻ in pure water and food samples.
- 4. H-1 could serve as a potential recyclable component by fluorescent changes in

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