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Selective "Turn-On" Fluorescent Sensor for Cyanide in Aqueous Environment and Test Strips

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Abstract

A new very sensitive and selective fluorescent phenothiazine probe for the recognition of cyanide ions in an aqueous environment was prepared. The detection mechanism depends on the nucleophilic addition of cyanide ions to the fluorescent probe to result in fluorescent change go together with color change from purple to yellow. The prepared phenothiazine sensor was employed for invention of test strips able to recognize cyanide in aqueous media. It was found that the phenothiazine probe could selectively detect cyanide ions. When adding cyanide anions, the color of the yellow phenothiazine solution in dichloromethane changed to yellowish green, while a stronger green emission was monitored under UV lamp. Furthermore, the existence of 10 equivalents of other anions, including AcO^-, HSO_4^-, Cl^-, Br^-, I^-, $H_2PO_4^-$, did not result in apparent variations in the UV-Vis absorption and fluorescent emission spectra. The recognition limit of phenothiazine probe to cyanide aions was 7.2×10^{-8} mol/L in dichloromethane.

Keywords Fluorescence · Sensor · Cyanide · Nucleophile

Introduction

Determination of anions has received significant attention owing to their vital roles in biological systems, environment and industry [1–9]. Anions have potential harmful effects to both of environment and human. Cyanide anions particularly have recently drawn the consideration of researchers. Cyanide is one of the highest lethal toxic materials because it has the capability to suppress the transportation process of oxygen in human body. Cyanide anion played an important role in the reason of human death in fire accidents, and therefore reporting the blood cyanide levels in fire victims has also been an active research field [10–20]. In addition to being exist in numerous foodstuffs and plants, cyanide is also generally employed in a variety of global industrial purposes, such as metal plating and mining, and the production of plastic-based materials [21–28]. As a result, accidental cyanide discharge in effluent or water streams may lead quickly to severe pollution of drinking water. Therefore, it is significantly to find an efficient and dependable sensing process to monitor cyanide anions. Developing fluorescent and colorimetric probes for cyanide has received substantial attention as cyanides are necessary trace element in biological systems and also an important ecological contaminant. Fluorescent and colorimetric probes merge both of sensitivity of fluorescence with the convenient and aesthetic appeal of a colorimetric assay [29–33].

There are different analytical techniques have been developed to sense cyanide, such as chromatography, colorimetry, fluorimetry, and electrochemical analyses. Among the diverse described analytical approaches, fluorescent probes introduce numerous appealing advantages, such as higher sensitivity, remote control, lower prices, simple processing and in particular the appropriateness as a diagnostic device for biological concern. Whilst a range of synthetic organic fluorescent probes for cyanide ion have been designed, only a small number of such sensors could function in aqueous environment and also few of them are able to demonstrate high selectivity over other anions. Moreover, such organic fluorophores may suffer from poor quantum efficiency and low photostability, which therefore add restrictions to their practical applications [34–40].

Herein, we describe the preparation and characterization of new phenothiazine fluorophore chemical probe of a donor-π-acceptor (D-π-A) structure in which one terminal

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oxoindenylidenemalononitrile vinyl moiety acts as an acceptor moiety, while a phenothiazine as a donor. Upon the nucleophilic Michael addition of cyanide anion to the phenothiazine probe, the intramolecular charge transfer (ICT) amid D-π-A gets broken to result in a fast optical responding accompanied by high sensitivity and selectivity.

Experimental

Methods and Apparatus

All solvents and reagents (spectroscopic grade) were purchased from Sigma-Aldrich. ^{1}H and ^{13}C NMR were studied in $CDCl_3$-d_6 using Bruker Advance 600 MHz. Infrared spectra were made on PerkinElmer spectra 100 spectrometer. Mass spectroscopy was made by Agilent GC 7000 spectrometers. UV-visible absorption spectra were studied on Shimadzu UV-visible Spectrophotometer, while fluorescence spectra were reported on PerkinElmer LS 55 Spectrometer.

Synthesis and Characterization

10-Dodecyl-10H-Phenothiazine 1

Colourless oil, Yield 86.15%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.904 (3H,t, J = 7.2 Hz, CH_3),1.2–1. 4 (4H, m, CH_2), 1.449 (2H, m, CH_2), 1.825 (2H, m,CH_2), 3.858 (2H, t, NCH_2), 6.922 (2H,d,J = 7.8 Hz, Ar-H), 6.935 (2H,t, J = 7.8 Hz, Ar-H), 7.158 (2H,d,J = 7.8 Hz, Ar-H), 7.187 (2H,t,J = 10.2, 6.6 Hz, H-Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.21, 22.81, 26.88, 27.09, 31.69, 47.91, 115.83, 115.69, 122.69, 125.17, 127.65, 145.30. IR ν /cm^{-1}: C–H olefinic 3063.841 C–H aliphatic 2953.83, 2924.75, 2854.52 C=C stretch 1594.00, 1571.29, 1456.74.

10-Dodecyl-10H-Phenothiazine-3-Carbaldehyde 2

Yellow solid, Yield 91.18%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.912 (t, 3H, CH_2-N), 6.905(d, 1H, *J* = 8.4 Hz, Ar-H), 6.923 (d, 1H, *J* = 9.0 Hz, Ar-H), 6.99 (td, 1H, *J* = 7.2,1.2 Hz, Ar-H), 7.136 (dd, H, *J* = 7.8,1.8 Hz),7.187 (td, 1H, *J* = 6.6, 1.8 Hz, Ar-H), 7.61 (d,1H, *J* = 2.4 Hz, Ar-H), 7.663 (dd, 1H, *J* = 7.8,1.8 Hz, Ar-H), 9.818 (s, 1H, CHO). ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.97, 22.57, 26.52, 26.74, 31.38, 48.03, 114.75, 115.94, 123.57, 127.54, 127.57, 128.43, 130.06, 190.22 IR ν /cm^{-1}:C-H olefinic 3059.72, C-H aliphatic 2953.46,2926.49, C-H aldehyde 2725.48, 2854.75, C=O 1682.86.

2-(2-((10-Dodecyl-10H-Phenothiazin-3-Yl)Methylene) -3-Oxo-2,3-Dihydroinden-1-Ylidene)Malononitrile (C3)

A mixture of **2** (3 mmol) and 3-dicyanovinylindan-1-one (6 mmol) in basic ethanol solution (7 mL) was stirred at room temperature overnight, filtered off and crystallization from cyclohexane to afford 80% yield. M.p. 89–90 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.923 (t, 3H,CH_3), 1.4–1.55 (m, 6H, CH_2), 1.869 (q, 2H, CH2), 3.941 (t, 2H, CH_2-N), 6.90 (t, 2H, J = 6.0 Hz), 7.00 (t,1H, J = 6.0 Hz, Ar-H), 7.12 (d,1H, J = 6.0 Hz, Ar-H), 7.18 (t,1H, J = 6.0 Hz, Ar-H), 7.779 (m,2H, Ar-H),7.944 (d,1H, J = 7.2 Hz, Ar-H), 8.162 (d,1H,J = 8.4 Hz, Ar-H).8.200 (d,1H, Ar-H), 8.441(s,1H, Ar-H), 8.708(d,1H,J = 7.8 Hz, Ar-H). ^{13}C NMR (125 MHz, CDCl3) δ 14.19, 22.81, 26.73,26.98, 27.14, 31.59, 48.52, 70.52, 114.56, 114.84, 116.15, 124.22, 124.25, 124.34, 126.66, 127.77, 133.55, 134.80, 135.37, 136.67, 137.62, 139.91, 146.54, 150.69, 162.66, ESI-MS m/z [M] + calc 487.61 found 486. IR ν /cm^{-1}: C-H aliphatic 2925.49, 2851.27, CN 2214.23, C=O 1739.15, C=C 1693.96.

Spectroscopic Procedures

A solution of the prepared phenothiazine probe (2×10^{-5} mol L^{-1}) in acetonitrile-water (90:10) was titrated with increments of aqueous potassium cyanide (2×10^{-3} mol L^{-1}). Titration experiments were carried out in 10-mm quartz cell at room temperature. (λ_{ex} = 500 nm, λ_{em} = 588 nm). The selectivity experiment was made by monitoring the fluorescence intensity changes of the prepared phenothiazine probe (2×10^{-5} mol L^{-1}) in acetonitrile-water (90:10) at 588 nm (λex = 500 nm) upon addition of various anions at the concentrations indicated below the figure. The limit of detection was calculated by the equation; $\mathbf{3S}/\boldsymbol{\rho}$, where **S** is the standard deviation of blank measurements (10 times); $\boldsymbol{\rho}$, is the slope between intensity versus sample concentration.

Results and Discussion

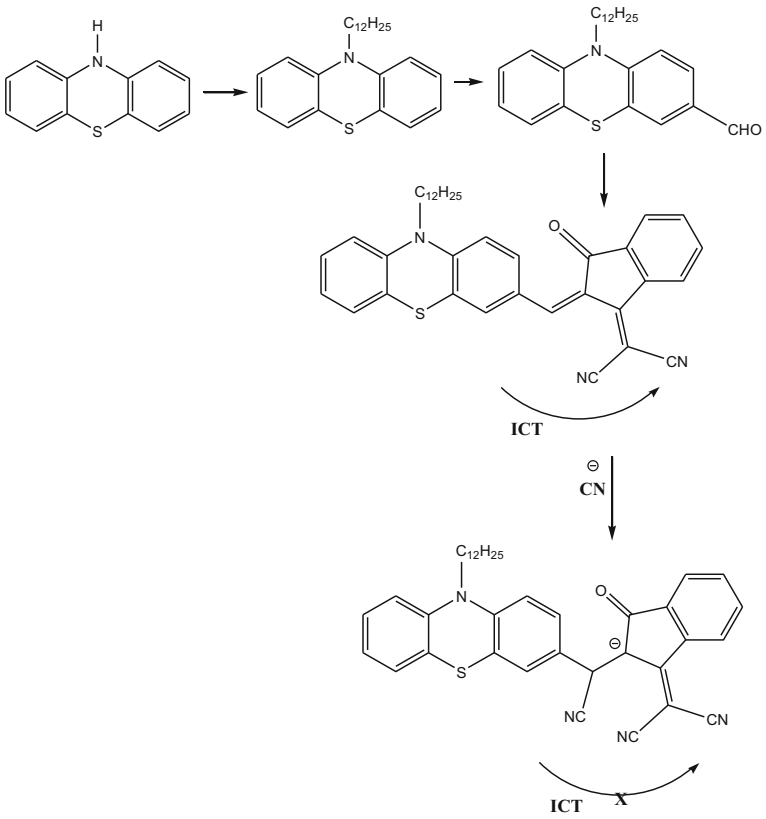
Synthesis of Phenothiazine Probe

The phenothiazine sensor was prepared as shown in Scheme 1. Initially, the phenothiazine moiety was *N*-alkylated and then subjected to Vilsmeier formylation to provide the corresponding formylated *N*-dodecyle phenothiazine in high yield. Knoevenagel condensation of formylated *N*-dodecyle phenothiazine with 2-(3-oxo-2,3-dihydro-1*H*-inden-1-ylidene)-malononitrile afforded the targeted phenothiazine sensor. The chemical structure of phenothiazine sensor was evidently confirmed by different analytical methods including ^{1}H- and ^{13}C NMR and FT-IR as demonstrated in the Supplementary Information.

Photophysical Properties

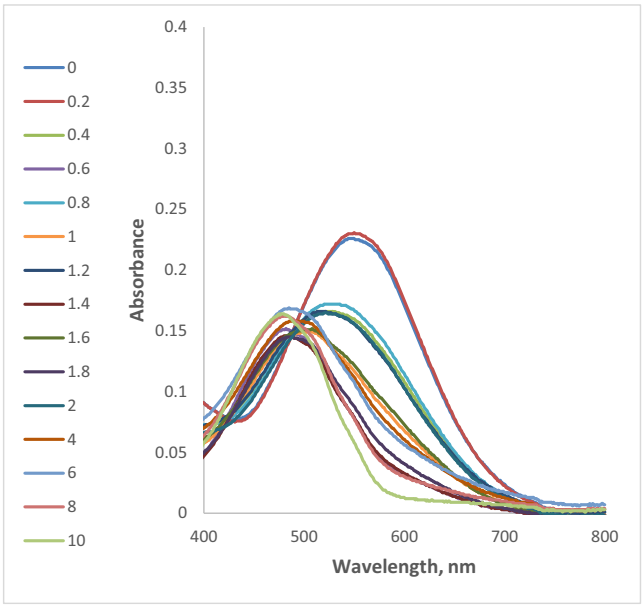
The UV-visible absorption and emission spectra of the prepared phenothiazine sensor were performed in a

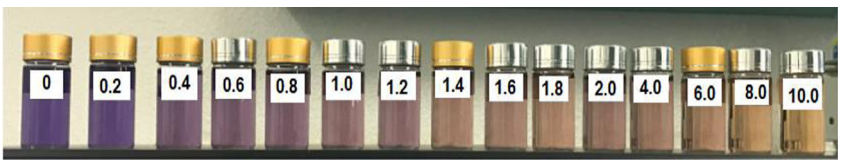
Scheme 1 Preparation of phenothiazine probe and its detection mechanism



mixture of acetonitrile/water (90:10) as shown in Figs. 1 and 2. It was monitored that the wavelength maxima of UV-Vis absorption and emission bands were reported at 500 and 576 nm, respectively. The visible band of the

Fig. 1 UV-Vis absorption spectra of phenothiazine probe (2×10^{-5} mol L^{-1}) in acetonitrile/water (90:10) upon raising cyanide concentration





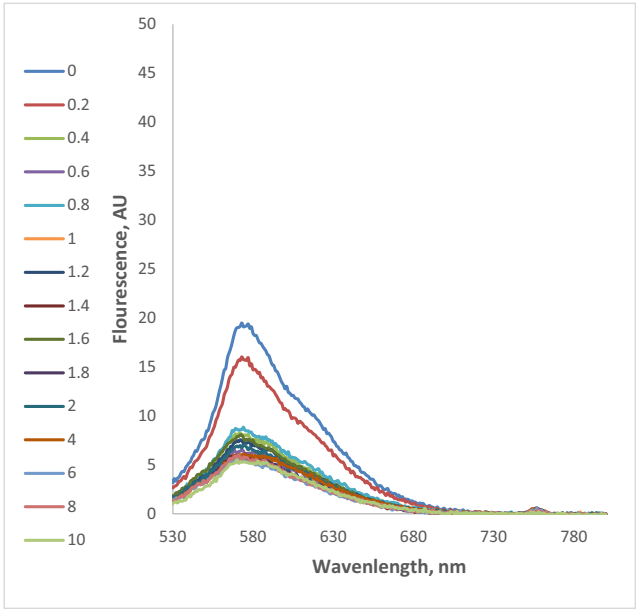


Fig. 2 Fluorescence emission spectra of phenothiazine probe (2×10^{-5} mol L^{-1}) upon adding cyanide at different concentrations in CH_3CN/H_2O (90:10). The emisison was recorded at 25 °C (ex = 500 nm, em = 588 nm)

absorption spectrum was owing to ICT with a relatively high molar extinction coefficient of about 1.5×10^4 mol^{-1} L cm^{-1}. In an acetonitrile (Fig. 1), the values of wavelength maxima were the same as in CH_3CN/H_2O, nonetheless, a slightly lesser extinction coefficient value was monitored at 1.4×10^4 mol^{-1} L cm^{-1}. On the other hand, the emission spectra showed a little blue shift to a lower wavelength by ~3 nm compared to that from acetonitrile/water. A responding period diagram for the phenothiazine sensor upon the addition of cyanide was examined following method A. The data shown in

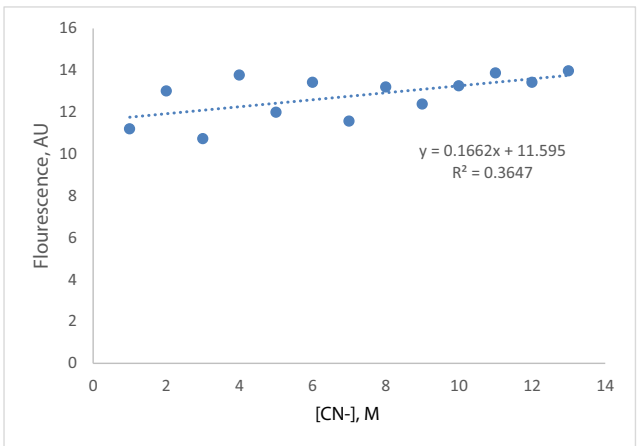


Fig. 3 Fluorescence emission intensity calibration curve of phenothiazine probe (2×10^{-5} mol L^{-1}) as a function of the cyanide concentration in CH_3CN/H_2O (90:10)

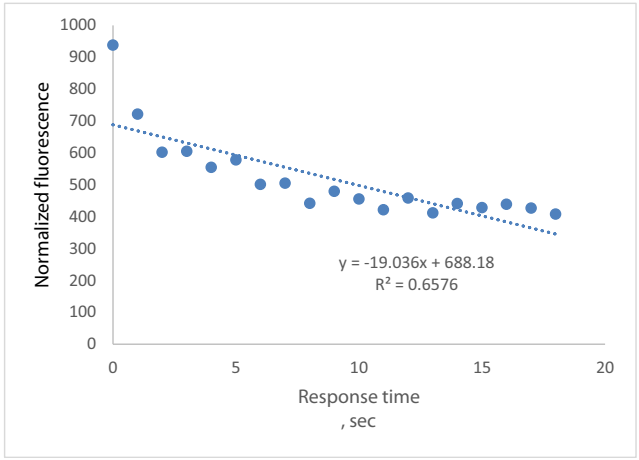
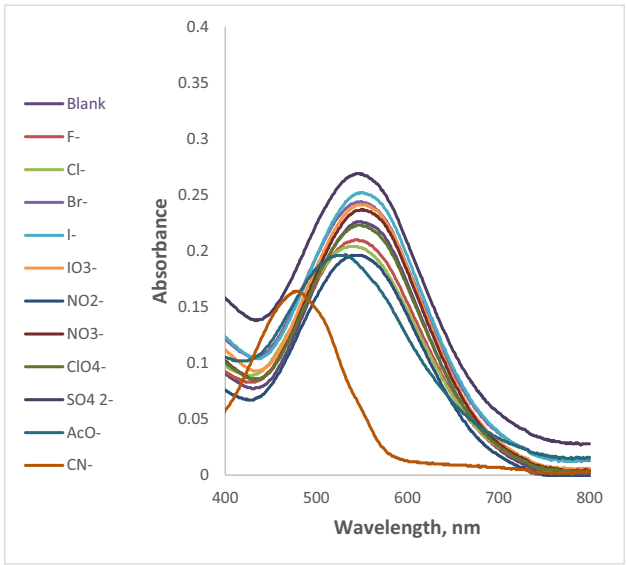


Fig. 4 Responding period for the recognition of cyanide in CH_3CN/H_2O (90:10)



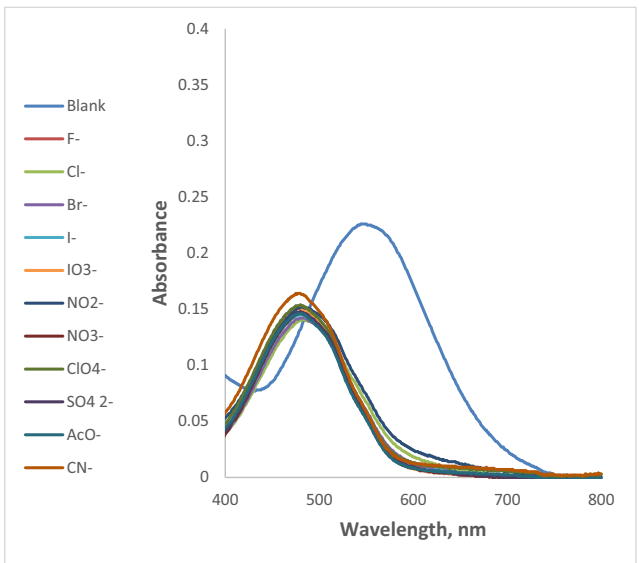


Fig. 5 UV-Vis absorption spectra of phenothiazine probe (2×10^{-5} mol L^{-1}) in acetonitrile/water (90:10) with different anions

Fig. 2 demonstrated that about 3 min was enough periods to verify the final value of the emission intensity. Thus, the value of 5 min was chosen as the best period during the titration of phenothiazine sensor with cyanide (Fig. 3). The detection limit (3.06×10^{-6} mol L^{-1}) of cyanide was achieved following method A. The responding period diagram for phenothiazine sensor upon the addition of cyanide was examined following method B. The data shown in Fig. 4 indicated that about 50 s is the requisite period to report the final value of emission intensity. Thus, we chosed about 3 min as the best period during the titration of phenothiazine sensor with cyanide (Figs. 5 and 6). The detection limit (3.2×10^{-9} mol L^{-1}) of cyanide was recorded following method B. This recognition limit is far away less than the allowable standard limit value at 1.9×10^{-6} mol L^{-1}.

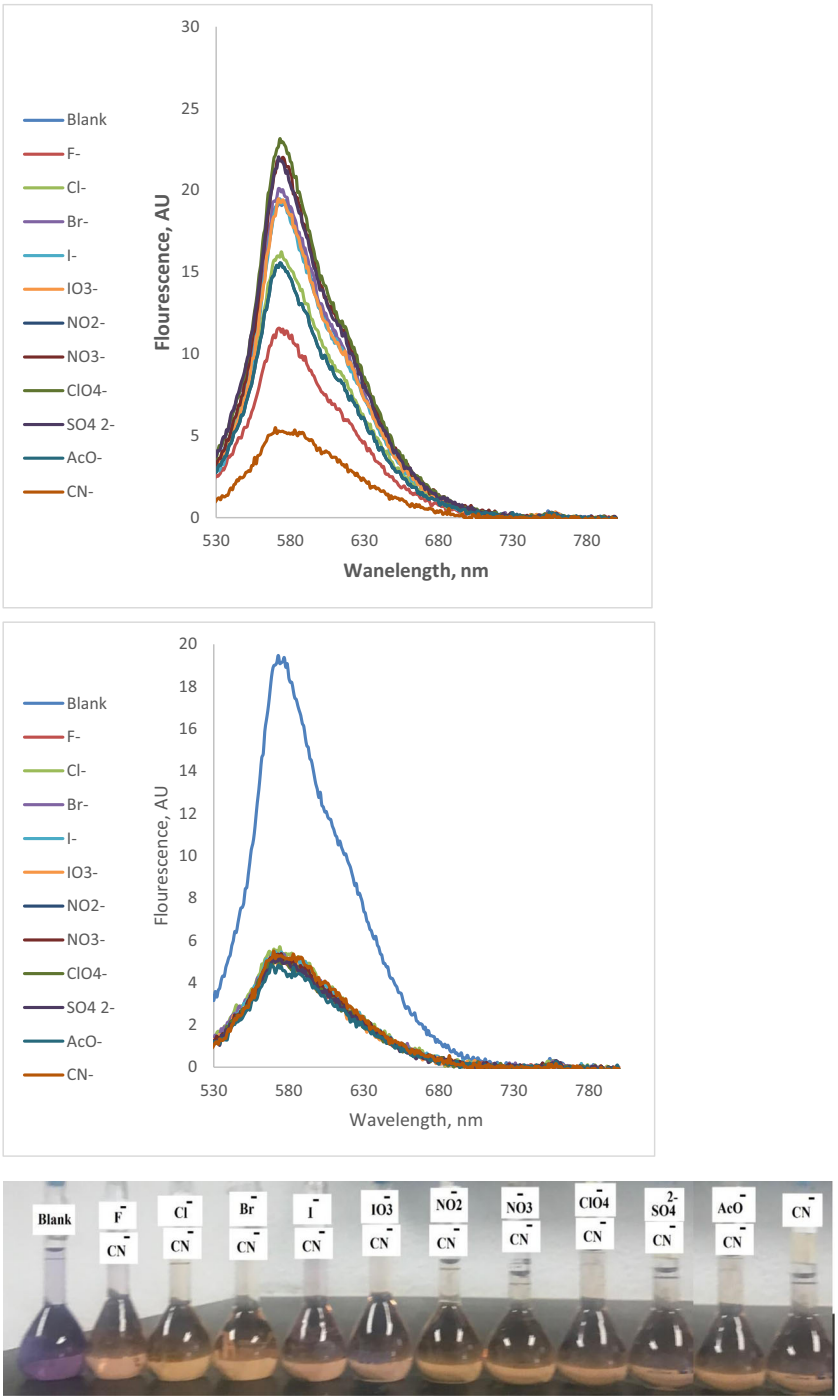


Fig. 6 Emission spectra of phenothiazine probe (2×10^{-5} mol L^{-1}) in acetonitrile/water (90:10) with different anions

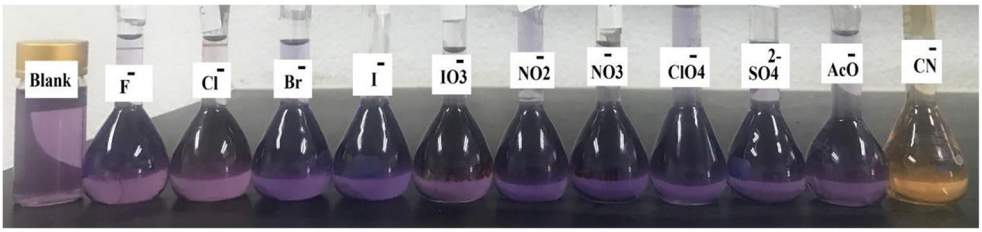


Fig. 7 The color variations with adding different concentration of cyanide (A) 10 equivalents of different anions (B) and mixing 10 equivalents of cyanide and other competing anion (C). (A) image of cyanide responding A2 (from left to right: 0equivalent,); (B) in the presence of 10 equivalent of different anions (from left, blank, Br^-, I^-, F^-, Cl^-, NO_3^-, NO_2^-, SO_4^{-2}, AcO^-, ClO_4^-, CN^-); (C) in the presence of 5 equivalent of CN- and 5 equivalent. of different anions (from left, blank, Br^-, I^- F^-, Cl^-, NO_3^-, NO^{2-}, SO_4^{-2}, AcO^-, ClO_4^-)

Selectivity of Phenothiazine Probe

To evaluate the selectivity of the phenothiazine probe to cyanide, a diversity of analytes, including NO_2^-, $S_2O_3^{2-}$, F^-, Cl^-, Br^-, I^-, AcO^- and SO_4^{2-} were investigated in CH_3CN/H_2O (90:10). Unexpectedly, even four equivalents of the analytes shown above did not result in any considerable variation in the emission intensity as demonstrated in Figs. 6 and 7. On the other hand, the addition of the cyanide to the mixture resulted in a significant decrease in the emission intensity indicating that phenothiazine probe is selective to cyanide with no interference with other anions.

The polarizable double bond in the 2-(3-oxo-2,3-dihydro-1*H*-inden-1-ylidene)-malononitrile groups is highly reactive owing to the existence of high electron withdrawing cyano and carbonyl substituents, which resulted in the generation of an electron-deficient reaction site appropriate for nucleophilic reagents like cyanide. This course would rupture the ICT in the conjugated system of phenothiazine probe to lead to an optical response, proposing that the 2-(3-oxo-2,3-dihydro-1*H*-inden-1-ylidene)-malononitrile moiety of phenothiazine probe was reacted with cyanide by breaking C=C bond and forming a new aliphatic C-C bond.

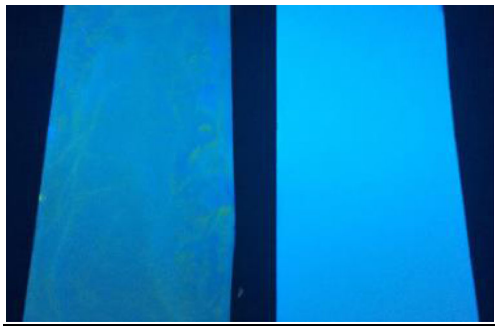


Fig. 8 Color variations of the dipstick test strips under ultraviolet irradiation containing A3 treated with cyanide (left) and untreated (right)

Dipstick Test

A dipstick test strip was immobilized by the phenothiazine probe via simple immersion of test strip in a solution of the phenothiazine probe in acetonitrile (10^{-3} mol L^{-1}) and then air-dried at atmospheric circumstances. This immersion-drying process was repeated three cycles for the same test strip. Figure 8 and 9 was taken under ultraviolet irradiation before and after dipping the coated test strip in cyanide solution. It was shown that phenothiazine probe could be used as a simple dipstick test strip for a quick detection of cyanide. Moreover, a qualitative detection was also potential under ultraviolet irradiation lamp.

Conclusions

A new phenothiazine fluorophore for detection of cyanide was designed, synthesized and characterized. The fluorophore demonstrated an excellent detection limit as low as 3.2×10^{-9} mol L^{-1}, which is realistic recognition limit for cyanide anion in an aqueous medium. The phenothiazine fluorophore

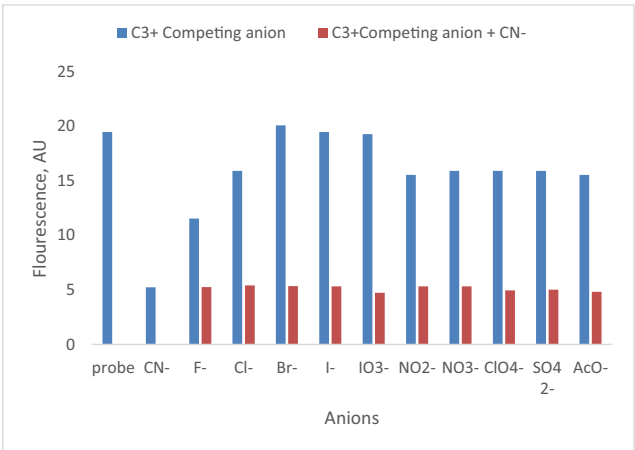


Fig. 9 Fluorescence intensity variations of A3 (2×10–5 mol L^{-1}) in CH_3CN/H_2O (90:10) in the presence of competing anions. (ex = 500 nm, em = 588 nm)

was applied to develop a simple and easy-to-use dipstick assay for fast recognition of cyanide.

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Compliance with Ethical Standards

Conflicts of Interest The author declares no conflict of interest.

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