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Carbazole based hemicyanine dye for both "naked eye" and 'NIR' fluorescence detection of CN^- in aqueous solution: from molecules to low cost devices (TLC plate sticks)†

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A hybrid carbazole hemicyanine dye (receptor CHD) was developed as a new visible and near infrared chemodosimeter type sensor with high ratiometric selectivity towards cyanide in the presence of other anions in aqueous solution. The chemosensor also showed excellent performance when used in the "dip stick" method, *i.e.* in solid phase (TLC plates).

Anion recognition is an area of growing interest in supramolecular chemistry due to its important role in a wide range of environmental, clinical, chemical, and biological applications.[1] Among the various anions, cyanide is one of the most concerning anions because of its extreme toxicity to mammals.[2] It inhibits cellular respiration in mammals by interacting strongly with a heme unit in the active site of cytochrome a3.[3] It could be absorbed through the lungs, gastrointestinal track and skin, leading to vomiting, convulsion, loss of consciousness, and eventual death.[4] However, the use of cyanide salts has remained widespread, particularly in electroplating, metallurgy, synthetic fibers, resins, herbicide and gold-extraction processes.[5] According to the World Health Organization (WHO), only water with cyanide concentration lower than 1.9 μM is fit to drink.[6] Thus, there is a need for an efficient sensing system that is cheap, simple, highly sensitive to cyanide in order to monitor cyanide from contaminant sources. Various kinds of colorimetric or fluorometric CN^- selective receptors have been proposed in organic media[7] and the receptors that act in aqueous media have also been proposed; however, many of these show insufficient selectivity towards CN^-.[8] The design of CN^- receptors with high selectivity in aqueous media is therefore currently the focus of attention. Compared to UV and visible light, a chemosensor with a near-infrared (NIR) region at around 650–900 nm has many obvious advantages, which can avoid or reduce the interference from background absorption, fluorescence and light scattering. Furthermore, they can penetrate deeply into tissues with less damage. The virtues of this particular spectral region make the NIR probe more suitable.[9,10] Heptamethine cyanine dyes[11] are some of the most important NIR dyes and have been widely used in various fields and have been employed as fluorescent labels in fluorescence imaging studies of biological mechanisms.

A colorimetric and ratiometric fluorescent chemosensor is of particular interest due to its simplicity.[12] In particular, ratiometric sensing provides a way of avoiding any misinterpretation of analyte-induced fluorescence quenching or enhancement due to photobleaching, sensor concentration, and medium effects.[13] A ratiometric method measuring the ratio of fluorescence intensities at two wavelengths provides an alternative approach. However, up to now, only a limited number of ratiometric fluorescence probes for cyanide have been reported in the literature.[14] In general, ratiometric probes can be designed to function by following two mechanisms: intramolecular charge transfer (ICT) and fluorescence resonance energy transfer (FRET). Although a good ratiometric response could frequently be achieved in some FRET-based probes, the long synthetic pathways as well as the requirement of a strong spectral overlap between the emission of the donor and the absorption of the acceptor are necessary. On the other hand, ICT-based ratiometric probes are structurally simple and have advantages such as being easy to make and exhibiting a large emission shift. Considering all the above facts for cyanide recognition, we present here a novel NIR ratiometric fluorescent sensor containing carbazole based hemicyanine dye (CHD) that exhibits a selective, sensitive and "naked eye" response to CN^- in aqueous solution. Due to the strong nucleophilic nature of cyanide, we have developed a reaction based receptor for cyanide ions to avoid the complication induced hydrogen bonding. This receptor also has a lower detection limit[15] (ESI†).

The target compound was synthesized through the series of reactions detailed in Scheme 1. The starting compound (A)

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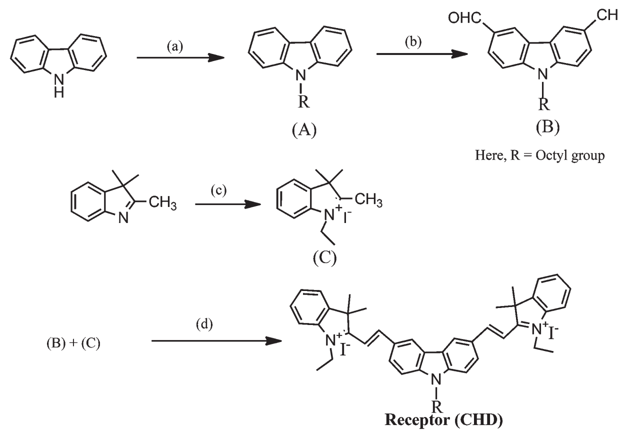
†Electronic supplementary information (ESI) available: Details of synthetic procedure with characterisation and spectral data. See DOI: 10.1039/c3dt51262b



Scheme 1 Synthetic route to CHD. Reagents and conditions: (a) octylbromide, K_2CO_3, KI, CH_3CN, heated at 80 °C, 4 h; (b) $POCl_3$, DMF, 100 °C, 20 h; (c) ethyl iodide, toluene, reflux, 8 h; (d) ethanol, piperidine (cat), reflux, 12 h.

(octyl derivative of carbazole) was prepared by protecting the NH group of the carbazole by treating it with octylbromide. The precursor di-aldehyde of carbazole (B) was prepared from the corresponding *N*-octyl-carbazole maintaining the Vilsmeier–Haack conditions (see the Experimental section). When 1,3,3-trimethyl-2-methyleneindoline was treated with EtI in acetonitrile as a solvent, the corresponding salt (C) was obtained.[16] Receptor CHD was obtained by Knoevenagel condensation.[17] The structure of the receptor was confirmed using ^{1}H NMR, ^{13}C NMR and ESI MS spectra (ESI†).

The changes of the UV-vis spectra for the receptor CHD in the absence and presence of CN^- in a CH_3CN–water solution (1 : 1, v/v) are shown in Fig. 1. The receptor CHD shows one main absorption band at 493 nm, which is attributed to intramolecular charge transfer (ICT) transition in CHD. The various anions tested are AcO^-, Cl^-, Br^-, I^- and F^- as their tetra butyl salts; SH^-, $H_2PO_4{}^{2-}$, $PO_4{}^{3-}$, S^{2-}, N^{3-}, $P_2O_7{}^{4-}$ and SCN^- as their sodium salts; and CN^- and ADP as their potassium salts in solution. In UV-vis absorption spectrometry, only CN^- is found to perturb the electronic behavior of CHD to a significant extent.

Incremental addition of CN^- clearly showed the CN^- induced changes in the absorption spectrum of CHD at two wavelengths, *i.e.* a decrease in absorbance at λ_{max} = 493 nm and an increase in absorbance at λ_{max} = 285 nm, along with a clear isosbestic point at 323 nm, indicative of anion binding. Essentially, these changes in the absorption spectrum stopped and the ratio of the absorbance intensities at 285 and 493 nm (A_{285}/A_{493}) became constant when the amount of CN^- added reached 2 equiv. It is noteworthy that the difference between the two absorption wavelengths is very large (absorption shift ΔA = 208 nm), which not only contributes to accurate measurements of the wavelengths of two absorption peaks but also results in a huge ratiometric value. In fact in the presence of 2 equiv. of cyanide, a 71-fold enhancement in the ratiometric value of A_{285}/A_{493} (0.30 to 21.32) is achieved with respect to the cyanide free solution (Fig. 1).

An apparent fluorescence enhancement was also observed upon the addition of 2 equiv. of cyanide (Fig. 2). The cyanide sensing fluorescence of CHD was monitored by titration in a mixed solution of 1 : 1 (v/v) CH_3CN–H_2O upon excitation at 250 nm. CHD showed two characteristic fluorescence bands at 382 and 600 nm in the emission spectrum, respectively. The emission band at 600 nm is attributed to the ICT emission band, while the emission band at 382 nm can be ascribed to the carbazole moiety. With the addition of cyanide ions, the fluorescence intensity of the solution at 600 nm gradually decreased, but at 382 nm it gradually increased, and most interestingly, a new peak was generated at 732 nm with a clear isoemission point at 715 nm (Fig. S2†).

The large hypsochromic shift of the ICT band from 493 to 285 nm suggests that the π-conjugation and the ICT progress of CHD were both inhibited by the nucleophilic addition of cyanide to CHD. The absorption stabilized after the amount of added CN^- reached 2 equiv. and a significant color change from red to colorless could be observed easily. In the time dependent absorption spectra, we see that the reaction is complete within 90 s with a rate constant of 3.68×10^{-2} s^{-1}, which strongly supports the high reactivity of the probe (Fig. 3 and S3†).

The carbazole moiety was itself fluorescent and when it was coupled with the ethyl salt of 1,3,3-trimethyl-2-methyleneindoline, *i.e.* in CHD, fluorescence was greatly reduced due to extensive π-conjugation and the ICT mechanism. The

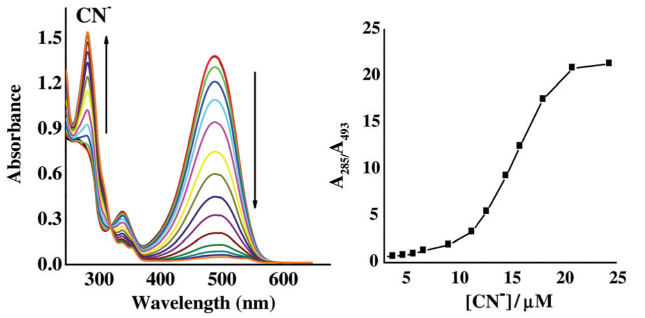


Fig. 1 UV-vis absorption titration spectra of CHD ($c = 2.0 \times 10^{-5}$ M) in the presence of 2 equiv. of CN^- ($c = 2.0 \times 10^{-4}$ M) at pH 7.1 in CH_3CN–H_2O (1 : 1, v/v) (left) and absorbance ratio changes (A_{285}/A_{493}) of CHD upon gradual addition of CN^- (2 equiv.) (right).

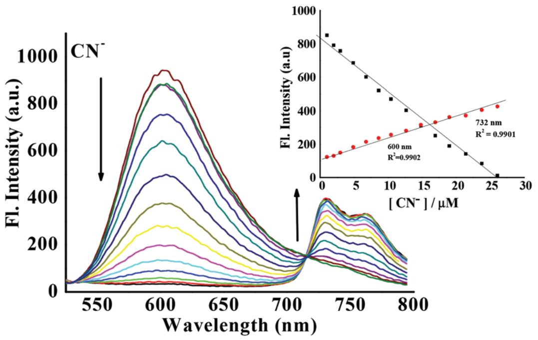


Fig. 2 Titration spectra of CHD ($c = 2.0 \times 10^{-5}$ M) in the presence of 2 equiv. of CN^- ($c = 2.0 \times 10^{-4}$ M) at pH 7.1 in CH_3CN–H_2O (1 : 1, v/v) and plot of $[CN^-]$ *vs.* Fl. Intensity at two different wavelengths.

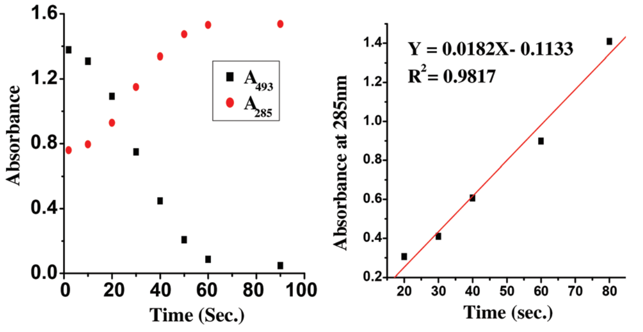


Fig. 3 Time-dependent absorption intensity of CHD at 285 nm (in red) and 493 nm (in black) in the presence of CN$^-$ (2 equiv.) (left) and the time *vs.* absorbance at a fixed wavelength (285 nm) plot using the first order rate equation (right).

addition of cyanide to the CHD results in the formation of new strongly fluorescent chromophores due to the breaking of the conjugation and the ICT (Fig. S4†).[18] Additionally, the absorbance intensity ratio at 285 and 493 nm and the emission intensity ratio at 732 and 600 nm showed drastic changes from 0.17 to 22.17 and from 0.12 to 11.43 in the presence of 2 equiv. of cyanide respectively (Fig. 4). The detection limit of CHD toward CN$^-$ was obtained as 0.54 µM (ESI†), which is sufficiently low for the detection of the CN$^-$ found in many chemical systems. Thus, CHD is a promising fluorescence ratiometric sensor for the detection of low levels of cyanide ions in samples and the selectivity of the fluorescence test was very high because the intensity enhancement was not observed in a solution containing all other anions before the addition of cyanide (Fig. 5).

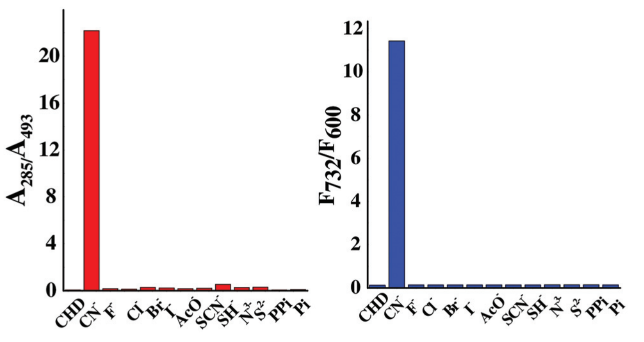


Fig. 4 Ratiometric response of CHD (2.0 × 10^{-5} M) towards anions (2 equiv.) with naked eye color changes and changes in emission spectroscopy (F_{732}/F_{600}).

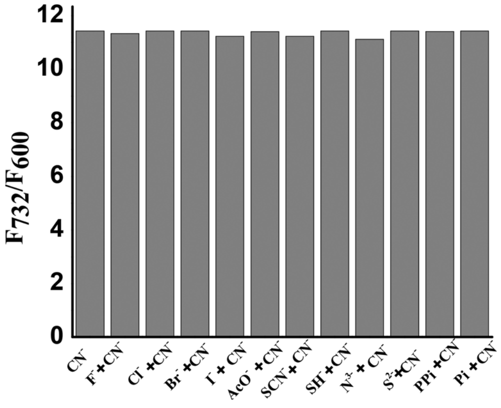


Fig. 5 Fluorescence responses of CHD (c = 2.0 × 10^{-5} M) to CN$^-$ (2 equiv.) containing 10 equiv. of various anions. λ_{ex} = 250 nm (right).

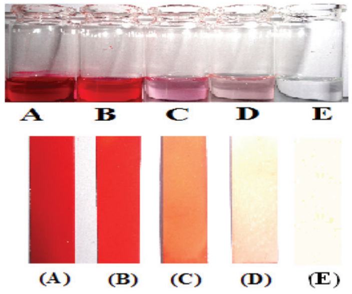


Fig. 6 Photograph of CHD towards various concentrations of CN$^-$ (×10^{-5} mol) of (A) 0, (B) 2, (C) 20, (D) 100, and (E) 200 in solution and in TLC plates.

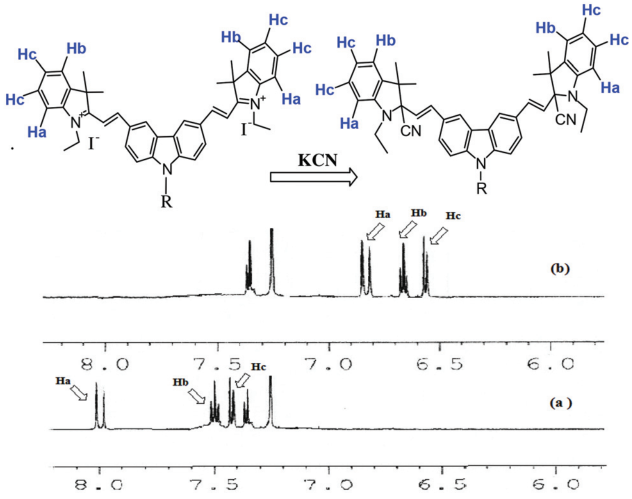


Fig. 7 ^{1}H-NMR spectra of (a) CHD and (b) CHD + CN$^-$ in CDCl$_3$.

Motivated by the favourable features of this system in solution, we prepared test strips by immersing TLC plates coated with CHD which were immersed in a CH$_3$CN–H$_2$O (1 : 1, v/v) solution of KCN in different concentrations and then dried in air to determine the suitability of a "dip stick" method for the detection of CN$^-$. We found that an instant bleaching of the red color was observed with increasing concentration of cyanide at concentrations as low as 20 µM of CN$^-$ (Fig. 6). Development of such dipsticks is useful as instant qualitative information is obtained without resorting to instrumental analysis.

Furthermore, we investigated the ^{1}H-NMR spectra of CHD in the presence of CN$^-$ and compared them with that of the sensor itself (Fig. 7). The protons of the benzene ring in hemicyanine dye in CHD at around δ 7.99 (Ha), 7.51 (Hb) and 7.42 (Hc) ppm dramatically shifted upfield toward δ 6.88 (Ha), 6.52 (Hb) and 6.51 (Hc) ppm respectively upon CN^- addition, indicating that the CN^- functions as a nucleophile. In ^{13}C-NMR spectra of CHD and a CHD cyanide adduct, we found that there is a new peak of cyanide at 116.62 and the peak at 180.28 (indolenine sp^2 C) is shifted to 70.35 (becomes sp^3 C), confirming the formation of the cyano adduct. In high resolution ESI TOF MS spectra, there is a peak at *m*/*z* 727.46 corresponding to a CHD–CN adduct, which confirmed the formation of a binuclear adduct of cyanide with CHD, *m*/*z* 675.45. In FT-IR spectra, there is a new peak at 2250 cm^{-1}, concluding the presence of cyanide in the CHD–CN adduct. All of these results are consistent with our proposed mechanism (ESI†).

In conclusion, we have successfully devised a novel NIR probe (CHD) towards cyanide in aqueous acetonitrile solution. CHD exhibits a unique colorimetric and fluorescence enhancement only with cyanide ions even in the presence of excess amounts of other anions, demonstrating its excellent selectivity compared to other anions. The detection limit of CHD was estimated to be 0.54 μM. The sensitivity is lower than the maximum permissive level in drinking water according to the World Health Organization (WHO). The significant changes in color can be observed with the naked eye.

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