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ARTICLE TYPE

Rapid 'Naked eye' responce of DCP, a nerve agent simulant: from molecules to low cost devices for both liquid and vapour phase detection

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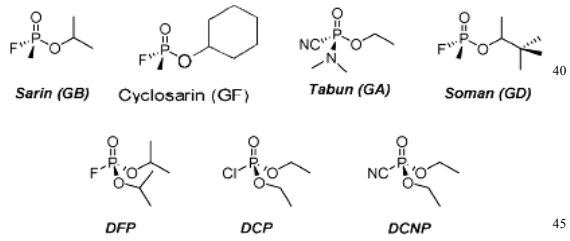
A rhodamine based new chemosensor, RHM has been designed and synthesized which react selectively with the organophosphate compound DCP, a well known nerve gas simulant (both liquid and vapour phase) with naked eye color and fluorescence change. The sensing phenomenon is supported by DFT calculation.

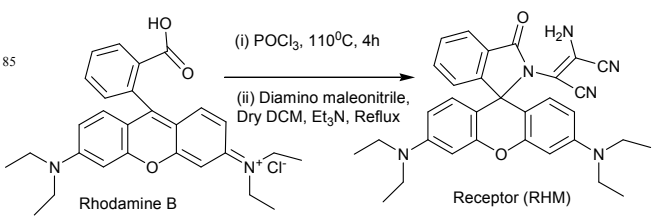
After the gas attack on the Tokyo subway in 1995, the detection of the ''nerve gases'' is important, but after the recent events in Syria it is particularly essential. The Nobel peace prize for 2013 has also been awarded to the Organisation for the Prohibition of Chemical Weapons (OPCW) "for its extensive efforts to eliminate chemical weapons". Use of these gases in terrorist attacks is somewhat silent; since they are colourless gases, their presence is not simply noticed until they have already been inhaled. Taking this benefit the Aum Shinrikyo group released Sarin gas in Tokyo, leading to thousands of wounded and 12 deaths.[1] Tabun (GA), Sarin (GB), Soman (GD) and cyclosarin (GF) known as nerve gases are chemically active organophosphates that can inactivate acetylcholinesterase (AChE), a critical central-nervous enzyme[2–5] via permanent modification of the catalytically essential serine residue in the enzyme active site[6] when inhaled[7] or absorbed through the skin. This triggers rapid and fatal consequences, such as paralysis of the central nervous system[8] and eventually death. The effectiveness of nerve agents stems from their amazing toxicity; a lethal dose can be as little as 0.70 mg for a normal 70 kg guy. Thus, due to moderately straightforward access by terrorists in present time, nerve gases are one of the most important and lethal classes of chemical warfare agents[9] which are a serious threat to countrywide and universal safety.



Scheme 1: Some nerve agents and their simulants.

Due to their tremendously hazardous nature of nerve agents, diethyl chlorophosphate (DCP) (Scheme 1) has been broadly used in laboratories as a safe simulant since it displays a parallel reactivity to those of nerve agents, such as Sarin, Soman, and Tabun, yet it lacks their toxicity. Systems that have been utilized to detect nerve agents contain enzyme based biosensors[10,11] interferometry,[12] surface acoustic waves,[13,14] electrochemistry,[15] and mass spectrometry[16,17] etc. There is some restriction for the predictable approch, such as lack of portability or storage/stability issues that limit their success in some circumstances. The challenge to the scientific society is to discover methods and devices that are compact, transportable and capable of real time detection. Sensors based on the chemical species i.e. chemosensors are beneficial from these views as they involve widely used instruments and put forward the opportunity to sense nerve agents with the bare eye. Chemosensors that exhibit notable fluorescence emission properties are attractive due to the highly sensitive, quick, simple and real time monitoring of the fluorescence or change of color in the existence of nerve agents which have been extensively investigated.[18]

Several chromogenic/fluorogenic and carefully planned probes[18(d,e,g,j,m)] in particular, fluorophores were covalently linked with fluorescence quenchers. After reaction with nerve agents with those quenchers, the covered fluorescence is in good health. Some probes react with nerve agent mimics to directly afford either detectable species,[18(l,n)] or to form intermediates, which could further undergo intramolecular transformations in situ to generate detectable species.[18(c,l,m,n)] Historically, nonfluorescent and colorless rhodamine derivatives with spirolactam moiety have been extensively utilized to detect metal ions by virtue of reversible ring-opening of the spirolactam, which gives rise to a highly fluorescent and colored rhodamine fluorophore.[19]



Scheme 2: Synthetic route of the receptor (RHM).

Herein, we have synthesized a Rhodamine B derivative as the fluorogenic and chromogenic probe for detection of diethyl chlorophosphate (DCP), a nerve agent simulant (Scheme 1) in liquid and vapour phase. The probe of Rhodamine B derivative is N-(rhodamine B)-lactam-di-amino maleonitrile (RHM), easily prepared from Rhodamine B (Scheme 2). Rhodamine B is treated with excess amount of $POCl_3$ in high temperature to produce Rhodamine B acid chloride which was then coupled with diamino maleonitrile in dry DCM to give the desired product (details of the procedure and spectra given in Electronic Supporting Information†: ESI†).

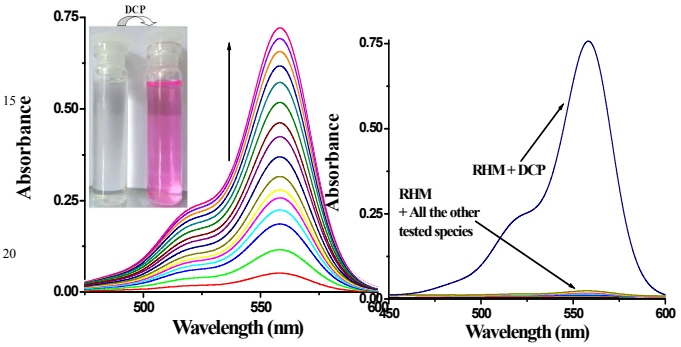


Figure 1: UV-vis absorption titration spectra of RHM (c = 1 x 10^{-5} M) in presence of 2.0 equiv. DCP (c = 2 x 10^{-4} M) in DCM (with 3% Et_3N) with the naked eye color change (left) and absorption spectra of RHM after addition of 2.0 equiv. each of the guest species (right).

In order to avoid the interference from inorganic acids, such as HCl or HBr, we decided to use a slight basic system to study the binding of nerve gas mimics. In addition, Sarin and Soman generate HF upon hydrolysis.[20] Therefore the use of the organic base into the solvent system for the sensing process is also necessary to show that sensors should interact with the nerve gas and not the nerve gas degradation products. We have used 3% Et_3N in DCM as the solvent for this process. The compounds were also tested with DMMP (dimethoxy methyl phosphate) to study the importance of leaving group (Cl) of DCP in binding with the sensors.

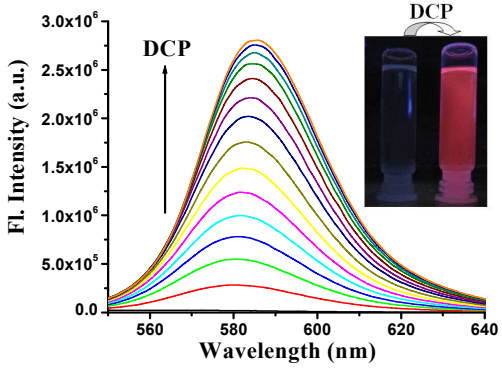


Figure 2: Fluorescence titration spectra of RHM (c = 1 x 10^{-5} M) in presence of 2.0 equiv. DCP (c = 2 x 10^{-4} M) in DCM (with 3% Et_3N) with the naked eye color change under UV-light (inset).

The photophysical properties of the receptor RHM was investigated by monitoring the absorption and fluorescence behavior upon the addition of nerve agent simulant DCP and its non reactive analogue DMMP, some common metal ions such as Co^{2+}, Cu^{2+}, Hg^{2+}, some reactive oxygen species (ROS) i.e. NaOCl, H_2O_2, ^tBuOOH, two common acid chlorides i.e. benzoyl chloride, acetyl chloride and some common pesticides i.e. BHC, chlorothalonil and phorate in DCM (3% Et_3N v/v).

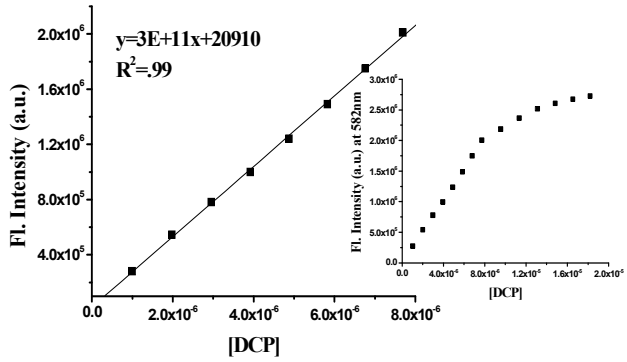


Figure 3: Binding isotherm of DCP with RHM at 582nm (with the addition of 1µM to 8 µM) from the titration spectra. The complete binding isotherm is in inset.

When we evaluated the changes in the absorbance of receptor upon treatment with DCP, the intensity of the absorption bands of receptor at 558 nm was increasing rapidly (figure 1) indicative of a clean conversion of the receptor, RHM into the RHM-DCP adduct. Thus with the strong binding by oxygen and nitrogen group present in RHM to DCP open the spirolactam ring to reproduce the pink color of rhodamine itself which is absent in addition of other species (figure 1). As the absorption band at 558 nm appears with a high absorption value, the "naked eye" detection of the nerve agent is possible (figure 1). Thus without using any instrument we can sense DCP using solution of the receptor.

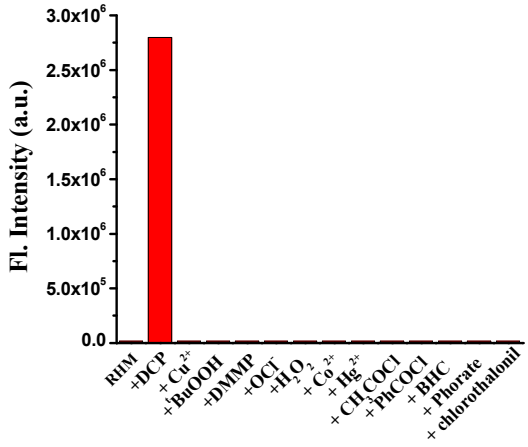
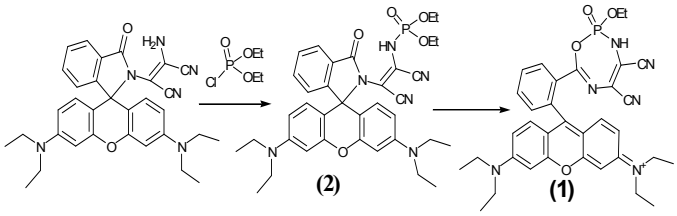


Figure 4: Emission intensity of RHM after addition of 2.0 equiv. each of the guest species as a bar diagram (right).

In emission spectroscopy, receptor exhibits an emission band arising at 582 nm which shows a remarkable enhancement during the gradual addition of DCP (figure 2). Moreover the

binding isotherm maintains a good linearity with the addition of 1μM to 8 μM of DCP solution towards RHM (figure 3). The detection limit of the nerve agent was found to be 0.2μM based on K × Sb1/S, where Sb1 is the standard deviation of blank measurements and S is the slope of the calibration curve (ESI†) suggesting that RHM is operable well below the reported lethal dose.[21]

The observed changes in the fluorescence emission are shown in a bar graph representation (figure 4). It seems that the fluorescence enhancement was derived from the more widespread formation of the RHM-DCP adduct with rhodamine based receptor, RHM. During the complex formation, the spirolactum ring of the receptor is opened up and gives the fluorescence emission of rhodamine itself upon gradual addition of DCP (0–2.0 equiv.).



Scheme 3: Probable reaction mode of RHM towards DCP.

To find the probable pathway of the sensing process (Scheme 3), the reaction solution was analyzed by mass spectrometry (figure 5). A major peak situated at 623.2573 was recognized, which is consistent with the theoretical molecular weight of the final compound **1** (MW: 623.2536) with the less intense peak at 668.2873 (2+ H^+) confirming formation of the intermediate compound **2** in the examined system.

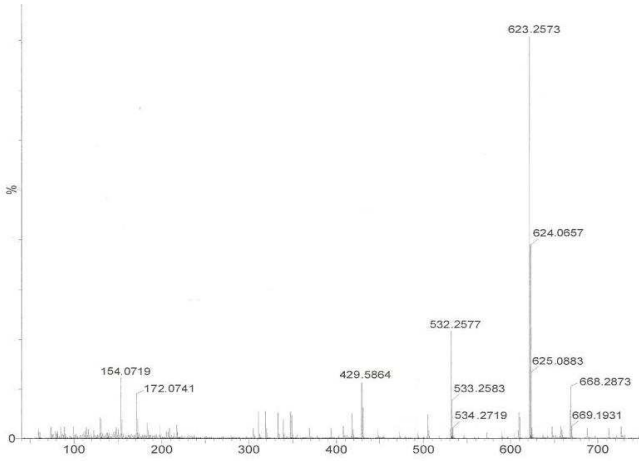


Figure 5: Mass spectra of mixture of RHM with DCP.

The DFT calculations with using DFT/B3LYP/6-31G* basis set level also support the sensing phenomenon with the formation of RHM-DCP adduct i.e. **1** from RHM. The Highest Occupied Molecular Orbital (HOMO) of product i.e. **1** is much more stable than RHM by about 0.10284 a.u.. The HOMO-LUMO energy gap also decreses from 0.12392 a.u. to 0.10338 a.u. with the formation of RHM-DCP adduct i.e. **1** from RHM (figure 6).

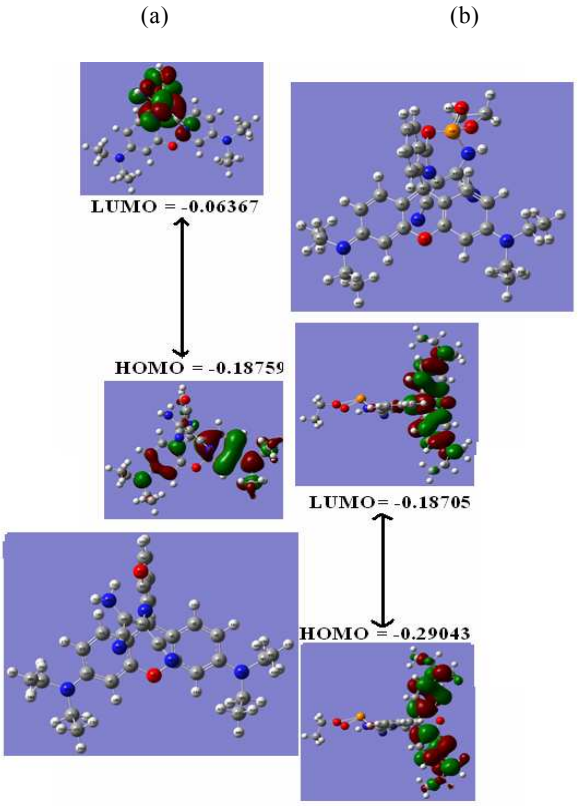


Figure 6: HOMO-LUMO energy levels and interfacial plots of the orbitals for RHM (a) and its DCP aduct (i.e. **1**) (b) with the energy minimised structure.

Interestingly, the sensing phenomena is very rapid and completed in about 8 minutes i.e. the receptor RHM rapidly reacts with DCP to give the opened-up form of rhodamine which gives the charecteristics color and fluorescence (figure 7).

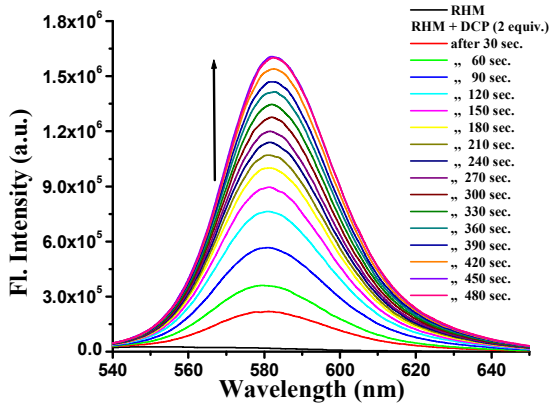


Figure 7: Time dependent fluorescence intensity spetra of RHM with addition of 2 equiv. DCP at a time.

It is noteworthy to mention that for rest of the species except DCP, neither pink coloration nor large emission intensity is observed demonstrating that the sensor is innocent towards the investigated metal ions, ROS and the other organo

phosphorus compound, DMMP. Interestingly, the inertness of the receptor RHM towards DMMP confirms that 'Cl' being a good leaving group in the reaction condition is the main reason for the higher reactivity of the DCP.

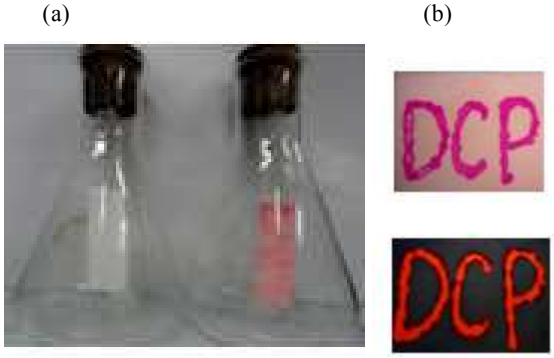


Figure 8: (a) Change of TLC plate (coated with RHM) in presence of DCP vapour and (b) Change of TLC plate (coated with RHM) in presence of DCP solution in sunlight (up) and under UV-light (below).

Provoked by its high sensitivity towards DCP, the practical application of RHM was also examined. Test strips were prepared to detect DCP both in liquid and its vapour phase. These test strips confirmed clear color changes under sun light (figure 8a and 8b) and also under the UV lamp (figure 8b). Thus, with the capability of 'naked-eye' detection of the nerve gas simulant in both (liquid and vapour) phase using coated sticks makes RHM a uniqe one and superior than the previous reports.

In conclusion, our designed receptor RHM can detect DCP selectively with high level of sensitivity with a unique mechanism. The mechanism was corroborated by mass spectrometry. Additionally, the reaction based sensing phenomenon was also supported by DFT calculations. More over, the test strips are also prepared for facile detection of both the vapour and liquid phase of DCP with naked eye.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [synthetic procedure, timedependent intensity and spectral data are available]. See DOI: 10.1039/b000000x/

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