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Rapid and ratiometric detection of hypochlorite with real application in tap water: molecules to low cost devices (TLC sticks)†

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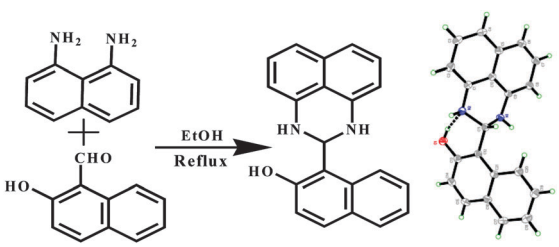
We have designed a chemodosimeter DPNO (weak fluorescence) which can be oxidized to HPNO (strong blue fluorescence) by OCl^- with high selectivity and sensitivity in a ratiometric approach with a noticeably lower detection limit. The sensor could be useful for the detection of hypochlorites in tap water.

Reactive oxygen species (ROS) are known to be crucial to several life functions.[1] They are important signaling molecules which control a wide range of physiological functions, but over production of ROS results in oxidative stress which is involved in the pathogenesis of many diseases, including Alzheimer's,[2] cardiovascular diseases,[3] neuron degeneration,[4] cancer,[5,6] arthritis,[7] ischemia-reperfusion injury in stroke,[8] inflammatory bowel disease,[9] myocardial infarction,[10] and organ transplant rejection.[11] Among the various ROS, hypochlorous acid (HOCl) acts as a dominant microbicidal mediator in the natural immune system. Production of the common ROS species is stimulated by heavy metal-catalyzed oxidation reactions and enzymatically by myeloperoxidase (MPO).[12] MPO is a heme-containing enzyme which produces hypochlorous acid ($HOCl/OCl^-$) from chloride ions (Cl^-) and hydrogen peroxide (H_2O_2). Regulated generation of hypochlorous acid is necessary for the host to control the invading microbes, while the HOCl formed can also react with amino acids, proteins, cholesterol, and nucleosides.[13] Moreover, to treat food preparation surfaces and water supplies in daily life[14] HOCl is used at a concentration in the range of 10^{-5}–10^{-2} M.[15] Such extremely intense hypochlorite solutions are a potential health hazard to humans and animals.[16] These significant results also motivate us to build a sensitive and specific probe for detecting HOCl in water samples. Compared with other detection methods, fluorescent probes have natural advantages including better sensitivity, quick response time and straight forwardness of implementation, offering application methods not only for *in vitro* assays but also for *in vivo* imaging studies.[17] On the other hand, ratiometric fluorescent probes should be able to enable the measurement of fluorescence intensities at two different wavelengths, providing a built-in rectification for environmental effects and rising the dynamic range of fluorescence measurement. This was considered as a high-quality approach to overcome the major drawback of intensity based probes, in which variations in the environmental sample and probe sharing were problematic for quantitative measurements. However, so far, the ratiometric fluorescent probes for HOCl are still very scarce. Recently, a number of organic fluorescent probes for HOCl sensing have been reported through modification of common fluorophores such as rhodamine,[18] fluorescein,[19] BODIPY,[20] quinoline,[21] triphenylamine[22] and carbazole[23] with $HOCl/OCl^-$ reactive groups.

In this work, naphthalene has been chosen as an ideal component of a fluorescent chemosensor due to its short fluorescence lifetime,[24] low fluorescence quantum yield[25] and ability to act as a donor as well as an acceptor.[26] Thus 1,8-diaminonaphthalene and 2-hydroxynaphthalene aldehyde is condensed to produce the receptor DPNO (Scheme 1). The structure of the receptor was confirmed by ^1H NMR, ^{13}C NMR, ESI TOF mass spectra and X-ray crystallographic analysis (ESI†).

The optical properties of the DPNO chemosensor were studied in a CH_3CN–H_2O solution (6 : 4, v/v, 10 mM HEPES, pH 7.4). As expected, in the absence of OCl^-, DPNO showed a weak fluorescence band at 354 nm upon excitation at 305 nm



Scheme 1 Synthetic scheme of DPNO with its ortep view.

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† Electronic supplementary information (ESI) available: Details of synthetic procedure, X-ray crystal structure and spectral data. CCDC 947173. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc47121g

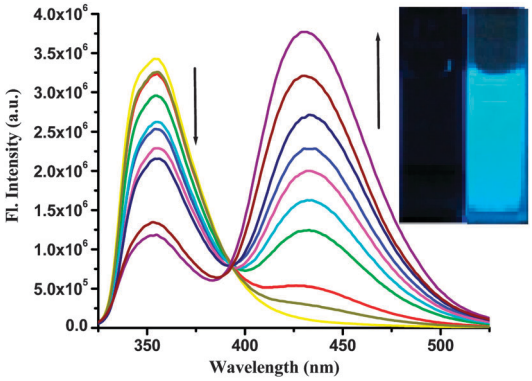

Fig. 1 Fluorescence emission spectra of DPNO ($c = 2.0 \times 10^{-5}$ M) with OCl^- ($c = 2.0 \times 10^{-5}$ M) at pH 7.4 in $CH_3CN:H_2O$ (6 : 4, v/v) with the naked eye fluorescence change (inset).

due to the effective photoinduced electron transfer (PET) process from the N atom to naphthalene. Upon the addition of increasing concentration of OCl^- (Φ = 0.35 from 0.01), the fluorescence intensity gradually increased significantly at 430 nm with a simultaneous decrease in intensity at 354 nm (Fig. 1) with a clear isobestic point at 392 nm. This fluorescence increase is caused by the selective oxidation by OCl^-. The non-fluorescent solution turned sky blue fluorescent upon gradual addition of OCl^- to the solution of DPNO (Fig. 1, inset). Thus, NaOCl triggered the oxidation of the chemodosimeter (DPNO). The possible oxidative product is shown in Fig. 2 (inset). From the time dependent fluorescence spectra, we observed that the reaction is completed within 1 min with a rate constant of 3.2×10^{-2} s^{-1}, which strongly supports the high reactivity of the probe (Fig. 3a).

Essentially, these changes in the fluorescence spectrum stopped and the linearly maintained ratio of the fluorescence intensities at 430 and 354 nm (F_{430}/F_{354}) (Fig. 3b) became constant when the amount of OCl^- added reached 1.2 equiv. The solution of DPNO exhibited a 6 fold enhancement of ratiometric fluorescence with the addition of 1 equiv. OCl^-. The detection limit was found to be 0.056 μM based on $K \times Sb1/S$, where Sb1 is the standard deviation of blank measurements and S is the slope of the calibration curve (Fig. S1, ESI†). The DPNO probe was treated with a wide variety of anions and oxidants to examine its selectivity. For the representative species including Cu^{2+}, Co^{2+}, Hg^{2+}, Mg^{2+}, Fe^{3+}, F^-, Br^-, Cl^-, I^-,

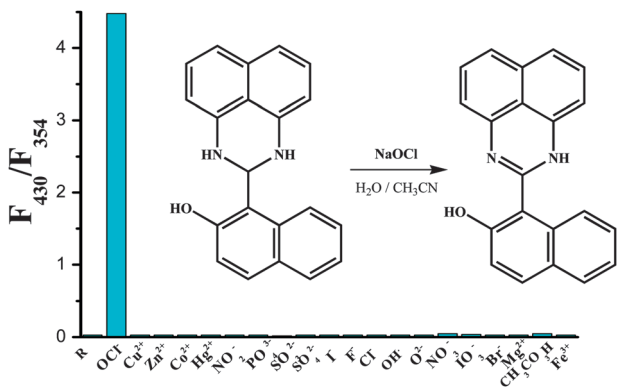

Fig. 2 Ratiometric response of DPNO ($c = 2.0 \times 10^{-5}$ M) towards metal ions and anions (1 equiv.) with the proposed reaction scheme from DPNO to HPNO.

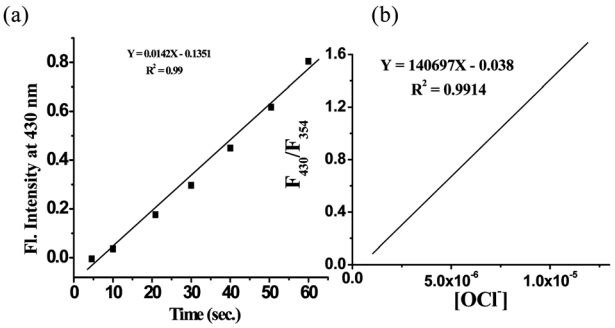

Fig. 3 (a) Plot of time *vs.* Fl. intensity at a fixed wavelength (430 nm) using the first order rate equation. (b) Titration curve of DPNO was plotted by F_{430}/F_{354} *versus* OCl^- concentrations.

NO_2^-, NO_3^-, SO_4^{2-}, SO_3^{2-}, IO_3^-, PO_4^{3-}, $OH^\bullet$, H_2O_2, O^{2-} and CH_3COOOH, the DPNO probe showed nearly no change in the fluorescence spectra upon the addition of those species indicating that our probe showed selective response towards OCl^- over other anions and oxidants (Fig. 2). The missing response in fluorescence intensity upon addition of other anions and oxidants indicated that fluorescence amplification occurred selectively upon reaction with OCl^-.

The selectivity of the DPNO probe was also evaluated by quantitative recording the fluorescence intensity of DPNO in the presence of 10 times excess of different anions and oxidants (Fig. S3, ESI†). Most of the other species exhibited no effect on DPNO detection of OCl^-. Thus, these results demonstrated that DPNO showed a sensitive response toward OCl^-.

To explore the sensing mechanism of the DPNO probe to OCl^-, the reaction of DPNO with 1 equiv. NaOCl was carried out in a CH_3CN–H_2O (v/v, 6 : 4) solution. After stirring at ambient temperature for 10 min, the product was separated and characterized by ^1H NMR and ESI-MS spectroscopy. A new peak appeared at m/z = 311.14 $[HPNO + H^+]^+$ in the positive-ion mass spectrum, suggesting that the transformation from the DPNO probe (m/z = 313.12, $[DPNO + H^+]^+$) to the corresponding product was induced by OCl^- (ESI†).

In order to investigate the structural change occurred due to the fluorogenic response of DPNO to OCl^-, DFT calculations were carried out for DPNO and HPNO using the DFT/B3LYP/6-31G* basis set (Gaussian 03 program). The corresponding HOMO–LUMO energy gap of DPNO and HPNO in the calculated structures (Fig. 4) also supports the observed ratiometric fluorescence. Prompted by its high sensitivity and selectivity, the practical application of DPNO was also investigated. Test strips were prepared to detect OCl^- with different concentrations. These test strips demonstrated apparent color changes under irradiation with a UV lamp, and the detectable OCl^- could be as low as 20 μM (Fig. 5).

Importantly, these strips could be conveniently used for the detection of OCl^-. One of the most important and useful practical application of the DPNO probe is the detection and quantification of the OCl^- anion under environmental conditions. Due to its excellent spectroscopic response, we tested the DPNO probe for detecting OCl^- in various types of water samples including pond water, river water, tube-well water, distilled water, rain water and tap water. Except for tap water, there was no significant fluorescence response found in addition with the other water samples (ESI†).

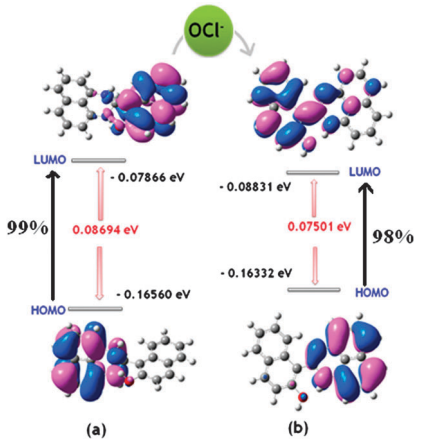

Fig. 4 HOMO–LUMO energy levels and interfacial plots of the orbitals for (a) DPNO and (b) HPNO with the calculated percentage of transition.

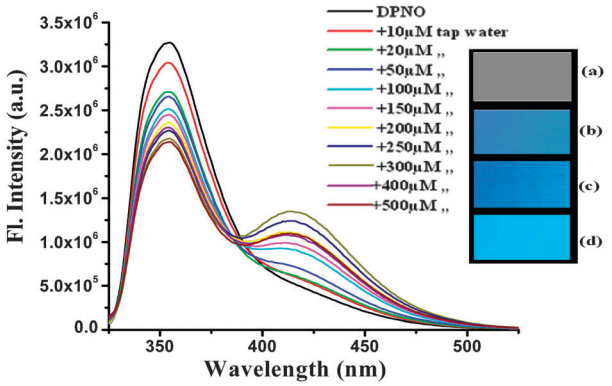

Fig. 5 Intensity change of aqueous solution of DPNO in addition with tap water (left) and color changes visualized under UV-light on TLC plate strips of (a) DPNO (c = 2.0 × 10^{-3} M) and during addition of NaOCl at (b) 2.0 × 10^{-6} M (c) 2.0 × 10^{-5} M and (d) 2.0 × 10^{-3} M in water (right).

Only the introduction of 100 μM tap water resulted in a remarkable enhancement of fluorescence. This result indicates that the DPNO probe can detect OCl^- in natural waters of significantly more complex composition as compared to laboratory conditions (Fig. 5). The concentration of hypochlorite in tap water was determined to be 0.04 mol L^{-1} by comparing the fluorescence titration data.

In summary, we have designed a fluorescence turn-on chemodosimeter DPNO for detection of OCl^- *via* analyte mediated oxidation of DPNO over other anions and oxidants in aqueous solution. This technique opens up new way for the recognition of OCl^-. The sensing phenomenon is supported by DFT and TD-DFT calculations. The sensor showed an excellent performance when used in the solid phase (TLC plate). The probe can be used for qualitative and quantitative detection of OCl^- in tap water.

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