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Journal Name

ARTICLE

High selectivity and sensitivity chemosensor based on 2, 3-Diaminophenazine hydrochloride for detection of cyanide in pure water and its application in plant seeds sample

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In this work, we prepared an efficient chemosensor base on hydrochloride of 2,3-diaminophenazine which has good dissolvability in water, and more importantly, it can be act as an efficient chemodosimeter approach for the selective detection of CN^- in pure water. Upon treating with CN^-, 2,3-diaminophenazine hydrochloride (**Q1**) displayed a remarkable naked-eye and fluorescent response, simultaneously with significant changes in absorption spectra and fluorescent spectra. Furthermore, the absorption and fluorescent detection limit of CN^- was 1.95×10^{-7} M and 1.13×10^{-9} M, respectively. As practical applications, the test strips based on the chemosensor could serve as convenient CN^- detected tools. And it also successfully applied to the detection of CN^- in plant seeds and several natural water samples.

Introduction

Prior to this, a large number of studies on the properties of phenazine and its derivatives have been reported[1]. However, there is no reports of the 2,3-diaminophenazine hydrochloride. As we all know, 2,3-diaminophenazine has a poor solubility which limits its scope of application. However, we have found that after acidified with hydrochloric acid it has a good dissolvability in pure water. More importantly, it can be used to act efficiently in a chemodosimeter approach for the selective detection of CN^- in pure water.

Cyanides received wide attention because of their extremely toxic effect towards humans and they were regarded as environmental inorganic pollutants[2]. A small amount of cyanide is present in certain seed, e.g., those of an apple, mango, peach and bitter almonds[3].There are also found in cigarette smoke, in vehicle exhaust and formed by the incomplete combustion of all nitrogen-containing substances[4]. Due to the wide range of application, the large amount of CN^- pollutants was produced by mining and jewelry industries into the environment[5], the World Health Organization (WHO) recommended a tolerable limit of CN^- at 1.9 μM[6]. Based on these, it is very important to find a highly efficient and quick identification of CN^- in pure water[7]. Although previously have a large number of document reports are used to detect CN^- [8], most of these methods are costly and time-consuming, the CN^- sensors often employ sophisticated structures and require complicated synthetic steps and high temperature, moreover, they have a poor solubility in pure water or the tests are carried out in organic solvents[9] (Scheme 1), thus limiting their applications. Hence, it is worthy of serious consideration to find a new method to rapidly, simple and highly selective and sensitive detection of CN^- in pure water. Different from the previous reports, here, it is concluded that the probe **Q1** must be a promising its selectivity, ease-of-use, rapid response (response time about 3 s), and the low, naked-eye discernible CN^-, and it can be used to act efficiently in a chemodosimeter approach for the selective detection of CN^- in pure water. What is more, to our knowledge, there is no published information that 2,3-diaminophenazine hydrochloride is highly selective and sensitive detection of CN^- in pure water.

We found the progress of recognition could be hydrogen bonding in the labile of primary amine salt on **Q1** and CN^-, and they formed through **Q1** and CN^- 1: 2 ratio. When exposed **Q1** to the CN^- solution of low concentration, there was a significant color change from yellow-orange to bright yellow in visible light and accompanied with a strong and broad blue shift. However, without any signal changes after the addition of F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, SCN^- and S^{2-}, the absorption spectra detection limit of **Q1** for CN^- was 1.95×10^{-7} M, and the fluorescent color change from dark red to bright yellow, the fluorescence spectra detection limit was 1.13×10^{-9} M and other common anions had almost no influence on the probing behavior of CN^-. Meanwhile, test strips based on sensor **Q1** were fabricated and it was also successfully applied to the detection of CN^- in bitter almonds and wild peach kernel. Besides, it has been tested in river water and lake water.

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† Electronic supplementary information (ESI) available: Complete experimental procedures and some of the spectroscopic techniques.
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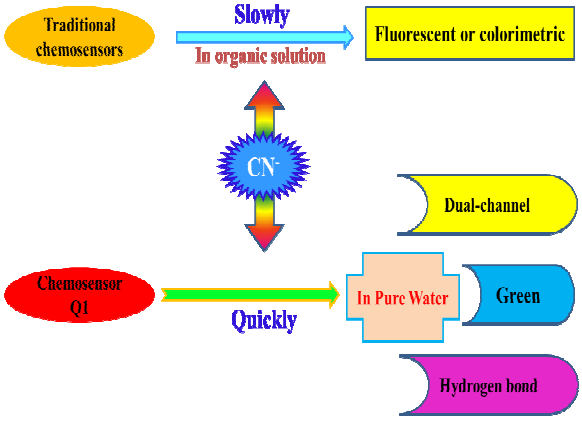

Scheme 1. Tactics for the sensing of CN^-.

Notably, this 2,3-diaminophenazine hydrochloride can be used as a green, non-toxic, pollution-free and high selectivity, sensitivity chemosensor to detection of CN^- in pure water on the basis of these observations and mechanistic supported analyses.

Synthesis and characterization of chemosensor Q1

2,3-diaminophenazine was prepared according to the literature procedure[10], chemosensor 2,3-diaminophenazine hydrochloride (**Q1**) were synthesized according to the Scheme S1†. All complexes were unambiguously characterized by ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy, ESI mass spectrometry, FT-IR (Experimental section for further details can be seen in Supporting Information). **Q1** as dark brown solid and has a good solubility in pure water, ^{1}H NMR (D_2O, 600 MHz) δ: 7.61 (s, 4H); 7.51 (s, 2H); 6.51 (s, 6H). ^{13}C NMR (DMSO-d6, 150 MHz) δ/ppm 149.53, 132.57, 129.11, 127.75, 123.02, 96.88. ESI-MS calcd for $C_{12}H_{12}N_4^{2+}$ 212.1051, found 212.1010.

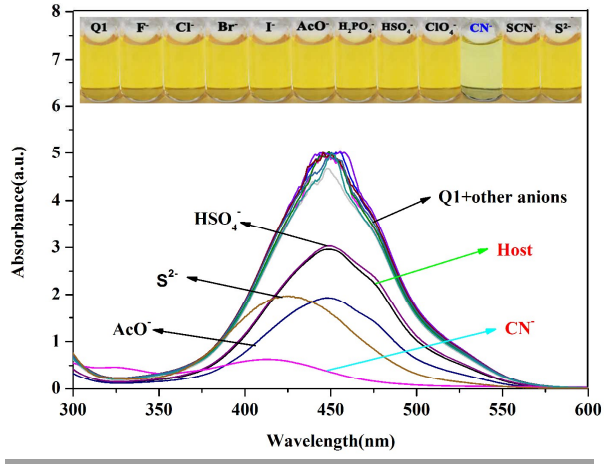

Fig. 1 Absorbance spectra of target compound **Q1** (2.0×10^{-5} M) in fresh distilled water presence of CN^- and other anions (5 equiv.). Inset: photograph showing the change in color of the solution of **Q1** in fresh distilled water after addition of CN^- and other anions in visible light.

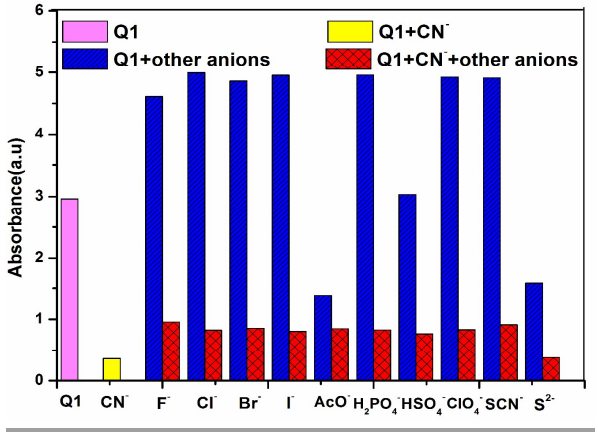

Fig. 2 Absorbance response of **Q1** (2.0×10^{-5} M) in the presence of 5 equiv. various anions containing 5 equiv. of CN^- in fresh distilled water at room temperature.

Results and discussion

In order to investigate the CN^- recognition abilities and progress of **Q1** in pure water, we carried out a series of recognition experiments. The recognition profiles of **Q1** (2.0×10^{-5} M) toward various anions, including F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^- and sodium salt (1.0×10^{-3} M) of anions (CN^-, SCN^- and S^{2-}) were primarily investigated using UV–vis spectroscopy in fresh distilled water. As shown in Fig. 1, in the absorbance spectrum, the maximum emission of **Q1** appeared at 448 nm. When 5 equivalents of CN^- was added to the solution of **Q1**, there was a significant color change from yellow-orange to bright yellow and accompanied with a strong and broad blue shift, which was visible to the naked eyes in

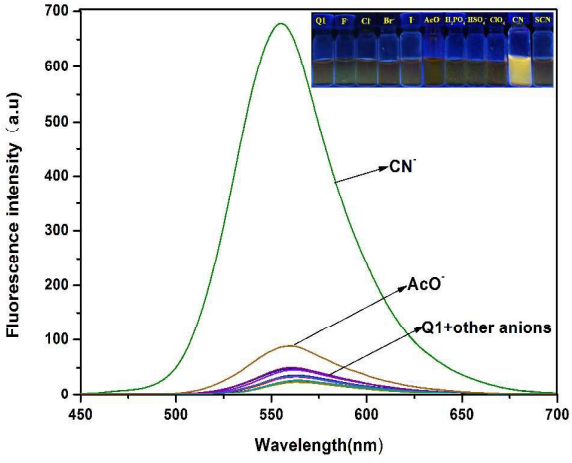

Fig. 3 Fluorescence spectra of target compound **Q1** (2.0×10^{-5} M) in fresh distilled water presence of CN^- and other anions (5 equiv.). Inset: photograph showing the change in color of the solution of **Q1** in fresh distilled water after addition of CN^- and other anions at UV lamp (excitation wavelength = 410 nm).

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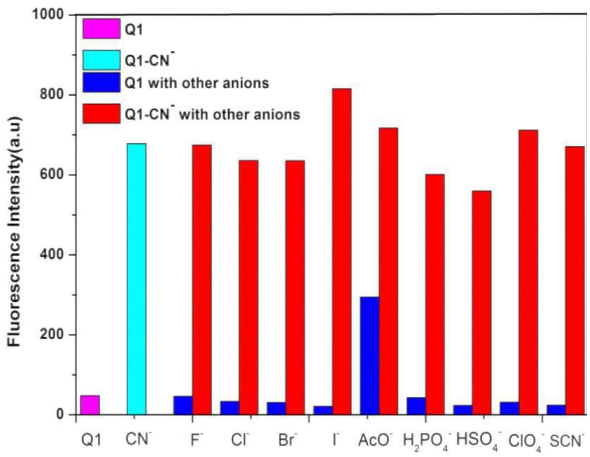

Fig. 4 Fluorescence response of **Q1** (2.0×10^{-5} M) in the presence of 5 equiv. various anions containing 5 equiv. of CN^- in fresh distilled water at room temperature.

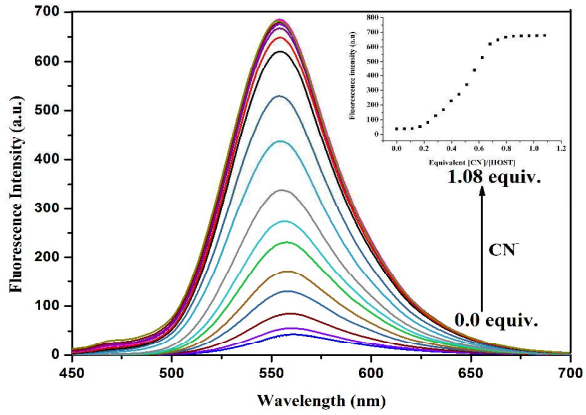

Fig. 6 Fluorescence spectra of **Q1** (2.0×10^{-5} M) in various concentrations of CN^- are acquired in fresh distilled water. Inset: a plot of fluorescent intensity depending on the concentration of CN^- in the range from 0 to 1.08 equivalents. Each measurement was done at room temperature of mixing for **Q1** and CN^- in fresh distilled water (excitation wavelength = 410 nm).

fresh distilled water. Furthermore, the selectivity of **Q1** for CN^- over other anions was examined. The results revealed that all potentially competitive anions (F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, SCN^- and S^{2-}) exerted no or little influence on the UV–vis detection of CN^- in fresh distilled water (Fig. 2). To evaluate the selectivity of **Q1**, we measured the fluorescence intensive of **Q1** in the presence of various anions (F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, CN^-, and SCN^-). As shown in Figure 3, in the fluorescence spectrum, the maximum emission of **Q1** appeared at 562 nm in fresh distilled water when λex = 410 nm. On the addition of 5 equivalents of CN^-, the fluorescence intensity of sensor **Q1** (20 μM) increased rapidly. The fluorescence maximum intensity of **Q1** was dramatically change almost 26 times after the addition of CN^-, whereas the fluorescence intensity changes were not monitored obviously by other anions but were just comparable to **Q1** itself. Meanwhile, the fluorescence color change from dark red to bright yellow and it could be distinguished by a UV lamp at 365 nm as shown in Fig. 3. Competitive assay also showed consistent evidence that **Q1** only was transformed by CN^-. The fluorescence intensity of **Q1** and other anions was restored as much as that of **Q1-CN** by addition of CN^- to the mixture of **Q1** and other anions (Fig. 4).

To further investigate the interaction between probe **Q1** and CN^-, the variation in the UV–vis absorption spectral of probe **Q1** (20 μM) in fresh distilled water was recorded during the titrations with different concentrations of CN^- from 0 to 1.96 equivalents. As shown in Fig. 5, an isosbestic point at 410 nm was clearly observed with increasing concentrations of CN^-. At the same time, fluorescence emission spectral variation of probe **Q1** (20 μM) in fresh distilled water was monitored during titrations with different concentrations of CN^- from 0 to 1.08 equivalents (Fig. 6). With an

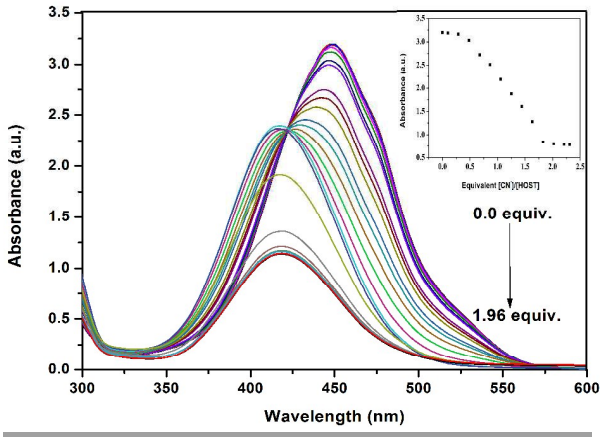

Fig. 5 Absorption spectrum of **Q1** (2.0×10^{-5} M) in various concentrations of CN^- are acquired in fresh distilled water. Inset: a plot of absorbance intensity (emission at 448 nm) depending on the concentration of cyanide in the range from 0 to 1.96 equivalents. Each measurement was done at room temperature of mixing for **Q1** and CN^- in fresh distilled water.

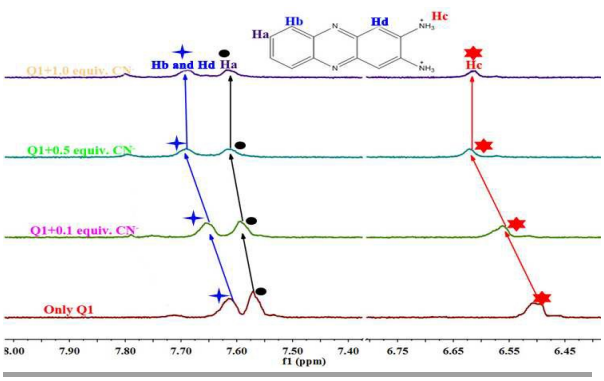

Fig. 7 Titration curves of ^1H NMR spectra of **Q1** (0.01 M) in D_2O upon addition of CN^- (1 M, D_2O).

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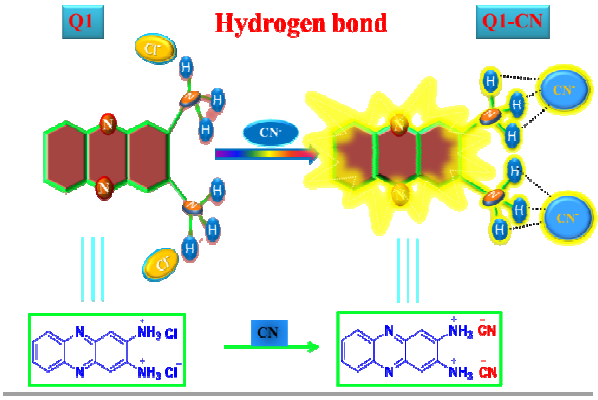

Fig. 8 Chemical structures of **Q1** and **Q1-CN** and the reaction mechanism in this system.

increasing amount of CN^-, fluorescence intensity of sensor **Q1** (λem = 562 nm) gradually increased. A plot of absorbance intensity depending on the concentration of CN^- in the range from 0 to 1.96 equivalents was drawn. Similarly, a plot of fluorescent intensity depending on the concentration of CN^- in the range from 0 to 1.08 equivalents (excitation wavelength = 410 nm) was drawn. Each measurement was done at room temperature of mixing for **Q1** and CN^- fresh distilled water. Furthermore, to determine the detection limit of **Q1**, the UV–vis absorption spectral and the fluorescent spectrums of blank tests were measured 20 times and the standard deviation of the blank measurements was determined. The linear fitting was performed according to the titrations curves, and the mean intensity was calculated to determine the slope.

The detection limit was calculated using the following equation: Detectionlimit = 3δ/S (δ is the standard deviation of the absorbance and emission intensity of **Q1** in the presence of CN^- and **S** is the slope times ten to the sixth between the absorbance/emission intensity and concentration). Obtained by calculation, the absorption spectra detection limit of the chemosensor for CN^- was 1.95×10^{-7} M (Fig. S6) and the fluorescence spectra detection limit

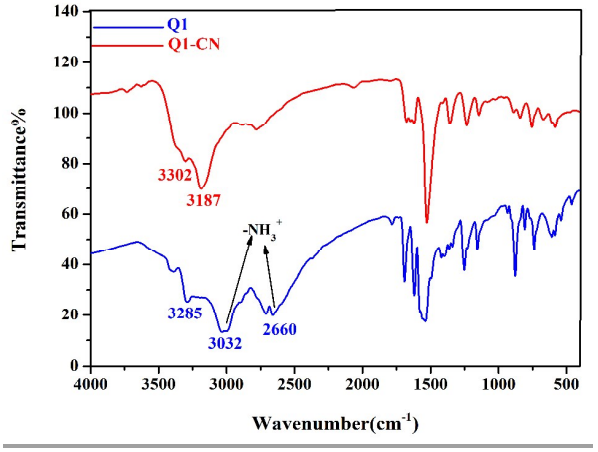

Fig. 9 FT-IR spectra of the compound **Q1** and **Q1-CN** complex powdered in KBr disks.

Fig. 10 Photographs of test strips: only **Q1** (2×10^{-4} M), **Q1** and other anions, **Q1-CN**, **Q1-CN** and other anions (from left to right).

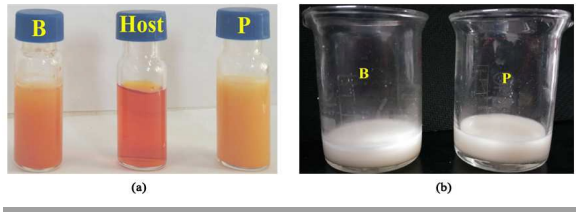

Fig. 11 Photographs showing: (a) **Q1**+Bitter almonds, only **Q1** (2×10^{-4} M), **Q1**+Wild peach kernel in visible light (from left to right). (b) Only bitter almonds filtrate, only wild peach kernel filtrate (from left to right).

was 1.13×10^{-9} M (Fig. S7). This data indicate that **Q1** can detect CN^- at very low concentrations in the environment.

To gain better insight into the sensing mechanism of chemosensor **Q1** to CN^-, the ^1H NMR titration, IR spectrum and ESI-MS were performed. The recognition mechanism was investigated by ^1H NMR titration of the probe **Q1** with CN^-. ^1H NMR titration displayed the chemical shift changes of **Q1** upon the addition of CN^-, as shown in Fig. 7, sensor **Q1** showed peak Ha at δ7.61 ppm, Hb and Hd at δ7.57 ppm and Hc at δ6.50 ppm in D_2O, after the titration we found that with the addition CN^- (concentration from 0 equivalent to 1.0 equivalent), Ha, Hb, Hc and Hd proton exhibited a downfield shift on different levels. However, the addition of more CN^- led to the absorbance decreased monotonously which suggest the decrease in the electron density in the benzene ring and primary amine salt via hydrogen bonds. Through UV-vis absorbance titration experiments, we can infer that this process through hydrogen bonds takes at least two steps. Upon addition of increasing amounts of CN^- to the solution of **Q1**, a decrease in the absorbance peak with a hypochromatic shift of **Q1** was observed. In this step, we consider that only one CN^- forms a hydrogen bond with the host, resulting in a blue shift of the absorption peak. After that, as the concentration of CN^- increases, the host bound two molecules of CN^- by hydrogen bonds. At this point, the system becomes stable and the absorption peak no longer changes (Fig. 8).

Further evidence for the formation of the desired complex was obtained by ESI-MS, revealing a peak of **Q1** at m/z 212.1010 (Fig. S4 in SI), corresponding to $[\mathbf{Q1\text{-}CN}+H]^+$ revealed a peak at m/z 265.1922, (Fig. S5 in SI), indicating a 1:2 stoichiometry between **Q1** and CN^-.

Meanwhile, in order to further illustrate the problem, we did the infrared spectroscopy experiment. In the IR spectrum of **Q1**, a stretching vibration absorption double peaks of primary amine salt

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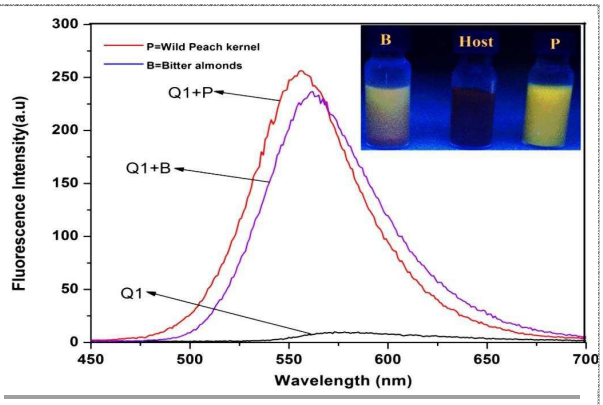

Fig. 12 Fluorescence spectra of target compound **Q1** (2.0 × 10^{-4} M) in the presence of bitter almonds and wild peach kernel in fresh distilled water on excitation at 410 nm. Inset: photograph showing the change in color of the solution of **Q1** in fresh distilled water after addition of bitter almonds and wild peach kernel under irradiation at 365 nm by UV lamp (from left to right).

($-NH_3^+$) appeared in 2660 cm^{-1}, and a broad peak appeared in 3032 cm^{-1}. However, compared with **Q1**, the **Q1-CN** absorptions shifted showed two new peaks at 3302 and 3187 cm^{-1}, respectively (Fig. 9), which indicates primary amine salt reacted with CN^- by hydrogen bonds when 2.0 equivalents of CN^- was added.

To investigate the practical application of chemosensor **Q1**, test strips were prepared and utilized to sense CN^-. As shown in Fig. 10, we prepared test strips by immersing filter papers into pure water of **Q1** (2.0 × 10^{-4} M) and then drying in air. The test strips containing **Q1** were utilized to sense CN^- and other anions. When CN^- and other ions were added on the test kit, respectively, the remarkable colour change was observed only with the CN^- solution under natural light and UV lamp at 365 nm. And potentially competitive ions exerted no influence on the detection of CN^- by the test strips. Therefore, test strips could conveniently detect CN^- in pure water.

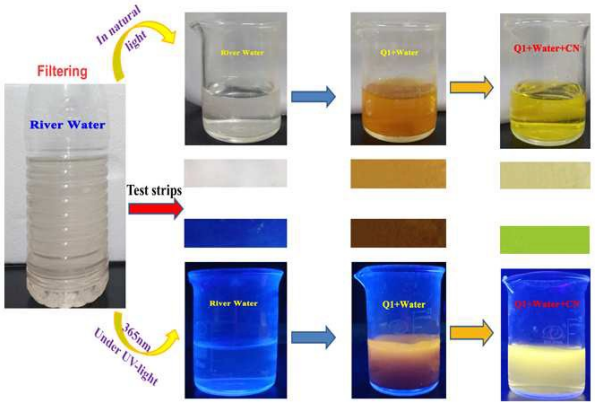

Fig. 13 Photographs showing: the sensing performances of **Q1** and test strips in river water.

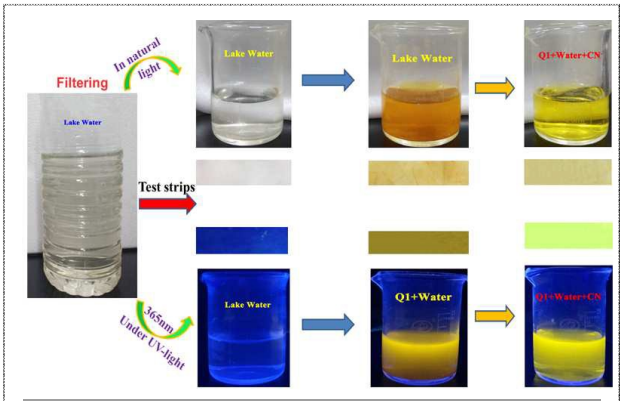

Fig. 14 Photographs showing: the sensing performances of **Q1** and test strips in lake water.

In order to further investigate the practical utilities of probe **Q1** in our lives, we prepared bitter almonds and wild peach kernel to implement this experiment. One gram of dry seed was crushed and pulverized and the obtained mixture must be vigorously stirred for 15 min. Then the mixture was filtered to obtain the cyanide-containing solution. Next, the bitter almond (1 g) was first mashed until the extract became turbid. The mixture was filtered and the filtrate was eluted to obtain the cyanide-containing solution (**B**). The wild peach kernel in cyanide-containing solution (**P**) obtained in the same way. We diluted the **B** and **P** with fresh distilled water, which ensured this experiment in neutral water, upon the addition of the two different CN^- solutions to **Q1** (2.0 × 10^{-4} M), the fluorescence intensity of sensor **Q1** increased rapidly. The color change from red-orange to bright yellow could be distinguished by the naked eyes under natural light (Fig. 11), and the color change from dark red to bright yellow could be distinguished at 365 nm by UV lamp (Fig. 12). Finally, in order to expand the scope of application of this chemical sensor, we have increased the experiment of detecting CN^- in natural water by **Q1**, and we can also detect CN^- in river water and lake water, which also can obtain good results (Fig. 13 and Fig. 14).

Conclusions

In conclusion, we have found that hydrochloride based on 2,3-diaminophenazine has a good dissolvability in water and it can be used to act efficiently in a chemodosimeter approach for the selective detection of CN^- via hydrogen bonding in pure water. Furthermore, an observable color change of dilute solutions of **Q1** under the visible light and fluorescent light when exposed to low concentrations of CN^-. In addition, test strips based on sensor **Q1** were fabricated and it was also successfully applied to the detection of CN^- in bitter almonds and wild peach kernel. Meanwhile, in order to expand the application field, we also detected CN^- in a more complex natural water system. The results show that it can detect CN^- in more complex water systems. Thus, it will be regarded as a simple and highly efficient chemosensor for detecting CN^- and the chemosensor which can be recognized as an ideal, green probe of CN^- in pure water.

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Graphical Abstract

High selectivity and sensitivity chemosensor based on 2,3-Diaminophenazine hydrochloride for detection of cyanide in pure water and its application in plant seeds sample

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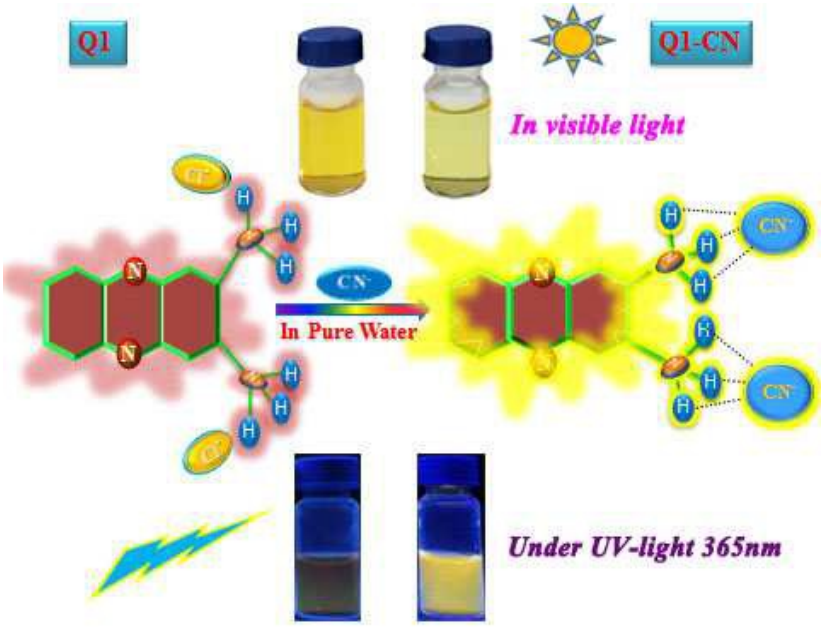

In this work, we have found that hydrochloride based on 2, 3-diaminophenazine has a good dissolvability in pure water, and more importantly, it can be used to act efficiently in a chemodosimeter approach for the selective detection of cyanide in pure water. Upon treatment with cyanide, 2, 3-diaminophenazine hydrochloride (**Q1**) displayed a remarkable naked-eye and fluorescent response. It can detection of cyanide (CN^-) rapidly, simply and has a highly selective and sensitive ability in pure water. And it also successfully applied to the detection of cyanide in bitter almonds and wild peach kernel.