ORIGINAL ARTICLE



Cyanine-based Fluorescent Probe for Cyanide Ion Detection

Mahesh Gosi¹ · Nagaraju Marepu¹ · Yeturu Sunandamma¹

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Abstract

Cyanine-based probe-possessing indolium iodide and indole unit were synthesized in two-step with easy available raw material: a potential probe for the cyanide ion detection. The detecting ability of the probe was investigated and confirmed by a visual and instrumental approach. A noticeable color change from orange to colorless obtained only for cyanide ions and other added ions does not impart any changes visually and through UV and Fluorescence technique. To confirm the mechanism of sensing 1H-NMR recorded. From the result, the peak belonging to *N*-methyl displayed an upfield shift from 4.01 δ ppm to 2.74 δ ppm due to the disappearance of indolium iodide ion and the olefin protons peaks were shifted from 7.19 to 6.17 and 8.70 to 7.20 δ ppm confirms the nucleophilic addition of cyanide ion to the probe. Test kit from filter paper prepared for the real-time monitoring cyanide ion. The prepared strip is effective in detecting cyanide ion with a visual color change.

Keywords Orange to colorless · Nucleophilic addition · Test strip · Fluorimetric probe · Cyanide ion

Introduction

Cyanide (CN⁻) is the primary pollutant found in our water surface due to its extensive industrial usage. It is involved in several chemical procedures such as silver extraction and gold extraction, metallurgy, tanning, plastic and medicine manufacturing, electroplating, etc. [1–5]. Besides, cyanide anion containing gases used in eliminating pests and insects and is used as a warfare chemical agent [6, 7]. The world health organization set a bearable amount of CN⁻ ions in drinking water and is nearly 500 µM for short-term and 70 µM for long-term exposures [8]. Several methods are currently reported to recognize CN⁻ ions, such as titrimetric, potentiometric, chromatographic, voltammetry, and electrochemical approaches [9-12]. However, many of these approaches are time consuming, expensive, and difficult for on-site recognition. Hence, it is essential to produce extremely selective, sensitive, and suitable methods for recognizing CN⁻ ions in real samples. The simple, economical, and quick executions of optical sensors (colorimetric/ fluorimetric) have been getting much attention recently [13-17]. In the optical chemosensors, various mechanisms

☑ Yeturu Sunandamma yeturuasnu7@gmail.com were reported in the literature, such as hydrogen bonding, nucleophilic addition (chemodosimeter), and supramolecular self-assembly [18–24]. The maximum of developed probes suffered by challenging synthetic procedures, inadequate color response, lack of selectivity, and sensing limits in a complete aqueous medium. However, some of the receptors overcome these drawbacks to detect CN⁻ ions reported in the literature [25-27]. Indolium moieties are also identified CN^{-} ions [28–30]. Thus, the progress of developing a useful probe, which could be proficient at recognizing cyanide ions in a maximum water-consuming medium, is getting extreme care. Considering these points, we have synthesized a simple cyanine-based probe (PI) having the indolium iodide and indole moieties connected with an olefin bridge. Hence, an intramolecular charge transfer transition (ICT) will take place between the electron-deficient indolium iodide and electron-rich indole moiety via olefin bridge. Therefore, it showed brilliant recognizing properties. Further, the CN⁻ ion reacts with probes' indolium iodide site by the nucleophilic attack that interrupts the π -conjugation and thus blocked the ICT from indole to indolium iodide group follow-on a substantial fluorescence and absorption variation can occur.

¹ Department of Chemistry, Acharya Nagarjuna University, Nagarjunanagar Guntur-522510 Andhra Pradesh, India

Scheme 1 Synthesis of probe (PI)



Experimental

Materials and Methods

2.3.3-Trimethyl-3*H*-indole, 1*H*-indole-3-carbaldehyde, iodomethane, anions in the form of tetrabutylammonium salts were purchased from Sigma Aldrich, Piperidine purchased from TCI chemicals. Toluene and ethanol HPLC grade solvents received from Rankem. ¹H NMR and ¹³C NMR spectra were reordered to confirm the compounds. Bruker AVANCE III spectrometer was used and operated at 600 MHz (¹H NMR), and 150 MHz (¹³C NMR) at ambient temperature, DMSO-d₆ is used as a solvent. BrukermicrOTOF QII mass spectrometer was used to record the HRMS. Agilent 8453 spectrophotometer was used to obtain UV-Vis absorption spectra and RF-5301PC spectrofluorophotometer was used for fluorescence emission spectra. For the fluorescence measurements, the excitation wavelength was fixed as 290 nm, and both the excitation and emission slit widths were 3 nm.

Synthesis

Fluorescent probe (**PI**) was easily synthesized in two steps, by using simple and inexpensive starting materials. In the first step, methylation of compound (**1**) with iodomethane gives compound (**2**) excellent yields. In the second step, compound (**2**) undergoes a simple aldol condensation reaction with indole aldehyde to provide a fluorescent probe (**PI**) in good yield. The synthesized compounds were confirmed by NMR (¹H and¹³C) and HRMS. Scheme 1 Synthesis of probe (**PI**).

Experimental Procedure for the Synthesis of 1,2,3,3-tetramethyl-3*H*-indolium lodide (2)

Compound **1** (0.5 g, 4.399 mmol) was dissolved in toluene (10 mL) and iodomethane (0.85 mL, 13.207 mmol) was added at room temperature and the reaction was refluxed for 6 h. during the reaction the solid precipitate was formed. The reaction mixture was cooled to room temperature and the solid was filtered and washed with diethyl ether (20 mL). The resulting white crystalline solid was used for next step without purification. Yield: (0.734 g, 96%), mp 258 – 260 °C, ¹H NMR (DMSO-d₆, 600 MHz) δ 7.94 – 7.92 (m, 1H), 7.86 – 7.83 (m, 1H), 7.64 – 7.61 (m, 2H), 3.99 (s, 3H), 2.80 (s, 3H), 1.54 (s, 6H); ¹³C NMR (DMSO-d₆, 150 MHz) δ 196.4, 142.5, 142.0, 129.7, 129.2, 123.7, 115.6, 54.4, 35.3, 22.2. HRMS m/z calculated for C₁₂H₁₆N⁺ (M)⁺: 174.1277, found: 174.1280.

Experimental Procedure for the Synthesis of 2-(2-(1*H*-indol-3-yl) vinyl)-1,3,3-trimethyl-3*H*-indol-1-ium lodide (Pl)

Compound **2** (0.5 g, 2.871 mmol) and indole-3-carbaldehyde (0.41 g, 2.871 mmol) was dissolved in ethanol (15 mL) and piperidine (3 μ L, 0.12 mmol) was added and refluxed for 3 h under nitrogen atmosphere. The orange solid was obtained, filtered and washed with diethyl ether (20 mL) to produce a bright solid. Yield: (0.95 g, 78%), mp 285 – 287 °C, ¹H NMR (DMSO-d₆, 600 MHz) δ 12.76 (brs, 1H), 8.70 (d, *J*=15.8 Hz, 1H), 8.69 (s, 1H), 8.28 (dd, *J*=5.8, 3.0 Hz, 1H), 7.81 (d, *J*=7.3 Hz, 1H), 7.76 (d, *J*=7.9 Hz, 1H), 7.62 (dd, *J*=5.8, 3.0 Hz, 1H), 7.57 (t, *J*=7.5 Hz, 1H), 7.50 (t, *J*=7.5 Hz, 1H),



Fig. 1 Color changes of PI with various anions A in day light, B under UV lamp (λ =365 nm)



Fig. 2 Absorption spectral changes of PI with several anions

7.39 (dd, J=6.0, 3.0 Hz, 1H), 7.19 (d, J=15.8 Hz, 1H), 4.01 (s, 3H), 1.81 (s, 6H); ¹³C NMR (DMSO-d₆, 150 MHz) δ 180.6, 149.3, 142.9, 142.5, 138.8, 129.7, 129.2, 129.1, 127.9, 125.0, 124.8, 123.6, 123.1, 121.9, 116.3, 115.5, 114.0, 113.9, 105.5, 54.3, 51.3, 22.1. HRMS m/z calculated for C₂₁H₂₁N₂⁺ (M)⁺: 301.1699, found: 301.1704.

Results and discussion

Visual Reaction of Probe PI

The binding interaction of the probe **PI** ($10 \times 10^{-5}M$) with various anions ($10 \times 10^{-5}M$) AcO⁻, SCN⁻, CO₃²⁻, SCN⁻, PF₆⁻, CNO⁻, HSO₄⁻, H₂PO₄⁻, CL⁻, Br⁻, NO₃⁻, CIO₄⁻, F⁻, SO₄²⁻ and N₃⁻ were inspected in DMF: water (2:8, v/v) through

colorimetric appreciation performance was exhibited in Fig. 1A. The synthesized probe **PI** display deep orange color which immediately changed to colorless with cyanide addition while the other added ions not generating such color variation. Similarly, the changes obtained were checked under UV lamp the probe and the added ion except cyanide ion showed little yellow fluorescence means no substantial change in the probe solution and with cyanide ion, it is showing blue fluorescence under UV light Fig. 1B. This primary examination exposed that the probes **PI** can selectively recognize CN⁻ ion with a discrete color variation.

Absorption Studies

The absorption modification of probe PI was examined with the existence of several anions as mentioned directly above. As seen from Fig. 2, the probe PI displayed a band pointed at 489 nm, this might be because of the ICT throughout the probes, and this transition is accountable for the color of probes. Further, the added ions did not produce the least alteration in the bands while the adding of cyanide ion showed a fresh band at 289 nm array with the disappearance of a band at 489 nm. The blue shift observed in the probe with CN⁻ ion interaction indicates that there was a strong binding between the probe and cvanide ion. To know the binding capability of probe **PI** $(1 \times 10^{-5} \text{ M})$, a titration was conducted with an increase in the addition of CN⁻ ion to the probe solution (Fig. 3A). With the gradual quantities of CN^{-1} ions $(0-1 \times 10^{-5} \text{ M})$ added, the fresh band placed on 289 nm detected with the growing intensity of the band, which is attributed by clear color transformation, which could be seen above. The limit of detection of PI for CN⁻ was deliberated as 1.73×10^{-5} M, based on 38/k (Fig. 3B).



Fig. 3 A Absorption changes of PI $(1 \times 10^{-5} \text{ M})$ with CN⁻ ion $(0-1 \times 10^{-5} \text{ M})$ addition in 2:8 v/v DMF: Water, **B** Plot for limit of detection calculation



Fig. 4 Fluorescence spectral changes of PI with various anions

Fluorescence Spectral Studies

Similarly, the recognizing capability of **PI** with chosen anions has been inspected by emission spectral technique when exiting at 289 nm. As seen from Fig. 4, the probe **PI** exited at 289 nm. Then the emission peak appeared at 373 nm and was enhanced with the added CN^- ion, whereas no changes in the spectra were spotted with the other chosen anions. Thus, the above observation states that there is an interaction among the probes with cyanide ions.

The 'On–Off' response of fluorescence is effectively monitored with the steady addition of CN^- ion $(0-1 \times 10^{-5} \text{ M})$ to the solution of probes. Figure 5A displayed that the probes shown its emission at 373 nm for **PI** were in progress to increase progressively with the CN^- ion addition. The emission peak of the probe enhanced with the added cyanide



Fig. 6 The pH effect of probe PI and its cyanide complex

ion $(0-1 \times 10^{-5} \text{ M})$. The color variations of **PI** with the added cyanide ion under UV lamp was shown in Fig. 5A (inset). With the added CN⁻ ion, the probes turned their less fluorescent yellow color to deep blue fluorescent. From the obtained data from the fluorescence spectra, the binding constant can be calculated using the equation for cyanide ion $(F_{\alpha} - F_0)/(F_x - F_0) = 1/K[CN^-]$ it is $1.17 \times 10^5 \text{ M}^{-1}$ (Fig. S7). The calculated detection limit by emission profile was found to be $1.8 \times 10^{-8} \text{ M}$ for **PI** by means of $3\sigma/k$ (Fig. 5B).

pH Effect of Probe Towards CN⁻ Ion

The recognizing aptitude of **PI** for cyanide ion was further checked by the effect of pH by absorption study. As seen in Fig. 6, in acidic and neutral pH (2–7), the free probes exhibited absorbance at 489 nm while the absorbance is reduced



Fig. 5 A Fluorescence spectral changes of PI (1×10^{-5} M) with CN⁻ ion ($0-1 \times 10^{-5}$ M) addition in 2:8 v/v DMF: Water, B Plot for limit of detection calculation



Fig. 7 Competitive study of probe PI with cyanide ion in presence of different anions

with CN⁻ ions. At basic pH (8–10), the probes absorbance gently weakened and touched the lowermost λ_{max} (pH 10), whereas no absorbance was detected with the added CN⁻ ions. The changes in the absorbance peak in basic pH are primarily because of the OH⁻ion interference, which binds intensely with the probes.

Anti-interference Study of Probes

Moreover, to inspect the anti-interference study of probe **PI** with CN^- ion in the existence of added interfering anions, a competitive investigation has been carried out (Fig. 7). The emission of probe **PI** (1×10^{-5} M) with several chosen ions (1×10^{-5} M) excepting CN^- were initially monitored. Afterwards, the emission was measured for a second time with the same samples with the addition of 1×10^{-5} M of CN^- ions. The figure pointed out that there is no or weak emission intensity for free probes and probes with the chosen ions, but the peak intensity increased with the added cyanide ion to the probe with other ion. These observations suggested that the anti-interference of the probes for cyanide ion possibly would not be inclined by chosen competing ions. Hence, these probes confirm the exceptional selectivity for cyanide ions.

Probable Mechanism by ¹H NMR Study

In the synthetic scheme, *N*-methylation step (step-1) was confirmed by the appearance of sharp singlet at 3.99 (¹H NMR) and 54.4 δ (¹³C NMR) ppm, which corresponds to the methyl protons. These protons are more deshielded due to the



Fig. 8 The partial ¹H-NMR spectra of A PI and B PI-CN in DMSO-d₆





formation of indolium iodide ion (strong electron-withdrawing ability (N⁺-CH₃). The HRMS spectra also further proved the *N*-methylation. In the second step, the fluorescent probe (**PI**) was confirmed by the disappearance of a sharp singlet peak of methyl protons at 2.80 (¹H NMR) and 35.3 δ (¹³C NMR) ppm, and the appearance of indole protons as well as olefin bridge protons in the ¹H NMR spectrum. The stereochemistry of the double bond (trans or "Entagagen") was also confirmed by the ¹H NMR coupling constant of olefin protons (*J*=15.8 Hz). This probe **PI** is used for the detection of CN⁻ anion. The nucleophilic addition of CN⁻ anion to the C=N bond in the indolium iodide ring was also confirmed by the in situ analysis (NMR) of the sample.

In the probe **PI**, the chemical shift values of *N*-methyl protons appeared at 4.01 (HX) δ ppm and the olefin protons were observed at 7.19 (HY) 8.70 (HZ) δ ppm. In the next step, we dissolved this probe PI and TBACN in DMSOd₆ solvent and recorded ¹H NMR spectra. After CN⁻ anion attack, the chemical shift values were shifted to upfield region on the ¹H NMR scale (Fig. 8). Moreover, a remarkable difference was observed in the chemical shift values of HX, HY and HZ. The peak belonging to N-methyl displayed an upfield shift from 4.01 δ ppm to 2.74 δ ppm due to the disappearance of indolium iodide ion. Further, the olefin protons peaks were shifted from 7.19 to 6.17 (HY) and 8.70 to 7.20 (HZ) δ ppm. From the above data, it is confirmed that the PI molecule underwent CN⁻ nucleophilic addition reaction that cleanly converted the indolium iodide ion to indoline-2-carbonitrile (PI-CN).

Practical Application

The probes' practical use was monitored by making test strips for the immediate recognition of CN^- ions in aqueous solutions. Whatman paper was immersed into DMF solution $(1 \times 10^{-3} \text{ M})$ of **PI** followed by drying. These strips were then tested with an aqueous solution containing different CN^- ion concentrations and thus were displayed in Fig. 9, where the strips of probes slowly decolorized. Therefore, the test kit prepared from filter paper appropriately senses cyanide ions in a water medium without the requirement and the usage of any additional equipment. This experimentation creates that the probes are reasonably helpful for quick on-site recognition of CN^- ion.

Conclusions

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To conclude, we have developed a cyanine-based conjugated system of probes for the recognition of cyanide ions by nucleophilic addition mechanism. The recognition method of cyanide ion is visualized by a notable color variation to colorless from orange color. The lowermost limit of detection for CN^- ion was proven by both fluorimetric approaches and thus below the tolerable limit set by WHO. The ¹H-NMR investigation was established the proposed mechanism of probes with cyanide ion. Additionally, the prepared strips were useful for the recognition of CN^- ions short of resorting to additional equipment.

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Data Availability All data available.

Declarations

Consent to Participate Informed consent obtained from all individual participants included in the study.

Competing Interests The authors declare that they have no conflict of interest.

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