

An interesting spectroscopic method for chromofluorogenic detection of cyanide ion in aqueous solution: Disruption of intramolecular charge transfer (ICT)

ABDOLHAMID ALIZADEH^{a,b*} , SOHRAB GHOUZIVAND^a, MOHAMMAD M KHODAEI^{a,*} and MEHDI ARDALANI^c

^aDepartment of Organic Chemistry, Faculty of Chemistry, ^bNanoscience & Nanotechnology Research Center (NNRC), ^cDepartment of Analytical Chemistry, Faculty of Chemistry, Razi University, Kermanshah 67149, Iran
e-mail: ahalizadeh2@hotmail.com

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Abstract. 5-[4-(dimethylamino)benzylidene) pyrimidine-2,4,6(1H,3H,5H)]-trione (DMP-3H), the receptor was synthesized which played a chemosensor role for cyanide ion (CN⁻) in aqueous solution with colorimetric and fluorescence turn-off responses. Upon addition of CN⁻ ion into the solution containing the receptor, a color change visible to the naked eye was observed from yellow to colourless and also, the fluorescence of the solution was immediately quenched. Moreover, DMP-3H exhibited a selective response to cyanide ion over many other anions such as F⁻, Cl⁻, Br⁻, I⁻, SO₃²⁻, OCN⁻, ClO₃⁻, CO₃²⁻, IO₃⁻, N₃⁻, C₂O₄²⁻ and SCN⁻. The detection limit toward CN⁻ was 8.1×10^{-7} mol.L⁻¹, which is satisfactory enough for monitoring CN⁻ levels in physiological and environmental systems.

Keywords. Cyanide ion; naked-eye sensor; colorimetric detection; selective sensing; fluorescent probes.

1. Introduction

Focus on the monitoring of hazardous materials and toxic pollutions in air and water sources have greatly increased in the past few years. Numerous organic and inorganic pollutants such as organophosphorous compounds, pesticides, herbicides, metal ions or anions are the main target of sensing studies. Due to its effect on biological functions, cyanide anion is an extremely toxic ion and deadly to humans.¹ It is proved that 0.5–3.5 mg of cyanide per kilogram of bodyweight is fatal to humans.² By the way, it is widely used in numerous chemical processes, for instance in gold and silver extraction, plastic manufacturing, tanning, electroplating and metallurgy.^{3–7} As a result, selective, simple and cost-effective sensors for monitoring of CN⁻ ion with considerable ease in preparation are of great interests.^{8–10} A variety of cyanide sensors have been designed and reported based on their mechanism of detection,^{11–14} such as electroanalytical,^{15,16} hydrogen-bond interaction,¹⁷ supramolecular self-assembly,^{18,19} nucleophilic addition reactions,^{20–23} and spectroscopy.^{24–26} Among these, reaction-based sensors show both selectivity and high sensitivity to the cyanide ion. However, most of these sensors often need high

temperature or long reaction time, have complex structures that require complicated synthetic procedures or they can only be used in pure organic solvents.

Therefore, developing feasible and efficient naked-eye sensors for CN⁻ detection in aqueous solution under mild condition is essential. With these considerations, we proposed an efficient optical sensor (scheme 1) for CN⁻ in aqueous solutions. The sensor has been already synthesized *via* various experimental methods and its structure is well characterized.^{27,28} However, in the present study, the sensor was prepared in excellent yield through a simple, efficient, and environment-friendly route without using any catalyst. Our design focuses on utilizing the nucleophilic addition reaction of CN⁻ to an α , β -unsaturated group on receptor as the strategy for sensor design for trace CN⁻ levels. The α , β -unsaturated acceptor group as electron-withdrawing group in DMP-3H can be modulated by CN⁻, which ruptures π -conjugation resonance and causes intramolecular charge transfer (ICT)^{23,29–32} blocking through nucleophilic addition reaction. In the present study, 5-[4-(dimethylamino)benzylidene)pyrimidine-2,4,6(1H,3H,5H)]-trione (DMP-3H) has been synthesized by a simple method and used as receptor for CN⁻ detection through colorimetric and fluorometric analyses.

*For correspondence

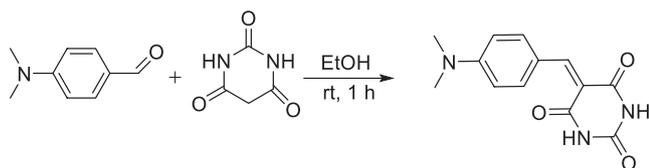
2. Experimental

2.1 Materials and Methods

Fresh, double-distilled water was used throughout the experiments. Barbituric acid and 4-(*N,N*-Dimethylamino)benzaldehyde were obtained from Merck and Sigma-Aldrich Co., respectively. Inorganic salts were obtained from Merck Co. The ^1H and ^{13}C NMR spectra were recorded on Bruker BioSpin 400 spectrometer at 400 and 100 MHz, respectively, using tetramethylsilane (TMS; $\delta = 0$ ppm) as internal standard and DMSO- d_6 as solvent. UV-Vis spectra were recorded by Perkin-Elmer lambda 25 spectrometer. Photoluminescence spectra were obtained by Varian Cary Eclipse fluorescence spectrophotometer. The FTIR analysis was carried out by Shimadzu 8300 spectrometer in the range 400-4000 cm^{-1} .

2.2 Synthesis of the receptor

General procedure for the synthesis of DMP-3H: 4-(*N,N*-Dimethylamino)benzaldehyde (0.60 g, 4 mmol)



Scheme 1. Synthetic procedure for receptor DMP-3H.

and barbituric acid (0.51 g, 4 mmol) were combined in 30 mL ethanol and the solution was stirred at room temperature (scheme 1). After 1 h, an orange precipitate was formed which was filtered and washed with ethanol several times and recrystallized in ethanol to obtain an orange powder. Yield: 90%; M.p. 276°C, ^1H NMR (400 MHz, DMSO- d_6 , 26°C), δ (ppm) = 11.07 (s, 1H), 10.94 (s, 1H), 8.43~8.41 (d, $J = 8$ Hz, 2H), 8.14 (s, 1H), 6.80~6.78 (d, $J = 8$ Hz, 2H), 3.12 (s, 6H), ^{13}C NMR (100 MHz, DMSO- d_6 , 26°C) 164.56, 162.58, 155.47, 154.13, 150.19, 150.10, 139.06, 119.91, 111.16, 109.38, 40.34.; IR (KBr) cm^{-1} : 3425, 3193, 3072, 2808, 1730, 1653, 1513, 1609, 1401, 1305, 1203.

2.3 Preparation of anion solution

In 100 mL of distilled water, 1 mmol of inorganic salt (NaF, NaCl, LiBr, NaI, Na_2SO_3 , KOCN, NaClO_3 , NaCN, Na_2CO_3 , NaIO_3 , NaN_3 , $\text{K}_2\text{C}_2\text{O}_4$, and NaSCN) was dissolved to obtain 1×10^{-2} mol/L aqueous solution. Then, the stock solutions were diluted to desired concentrations with water when needed.

2.4 UV-Vis spectral analysis

All UV-Vis experiments were performed in solution [0.2 M Sodium dihydrogen phosphate and disodium hydrogen phosphate $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer, pH 7.15, 50% tetrahydrofuran (THF)]. According to figure 1

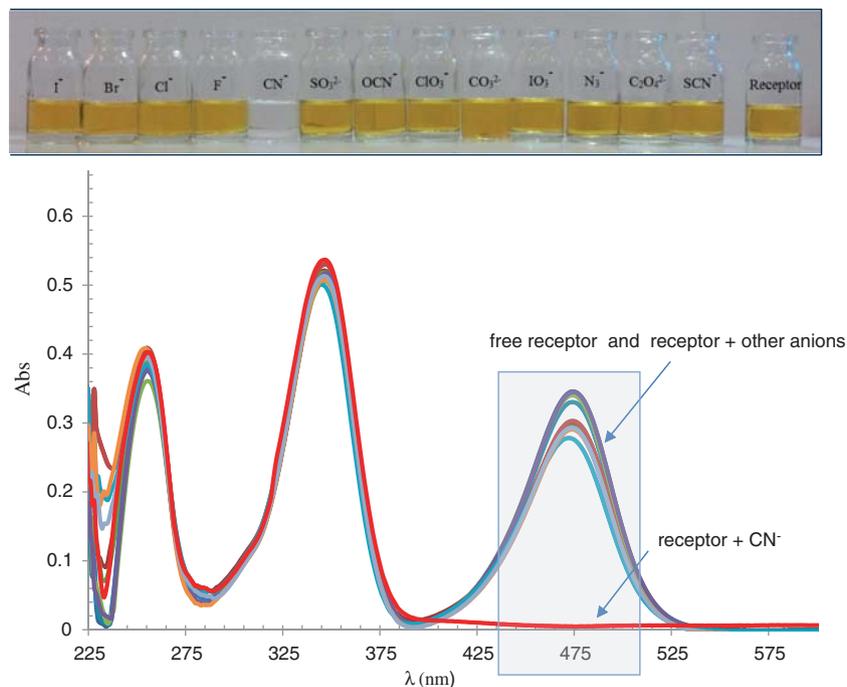


Figure 1. UV-Vis spectra of DMP-3H (24 μM) in the absence and presence of various anions (0.001 M) in THF/ H_2O solution (1:1 v/v), pH = 7.15. Inset: Absence of color changes of DMP-3H with various anions except CN^- .

any change in the UV-Vis spectrum of the synthesized compound was recorded upon the addition of anions (F^- , Cl^- , Br^- , I^- , SO_3^{2-} , OCN^- , ClO_3^- , CN^- , CO_3^{2-} , IO_3^- , N_3^- , $C_2O_4^{2-}$, and SCN^-) while keeping the receptor concentration constant ($24 \mu M$) in all experiments.

2.5 Fluorescence analysis

Fluorescence spectroscopy was performed in solution (Na_2HPO_4/NaH_2PO_4 buffer (0.2 M, pH 7.15) and 50% tetrahydrofuran (THF) on a Varian Cary Eclipse fluorescence spectrophotometer. Any change in the fluorescence spectrum of the synthesized compound was recorded upon the addition of anion while keeping the receptor concentration constant ($24 \mu M$) in all experiments. Anion salt in 1×10^{-3} M concentration was used for the fluorescence experiments too.

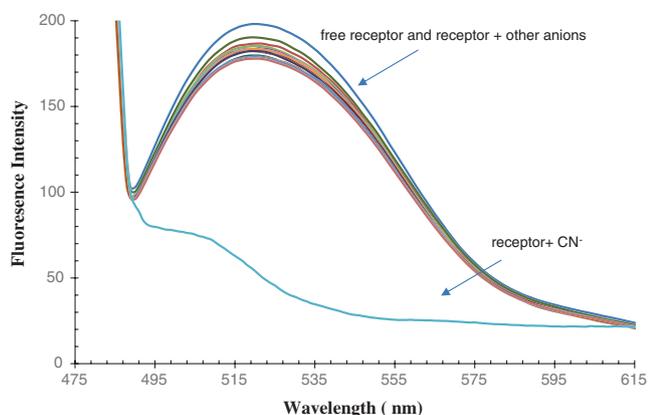


Figure 2. Fluorescence spectra of DMP-3H ($24 \mu M$) in the absence and presence of various anions (0.001 M) in THF/ H_2O solution (1:1 v/v, pH = 7.15, λ_{ex} = 470 nm).

3. Results and Discussion

To evaluate the CN^- detection ability of DMP-3H molecule, a series of sensing experiments were performed. Colorimetric and fluorimetric sensing abilities were probed by adding aqueous solution of anions to a THF/ H_2O (1:1 v/v; pH 7.15) solution containing of DMP-3H. As seen in figure 1, when aqueous solution of CN^- was added to the DMP-3H solution at room temperature, the sensor immediately responded with visible color changes from yellow to colorless, whereas other anions caused neither change in the color of the solution nor in the absorption spectrum of DMP-3H. The absorption peak at 473 nm in the UV-Vis spectrum decreased when CN^- solution was added but remained unchanged in the presence of other anions.

Meanwhile, as shown in figure 2, an emission band centered at 528 nm appeared in the fluorescence spectrum when the solution of DMP-3H was excited at

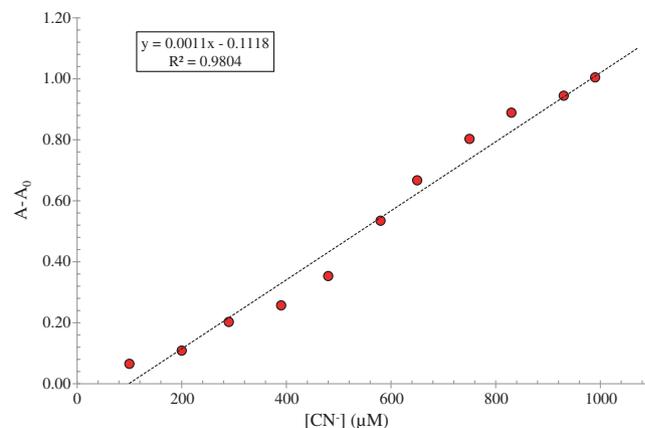


Figure 4. Plot of $A-A_0$ of DMP-3H ($24 \mu M$) in THF/ H_2O solution (1:1 v/v, pH = 7.15) versus concentration of CN^- .

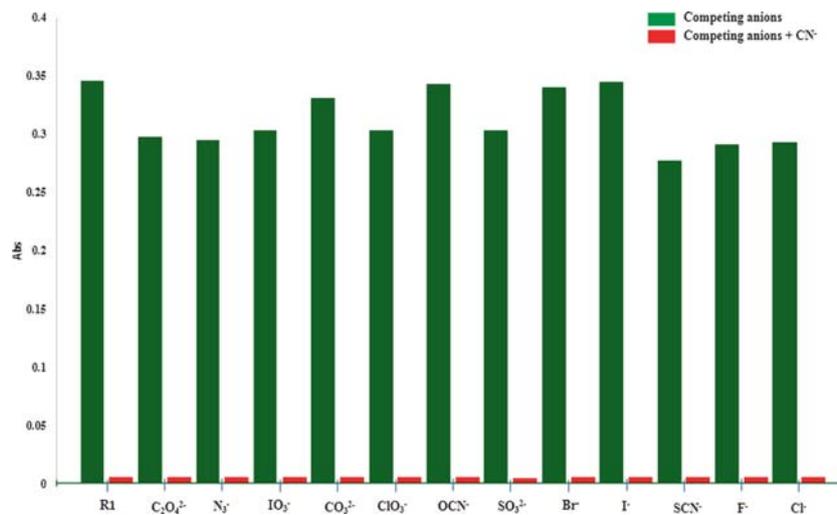


Figure 3. Absorbance of DMP-3H ($24 \mu M$) in the presence of various anions (0.001 M) (green bars) in THF/ H_2O solution (1:1 v/v, pH = 7.15) and in the presence of competing anions and CN^- (0.001 M) (red bars).

470 nm. By addition of CN^- solution, the fluorescence emission band decreased remarkably. However, when solutions of other anions (such as F^- , Cl^- , Br^- , I^- , SO_3^{2-} , OCN^- , ClO_3^- , CO_3^{2-} , IO_3^- , N_3^- , $\text{C}_2\text{O}_4^{2-}$, and SCN^-)

were added to the sensor solution, neither significant color nor fluorescence change was observed. DMP-3H was confirmed to be capable of selectively and instantly detecting CN^- in THF/ H_2O binary solution.

An important feature of the sensor is its specific selectivity toward the analyte over other competitive species. The variations of UV-Vis absorbance and the fluorescence of DMP-3H in THF/ H_2O solutions caused by the anions (F^- , Cl^- , Br^- , I^- , SO_3^{2-} , OCN^- , ClO_3^- , CN^- , CO_3^{2-} , IO_3^- , N_3^- , $\text{C}_2\text{O}_4^{2-}$, and SCN^-). As shown in figure 3, various competitive anions did not lead to any significant interference and in the presence of these ions, CN^- still led to similar color and optical spectral changes. The results showed that sensor DMP-3H selectivity toward CN^- was not affected by the presence of other anions.

A plot of $(A-A_0)$ versus the cyanide concentration in THF/ H_2O solution gave a linear relationship (figure 4) and the detection limit was calculated on the basis of $3\sigma/k$.³³ The term σ is the standard deviation of the blank measurements and k is the slope of the plot of intensity ratio versus the sample concentration. It was found that the detection limit is $0.81 \mu\text{M}$. This value is far below the WHO guidelines of drinking water ($1.9 \mu\text{M}$),³⁴ which makes the receptor DMP-3H a powerful tool for detecting cyanide ion.

We also investigated the interaction mechanism between DMP-3H receptor and cyanide ion. As shown in figure S1 in SI, free DMP-3H has three absorption bands appearing at 255, 346 and 473 nm. According to the UV-Vis spectra of the starting materials, the first absorption band is the result of transition $n \rightarrow \pi^*$ orbitals of carbonyl groups in receptor. This absorption band appears in spectrum of barbituric acid at 257 nm. The second band at 346 nm is attributed to $\pi \rightarrow \pi^*$ transition of the aromatic ring. The third band involves $\pi \rightarrow \pi^*$ transition of double bond in the vinyl group at

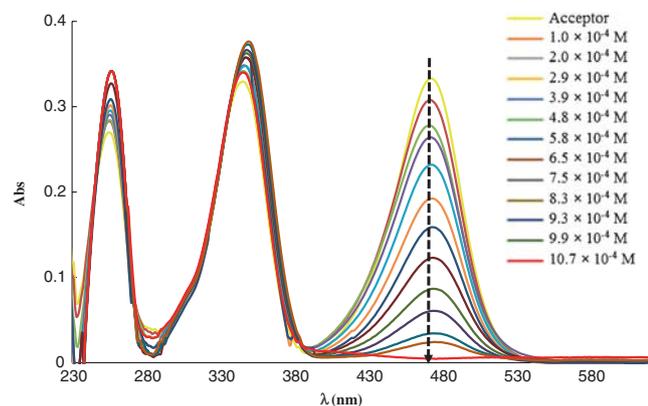


Figure 5. UV-Vis spectra of DMP-3H ($24 \mu\text{M}$) in THF/ H_2O solution (1:1 v/v, pH = 7.15) upon increasing concentration of CN^- .

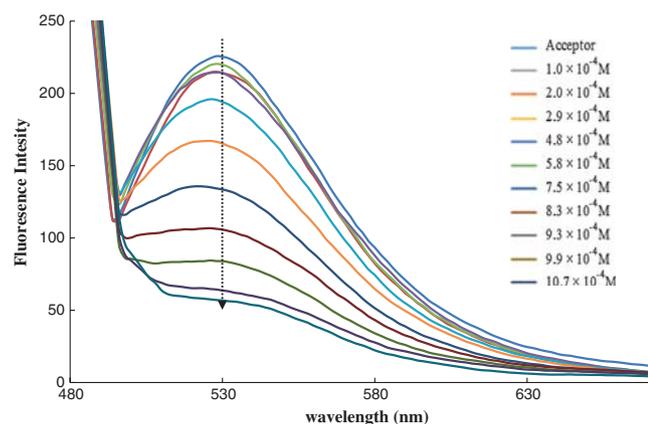
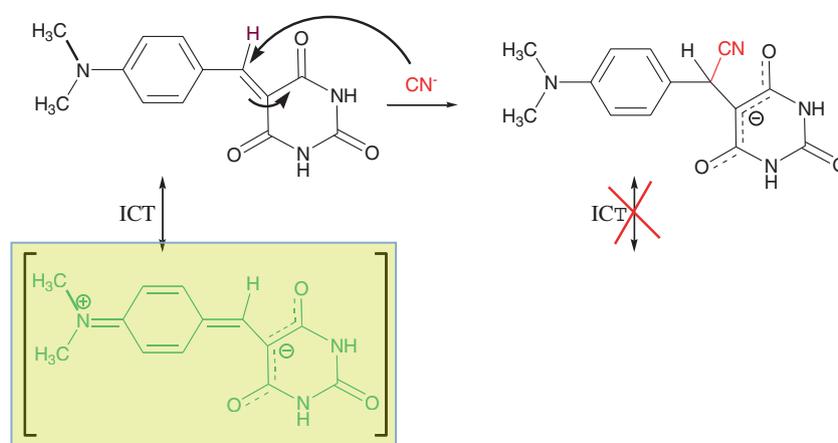


Figure 6. Fluorescence spectra of DMP-3H ($24 \mu\text{M}$) in THF/ H_2O solution (1:1 v/v, pH = 7.15) upon increasing concentration of CN^- .



Scheme 2. Proposed mechanism of ICT-disruption of DMP-3H caused by nucleophilic addition of cyanide ion to the unsaturated moiety of DMP-3H.

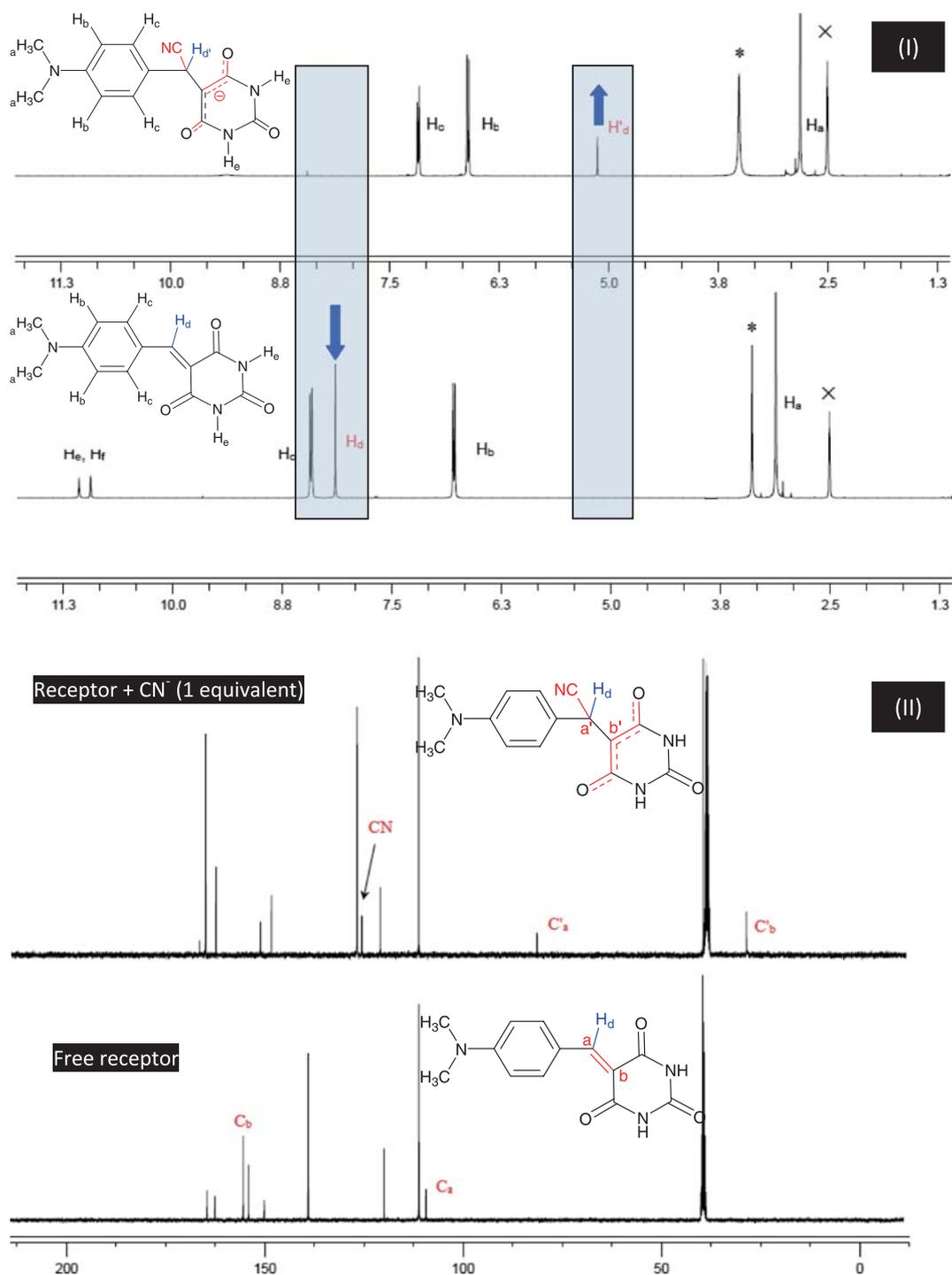


Figure 7. (I) ¹H NMR and (II) ¹³C NMR spectra of free DMP-3H(DMSO-d₆) and in the presence of one equivalent of CN⁻ ion. × -residual solvent; *-residual water.

α , β -unsaturated part of DMP-3H. There is a band appearing at 245 nm in the spectrum of 4-(N,N-dimethylamino)benzaldehyde which disappears in the receptor spectrum. This band is relevant to $n \rightarrow \pi^*$ of carbonyl group in 4-(N,N-dimethylamino)benzaldehyde which after the formation of DMP-3H, the carbonyl group converts to a vinyl group as in α , β -unsaturated moiety. Due to the strong resonance by two carbonyl groups on the barbituric acid and benzene ring, a

bathochromic phenomenon happens for this band which leads to a shift to upper wavelength.³⁵ According to UV-Vis spectra shown in figure 5, first two bands of DMP-3H (24 μ M) in THF/H₂O solution showed no change during the titration by cyanide ion but the band related to vinyl moiety at 473 nm ($\epsilon = 1.4 \times 10^4$ M⁻¹.cm⁻¹) was considerably decreased. This clearly reveals that the active site of the receptor that reacts with CN⁻ ion is only the vinylic site.

Table 1. Comparison the proposed method with other spectrophotometric (a) and electroanalytical (b) methods for cyanide determination.

Reagents and Methods	pH	Detection limit ($\mu\text{ mol.L}^{-1}$)	Reference
Nickel (2+) murexidecomplex ^a	9	40	36
AuNPs/Cu ²⁺ /phenanthroline Complex ^a	7	14	37
Amine-substituted heptamethine cyanine dye ^a	7.4	5	38
Glycoconjugated N-acetylamino aldehyde hydrazoneazodye ^a	7	1.29	39
Poly (vinyl alcohol) (PVA) derivative ^a	–	10	40
2-(4-((4-Bis(2-hydroxyethyl)amino)phenyl)- diazanyl)benzylidene)malononitrile ^a	–	1.1	23
Salicylideneaniline ^a	7.3	2.4	41
Cyanine dye Cy5 ^a	9.5	2	42
Photochromic phenoxyquinone ^a	7.4	27.5	24
Optical sensor ^a	7	63	25
BF2-curcumin dyes ^a	–	22	26
Amperometric sensor ^b	11.4	3.8	15
Differential pulse polarography (DPP) ^b	8	1.54	16
5-(4-(dimethylamino)benzylidene)pyrimidine-2,4,6(1H,3H,5H)-trione ^a	7.15	0.81	This study

Further, fluorescence titration of receptor with CN^- in various concentrations was also carried out. As shown in figure 6, with increasing concentration of CN^- , the emission peak at 528 nm gradually decreased in intensity and the fluorescence of DMP-3H was essentially quenched. These results are in a good agreement with the reaction of CN^- anion as a nucleophile with DMP-3H to form a stable adduct. With an α - β unsaturated structure, the receptor can undergo Michael-type nucleophilic addition. It is proposed that the mechanism of interaction of DMP-3H with CN^- anion as a well-known nucleophile is the attack of CN^- to the α - β unsaturated moiety of DMP-3H (scheme 2).

Further evidence for the mechanism of ICT-disruption of DMP-3H was obtained based on the FT-IR and NMR spectroscopic studies. The FT-IR spectrum of receptor after addition of cyanide (figures S2 and S3, in SI) shows a new band at 2090 cm^{-1} that does not exist in the FT-IR spectrum before anion addition. This presumably might be due to $\text{C}\equiv\text{N}$ stretching frequency. Additionally, the band at 1653 cm^{-1} which is attributed to $\text{C}=\text{C}$ bond disappears after addition of cyanide ion. This simply indicates the reaction between cyanide ion and vinyl part of the receptor and supports the proposed mechanism in scheme 2.

The NMR study of the recognition process also was informative. As shown in figure 7(I), upon addition of one equivalent of CN^- ion to the receptor, ^1H NMR peak of the vinylic proton (H_d) at 8.14 ppm completely disappeared. In contrast, a new signal emerged at 5.13 ppm (H'_d). Further, chemical shift of H_c showed a shift from 8.42 ppm to 7.17 ppm which confirms that with the addition of CN^- group to vinylic moiety, its

anisotropic deshielding effect on H_c is removed and the signal of H_c shifts to lower ppm. ^{13}C NMR spectroscopy also supports the mechanism shown in scheme 2. After the addition of one equivalent of CN^- , a new signal appeared at 126.43 ppm which is attributed to carbon atom of CN^- group [figure 7(II)]. This proves the formation of bond between CN^- and C_a of vinyl group during nucleophilic addition reaction. In addition, other signals were observed to shift from 109.37 ppm (C_a) to 82.22 ppm (C'_a) and from 155.47 ppm (C_b) to 29.44 ppm (C'_b) which prove the breaking of the $\text{C}=\text{C}$ double bond. These observations indicate that the CN^- was bound to the vinyl group and are in good agreement with the proposed mechanism.

Table 1 shows a comparison of the detection limits of various reported methods and the method reported in this study.

4. Conclusions

In summary, a reaction-based disruption in intramolecular charge transfer of a receptor (DMP-3H) leads to design and synthesis of a simple and cost-effective CN^- sensor *via* a clean chemical pathway. The designed sensor can detect CN^- ions in aqueous solution at room temperature. Investigation of the recognition mechanism indicated that the sensor recognizes CN^- ion by a nucleophilic addition reaction, while other anions did not interfere with the CN^- recognition process. The detection limit of the sensor toward CN^- was found as $8.1 \times 10^{-7}\text{ M}$, which was satisfactory for monitoring CN^- levels in physiological and environmental samples. In comparison with other methods that were

reported for the determination of cyanide, this method has better detection limit (table 1).

Supplementary Information (SI)

Additional information pertaining to UV-Vis spectra of starting materials and receptor DMP-3H (figure S1), IR and ^1H - and ^{13}C -NMR spectra of the receptor before and after addition of cyanide ion (figures S2-S7) are given in the supporting information, available at www.ias.ac.in/chemsci.

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