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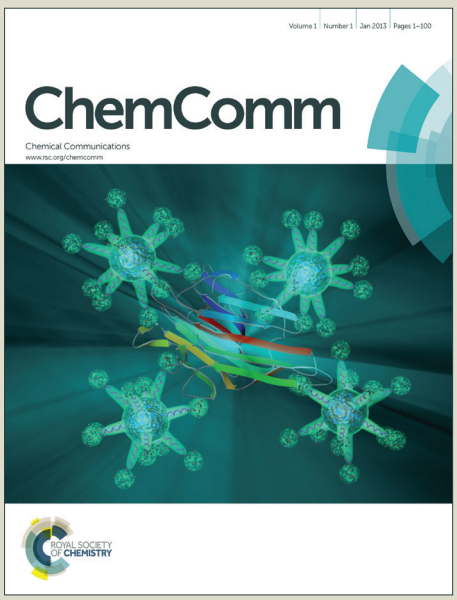

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Chromogenic and Fluorogenic Detection and Discrimination of Nerve Agents Tabun and Vx

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Our approach uses a squaraine (SQ) as the molecular-receptor as well as an indicator for chromogenic and fluorogenic detection and discrimination of nerve agents Tabun and Vx. To mimic a real-life senerio, the protocols were implemented in spiked water and soil samples, on surfaces, and in gas phase. The lower detection limit will be useful to protect human health and national security.

Nerve agents (Fig. 1)[1] such as Sarin, Soman, Tabun and Vx are the most nefarious class of chemical warfare agents (CWAs) which can lead to death within minutes if inhaled due to the inhibition of acetyl cholinesterase enzyme (AChE) and thereby overstimulation of the central nervous system.[2] Vx is the most toxic known compounds (10 times more so than sarin).[3] The lethal effect and ease of preparation make these agents weapons of choice by terrorist groups or rogue nations. Illegitimate use of chemical weapons has posed a grave concern among the international community, which has prompted an urgent need to develop a protocol/device for onsite/offsite detection.

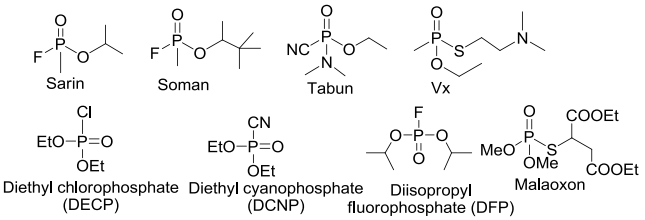

Fig. 1. Nerve agents and thier mimics

Several analytical techniques/methods using either instrumentation[4] or chemical approach[5] have been demonstrated. While many work well, they are non-portable and require experienced operators or had long detection times and require frequent calibration. As a result, detection by chemical methods has emerged; resulting in technologies such as chemically doped detection paper and residual vapor detection kits yet, these are non-specific, insensitive and give false positive signals.[6] Consequently, over last two decades the focused has shifted towards selective chromogenic and fluorogenic methods for nerve agents.[7] The general mechanism to detect nerve agents is a nucleophilic attack of the probe molecule on the electrophilic agent to form phosphate ester. The resultant phosphate ester either directly or via dephosphorylation responds a detectable optical signal[5a,b,7] (Fig. 2). However, interferences within CWAs including other organophosphorous compounds, and other reactive electrophiles still remain major concern. The detection of G-type agents such as Sarin, has been reported, but method for Tabun[8] and particularly V-type agents are rare.[9]

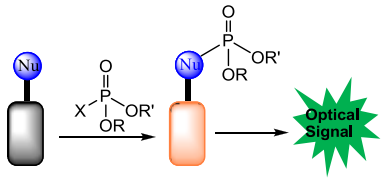

Fig. 2. General strategy for nerve agents' detection

In view of this, we took up a research programme to develop selective, sensitive, cheap and deployable methods for CWA detection. We postulated to target reactive functional groups in CWAs, which are also responsible for their toxicities. Using this hypothesis, a chromogenic and fluorogenic detection of sulfur mustard was sucessfully acheived.[10] We now focuss on the detection of nerve agents Tabun and Vx using **SQ**. Use of **SQ** in the detection of nerve agent was a part of our strategy for mainly two reasons. First, squaraine dyes are a class of organic electrophilic dye showing intense blue colour and fluorescence and having an electron deficient four membered ring which undergoes discoloration by nucleophilic attack.[11] Secondly, method will provide a single platform to the responders for the detection of real nerve and blistering agents using the same dye system.

In this strategy, the **SQ** dye in the presence of fluoride anion is allowed to interact with Tabun and VX. The dye is bleached with Tabun and Vx, and in the next step both bleached solutions are treated with thiophilic metals.[12] In case of Vx, the color of the dye

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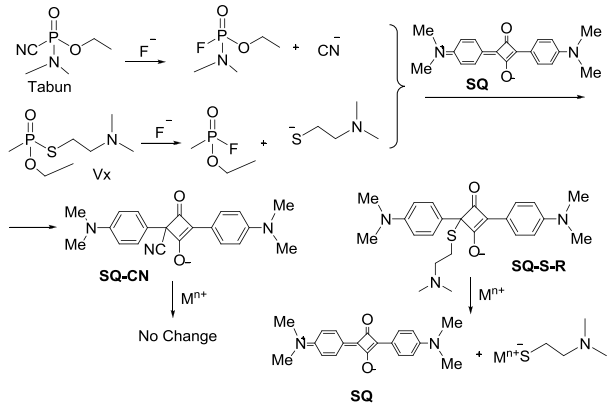
reappears while with Tabun no change occurs. Fluoride anion reacts with Tabun and Vx to generate cyanide and thiolate anions, respectively. Both being good nucleophiles, they attack **SQ** resulting in loss of color with formation of **SQ–CN** and **SQ–S-R** complexes (Scheme 1). In the next step, a thiophilic metal ion strips away the thiol from **SQ–S-R** complex leaving **SQ** in its original form. However, in case of **SQ-CN**, metals do not have ability to strip off cyanide from the complex, therefore no change is induced. This strategy not only detects Tabun and Vx but also discriminates them.



Scheme 1. Scheme showing detection of Tabun and Vx

In order to implement the approach first we sought a selective nucleophile which can react with nerve agents but not with **SQ**. Hence, we screened various nucleophiles, such as; tetrabutylammonium hydroxide, potassium hydroxide, potassium carbonate, pyridine, iodide, bromide, DBU, imidazole, acetate, ammonia, and oximes. But none could work successfully as per the requirement of our protocol. The literature reveals [13] that fluoride ion reacts with Vx to release thiolate group. Thus, we explored fluoride as nucleophile to react with nerve agents (Tabun and Vx) but not to **SQ**. To achieve the detection of Tabun and Vx, initially, DCNP and malaoxon therefore were chosen as simulants due to their lower toxicity but similar chemical reactivity. We conducted all the relevant studies using these mimics, and finally implemented our sensing protocol on the real agents *viz* Tabun and Vx.

DCNP (0.44 mM) and malaoxon (0.8 mM) were allowed to react separately with **SQ** (15.0 µL) containing tetrabutyl ammonium fluoride (TBAF) (0.44 mM and 1.2 mM respectively) in chloroform. In the case of DNCP the blue color of the dye disappears immediately while with malaoxon, it takes 2-3 minutes (Fig. 4a). This indicates the presence of both Tabun and Vx mimics, but the agents cannot be discriminated from each another. In the next step, addition of heavy metals such as salts of mercury(II) (0.08 mM) and silver(I) (0.08 mM) (*vide infra*) to both the solutions allows one to distinguish malaoxon from DCNP. With malaoxon, color regenerates instantaneously, while with DCNP, no change takes place even with the excess of metals ions (Fig. 4b). The complexes formation between SQ/DCNP and SQ/malaoxon in the reaction mixtures were analysed by mass spectrometry, which confirmed the formation of SQ-CN complex (exact mass: 446) and SQ-S-CH(COOEt)-CH2-COOEt (exact mass: 525) (Supporting Information, Fig. S1 and S2). Next, we aimed to discover the appropriate thiophillic metals which can respond to the **SQ**/malaoxon complex. In view of this, various metals such as salts of mercury (II), cadmium (II), silver (I) nickel (II) and copper (II) were screened. Hg(II) (0.08 mM), Cd(II) (0.08 mM) and Ag (I) (0.16 mM) were sufficiently thiophilic to scavenge the thiol from the complex and regenerates the color immediately. However, the metals Ni(II) (0.16 mM) and Cu(II) (0.24 mM) also responded, but require higher concentration to achieve saturation in the intensity of the color (Supporting Information, Fig. S3).

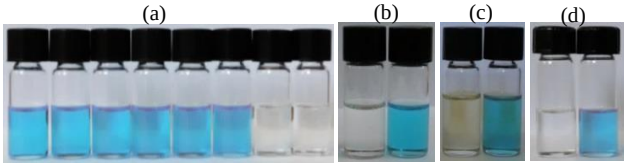


Fig. 4. (a) Chromogenic response of **SQ** (15.0 µL) with acetyl choride (0.88 mM), DECP (0.88 mM), BCEE (0.88 mM), **SM** (0.88 mM), sarin (0.88 mM), soman (0.88 mM), Tabun (0.44 mM), Vx (1.12 mM). (b) Chromogenic response of **SQ**/DCNP complex (0.44 mM) and **SQ**/malaoxon complex (1.12 mM) with Hg(II) (0.08 mM) (c) soil, (d) vapor phase.

To carry out fluorescence titrations, a solution of **SQ** (0.3 µM) in chloroform was titrated separately with DCNP (0.44 mM) and malaoxon (0.8 mM) in the presence of TBAF (0.44 mM for DCNP and 1.2 mM for malaoxon). The addition of DCNP and malaoxon to the solution of **SQ** gave a decrease in the intensity of fluorescence in both cases (Fig. 5 and 6) which results in complete quenching of the dye. Further, upon the addition of heavy metals to the **SQ/**malaoxon complex, the band at 640 nm increases with an increase in concentration, thus indicating the switch on of the signal with the regeneration of the dye. From the spectral data, it is evident that Hg(II) (Fig. 7) and Ag(I) (Supporting Information, Fig. S4) gave a better absorbance at lower concentrations of analytes as compared to Cd(II), Cu(II) and Ni(II) (Supporting Information, Fig. S5-S7). As expected, no response was observed upon the addition of heavy metals to the **SQ/**DCNP complex. It is important to note that when interaction between nerve agents and **SQ** in the presence of TBAF was carried out at 60 ^{o}C then we observed very fast color change. Thus, the detection at low concentration (0.22 mM for DCNP and 0.4 mM for malaoxon) can be realized by heating at 60 ^{o}C. The isotherms obtained for the titration experiments are sigmoid in shape for both Hg(II) and Ag(I). In the equilibrium conditions prevalent at the start of the titration, malaoxon complexion to **SQ** is not complete; a small fraction of the malaoxon is free in solution. The initial aliquots of metals ion added thus are first bound by the free malaoxon in the solution and then break down the **SQ**/malaoxon, causing switch on of the fluorescence signal.

Various possible interferences (slight excess) were tested that include acetyl chloride, DECP, BCEE, SM, sarin and soman. The two equivalents (0.88 mM) of these interferents were allowed to react with **SQ** (15.0 µM) in the presence of TBAF in a similar manner as described above. The results indicate that no interference from these analytes was observed, as blue color of dye persists with these interfering agents (Fig. 4a). Therefore, we are not only able to detect Tabun and Vx mimics selectively, but both can also be differentiated from each other.

Nerve agents, particularly tabun and Vx, are stable and relatively non-volatile liquid, and therefore are environmentally persistent (at neutral pH) for several weeks,[1] thus posing a great exposure hazard. Moreover, when deployed, these agents could be present in water, vapor phase, soil, and on a surface. To provide a robust sensing

system, it becomes mandatory to demonstrate detection in these matrices. For detection in a soil sample, 5µL of DCNP and malaoxon was spiked in 2 g of soil (prepared as mentioned earlier[10]). The spiked and unspiked samples (with no agents) were extracted separately with chloroform (2 mL). The dirt solution was filtered and the filtrate was treated with **SQ** (15 µM) and TBAF (1 mM). In the case of the Tabun and Vx mimics' spiked samples, the color of the dye disappears while in unspiked samples, the color persists. Further treatment of both the solutions with Hg(II) (0.08 mM) differentiates malaoxon from DCNP with the regeneration of dye color (*vide infra*).

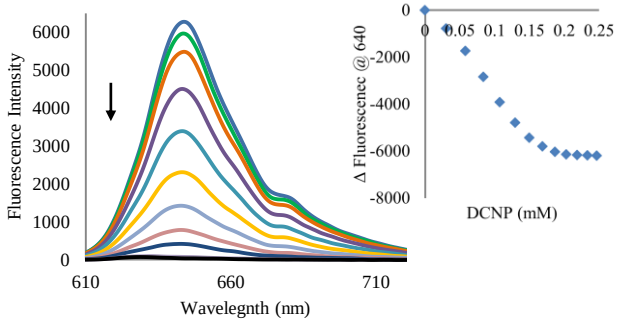


Fig. 5. Fluorescence intensity of **SQ** in $CHCl_3$ at 0.3 µM at 640 nm in the presence of increasing amounts of DCNP (stock solution conc. 0.44 mM) in MeOH (Excitation wavelength at 625 nm). Inset: Isotherm showing decrease in fluorescence intensity of **SQ** with the addition of DCNP.

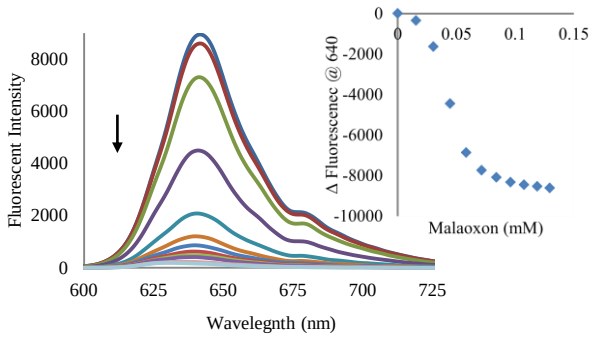


Fig. 6. Fluorescence intensity of **SQ** in $CHCl_3$ at 0.3 µM at 640 nm in the presence of increasing amounts of malaoxon (stock solution conc. 0.8 mM) in MeOH (Excitation wavelength at 625 nm). Inset: Isotherm showing decrease in fluorescence intensity of **SQ** with the addition of malaoxon.

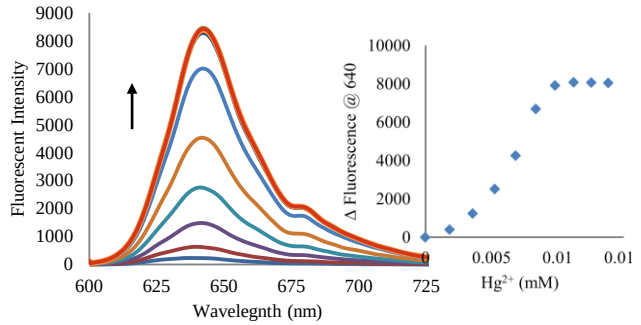


Fig. 7. Fluorescence intensity of a complex between **SQ** (0.3 µM) and Vx (0.1 mM) in $CHCl_3$ at 640 nm in the presence of increasing amounts of Hg(II) (stock solution conc. 76 µM) in MeOH (λex. = 625 nm). Inset: Isotherm showing increase in fluorescence intensity of **SQ** with the addition of Hg(II).

In an attempt to investigate the detection of these analytes in the vapor phase, the chromogenic detection was successfully achieved using a gas generation chamber. The agents (5µL) were spread over the heated surface (60 °C) of a chamber, the vapours formed were directly trapped into a solution of **SQ** (15.0 µL) containing TBAF (1 mM) in chloroform (2 mL). The dye color disappeared within 2-5 minutes with both agents. Rest protocols were followed as described above to confirm the presence of malaoxon.

Finally, we tested our detection strategy with real agents, viz Tabun and Vx. Chromogenic and fluorogenic detection were demonstrated in mixture analysis where the Tabun and Vx percentages were 42% and 62% respectively in the distillate (for details see Supporting Information, page S6). The main reasons for demonstrating the studies in mixtures are; 1) in real-life scenario, these chemicals have mostly been used in impure form, i.e. without purification. Purification makes the process of preparation cumbersome and expensive. 2) Because exposure hazards involved in a lab preparation process and in the analytical studies, it is recommended not using these agents in pure form, and 3) detection in complex background matrix has the added advantage to mimic a real-life scenario.

The detection with Tabun and Vx were performed following similar strategies and experimental protocols as in the case of DCNP and malaoxon. Tabun (0.65 mM) were allowed to react with a solution of **SQ** (15.0 µL) in the presence of TBAF (0.65 mM), and the dye color was bleached immediately. In a similar manner, Vx (1.12 mM) was allowed to react with a solution of **SQ** (15.0 µL) in the presence of TBAF (1.68 mM), and dye color was bleached in 2 minutes. Upon addition of Hg(II) (0.08 mM) to the bleached solutions of complexes between **SQ** and Tabun or Vx. The dye color in case of Vx regenerates while with Tabun no change was observed. Fluorescence data are shown in Fig. 8, and they respond in identical manner as the cases of their mimics. A solution

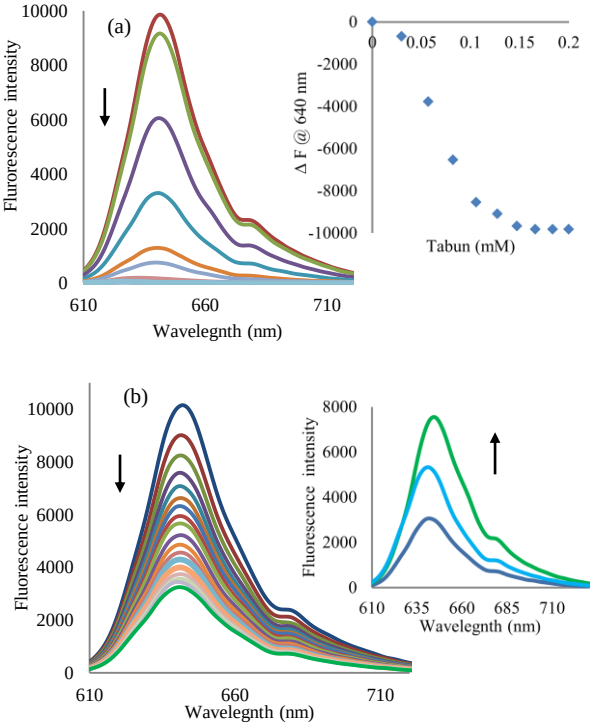


Fig. 8. (a) Fluorescence intensity of **SQ** in $CHCl_3$ at 0.3 µM at 640 nm in the presence of increasing amounts of Tabun (stock solution conc. 0.7 mM),

MeOH (Excitation wavelength at 625 nm). Inset: Isotherm showing decrease in fluorescence intensity of **SQ** with the addition of Tabun. **(**b) Fluorescence intensity of **SQ** in $CHCl_3$ at 0.3 μM at 640 nm in the presence of increasing amounts of Vx (stock solution conc. 0.37 mM) in MeOH (Excitation wavelength at 625 nm). Inset: Fluorescence intensity of **SQ**/Vx complex in $CHCl_3$ at 0.3 μM at 640 nm in the presence of increasing amounts of 25 μM and 50 μM of Hg(II) ($\lambda ex. = 625$ nm).

of Tabun (0.7 mM) and Vx (0.37 mM) containing TBAF (for Tabun 0.7 mM and for Vx 1.05 mM) were titrated with **SQ** (0.03 μL)). This gave a decrease in the intensity of fluorescence at 640 nm that resulted in a quenching of the dye. Upon the addition of incremental amount (25 μM and 50 μM) of Hg(II) to the **SQ/**Vx complex, the band at 640 nm increases and becomes saturated, thus indicating the switch on of the signal with regeneration of dye. The addition of mercury (II) in the **SQ/**Tabun complex shows no response.

Besides good selectivity, a crucial point in the realization CWAs sensor is to achieve high sensitivity by the visual inspection and fluorescence method. Therefore, Limit of Detection (LOD) in case of Tabun for visual and fluorescent detection was determined to be 50 μM and 8μM respectively and in case of Vx it was 80 μM and 8 μM respectively. These LOD were found to be much less than what can cause any hazards to human health and national security.[14]

In summary, we have demonstrated an innovative strategy where a squaraine dye system in combination with reagents afforded chromogenic and fluorogenic detection and discrimination of nerve agents (Tabun and Vx). The selective detection of individual agents could be desirable not only for the reason to reduce the false positive and negative signals but also from a medicinal chemistry point of view. Because specific antidote can be prescribed to the victim of specific CWA attacks as medical countermeasures. Since the different oximes are effective against different agents, for instance, PAM-Cl is effective against sarin and Vx while HI-6 and HIo-7 provide better protection against Tabun and Soman.[15] The developed protocol was also proven to be highly sensitive for the agents as LOD was much less than lethal doses in all cases. The detection of these agents in analytical settings such as in spiked water and soil samples, contaminated surfaces, and in gaseous phase will be useful in real-time monitoring.

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Electronic Supplementary Information (ESI) available: Experimetal details. See DOI: 10.1039/b000000x/

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