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Graphical Abstract

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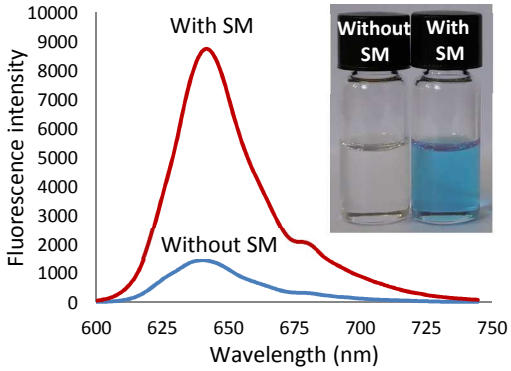
Abstract: We present a highly selective and sensitive detection protocol for the chemical warfare agent sulfur mustard. The chromogenic and fluorogenic system uses a squaraine dye (SQ) that not only detects and but also discriminates it from other chemical warfare agents (CWAs). With an aim to mimic real–life scenario for the onsite and offsite detection, the sensing protocol was implemented in spiked water and soil samples, on surfaces, and in gas phase. The lower detection limit (much lower than lethal dose) by the both visual inspection and fluorescence technique will be highly useful to mankind in order to avoid any eventuality.



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We present a highly selective and sensitive detection protocol for the chemical warfare agent sulfur mustard (SM). The chromogenic and fluorogenic system uses a squaraine dye (SQ) that not only detects and but also discriminates it from other chemical warfare agents (CWAs) and electrophilic potent interferents . A water soluble dithiol, 2-(3,5-bis(mercaptomethyl)phenoxy)acetic acid **1**, in the presence and absence of SM behaves differently towards a squaraine dye (SQ) to give different chromogenic and fluorogenic responses. With an aim to mimic the real–life scenario for the onsite and offsite detection, the sensing protocol was further implemented in spiked water and soil samples, on surfaces, and in gas phase. The lower detection limit (much lower than lethal dose) by the both visual inspection and fluorescence technique will be highly useful to mankind in order to avoid any eventuality.

1. Introduction

Chemical warfare agents (nerve and blister agents) are among the deadliest chemicals created by the humankind (Fig. 1).[1] The blister agent such as Sulfur Mustard (SM) or HD was used in World War I, World War II and more recently in the Iran–Iraq war.[2] SM is known to be extremely toxic, quite stable, and easy to synthesize, and therefore, named as the 'king of warfare agents'.[3] It causes severe skin as well as eye blistering and lung lesions upon exposure due to the alkylation of DNA.[4] After long term exposure, this can result in carcinogenic and mutagenic effects.[5] Apart from SM, nitrogen mustard (NM) also pronounces similar but remarkable blistering effect.[6] NM is even more toxic than SM but there is no report which certifies the use of NM as chemical weapons. This is probably due to its poor stability under normal conditions, hence is stored in hydrogen chloride salt form. Rather, it has been used in the treatment of cancer[7] and chemotherapy.[8]

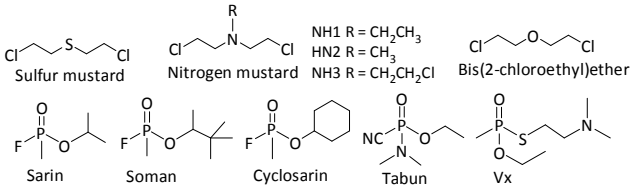

Fig. 1 Molecular structures of chemical warfare agents.

Destructive properties, the absence of an antidote[9] and simple preparation method make this agent a first choice to use as chemical weapons when a country or terrorist group decides to build up a capacity or intend to use CWA. Unlawful use of chemical weapons has posed a grave concern among the international community, which has prompted an urgent need to develop a protocol for SM detection.

Unfortunately, there are very few detection methods which often rely on the use of low toxic simulants to demonstrate the proof-of-concept rather than using a real agent. However, considerable efforts have been directed on the detection of nerve agents,[10] detection of SM is very rare. Nowadays, SM is detected either by the instrumentation methods[11] including hand-held detectors[12] or by the chemical methods[12a,13] like chemically doped detection paper and residual vapour detection kit. Though, selectivity, sensitivity and portability always remain the major issues. As an alternative to these chemical and instrumental methods, other approaches such as use of molecularly imprinted polymers,[14] immunochemical,[15] quartz crystal microbalance,[16] and platinum(II) pincer complexes,[17] dansyl-ligated gold nanoparticles[18] and rhodamine-thioamide,[19] have also been reported. Despite these elegant efforts, a 'full-proof detection' of SM is almost non-existent.

Using SQ-OH dye (Fig. 2), a proof-of-principle study for recognising and sensing 2-chloroethyl ethyl sulfide (CEES), a mustard simulant, has recently been demonstrated.[20] Unlike CEES, which on reaction with **1** form a podand **3**. SM is a bialkylating agent with two 'reactive groups' and is supposed to form a macrocycle **2**. (Scheme 1). With this change, the experimental conditions and the concentration of the molecular species involved may also change. Another consideration of showing detection of SM could be attributed to the fact that recently, we have demonstrated detection of real nerve agents tabun and Vx using SQ.[21] Hence, the present study along with previous one[21] will provide a single platform to the responders for the detection of three real warfare

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agents viz SM, tabun and Vx, thus fulfilling the detection of both the categories i.e. blistering and nerve agents. Both the reasons compelled us to demonstrate and provide accurate experimental settings for sensing of SM**.** So that in real situation**,** no further modification will be required.

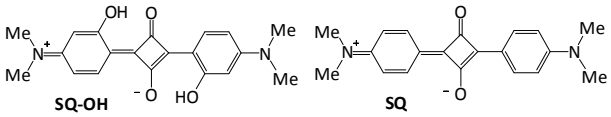
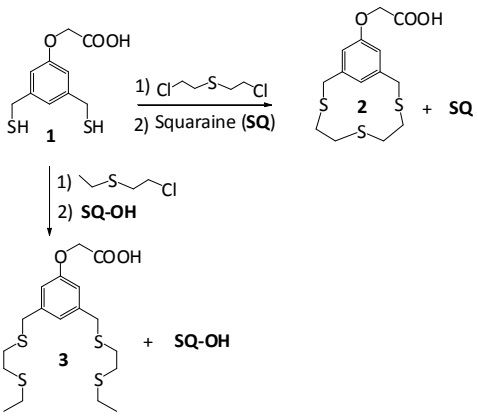


Fig. 2 Molecular structures of the squaraine dyes (**SQ-OH** and **SQ)**.



Scheme 1. Schematic presentation of the reaction **1** with SM and its simulant (CEES).

2. Result and discussions

2.1 Chromo-fluorogenic detection of sulfur mustard

Here we briefly summarize the approach. In the absence of SM, a dithiol **1,** will react with **SQ** resulting in the bleaching of the dye. However, in the presence of analyte, **1** reacts with SM to form a macrocycle **2** (Scheme 1), and then added to the solution of **SQ**. This results in the persistence of blue colur of dye with no loss of the chromogenic and fluorogenic properties of **SQ**. The formation of **2** was confirmed by mass spectrometry of the reaction mixture (Fig. 3). **SQ** is an organic electrophilic dye showing intense blue colour and fluorescence which undergoes discoloration by nucleophilic attack.[22] Thiol groups in **1,** in the presence of K_2CO_3, are strong nucleophile, and react rapidly at the central cyclobutene ring of **SQ**. This breaks the conjugation of the dye resulting in the loss of chromogenic and fluorogenic properties. When SM is present, it reacts with **1** to form **2**, which is not sufficiently nucleophilic to teact with **SQ,** thus dye retains its optical properties.

Since, dye used in earlier method[20a] was **SQ-OH** (Fig. 2) that was found to be sensitive under operating basic conditions due to the presence of two phenolic functional groups. A slight excess of base (K_2CO_3) could change the optical response while working in real scenario. This has prompted us to first explore to a non-responsive dye to basic medium. Hence, we used **SQ** in our present study (Fig. 2).

In order to establish the sensing protocols for SM and to determine the necessary concentrations of **SQ** and **1**, we explored the responses of **SQ** towards **1**. A solution of **1** containing 3.0 equivalents of K_2CO_3 reacts with **SQ** resulting the bleaching of the dye as indicated by a visual change and a fluorescence study (Fig. 4). Next, a visual detection of SM was performed using **1** and **SQ**. A solution of **1** (0.2 mM) in methanol containing 3.0 equivalents of K_2CO_3 was allowed to react with 1.4 equivalents (59.0 µM) of SM (optimized equivalent quantity) at 80 ^{o}C for one minute in a closed vial. The solution of **1** (42 µM) was then treated with **SQ** (15.0 µM) in chloroform. The blue color of the dye persists indicating the presence of SM (Fig. 5). Without SM, **1** bleaches the dye and hence the color disappeared. Using solutions of **1** (40 µM), SM (55 µM), and **SQ** (0.3 µM), a fluorescence titration was also performed (Fig. 6). The presence of analyte displays a large enhancement in intensity at 640 nm, while in the absence, the intensity remains completely quenched.

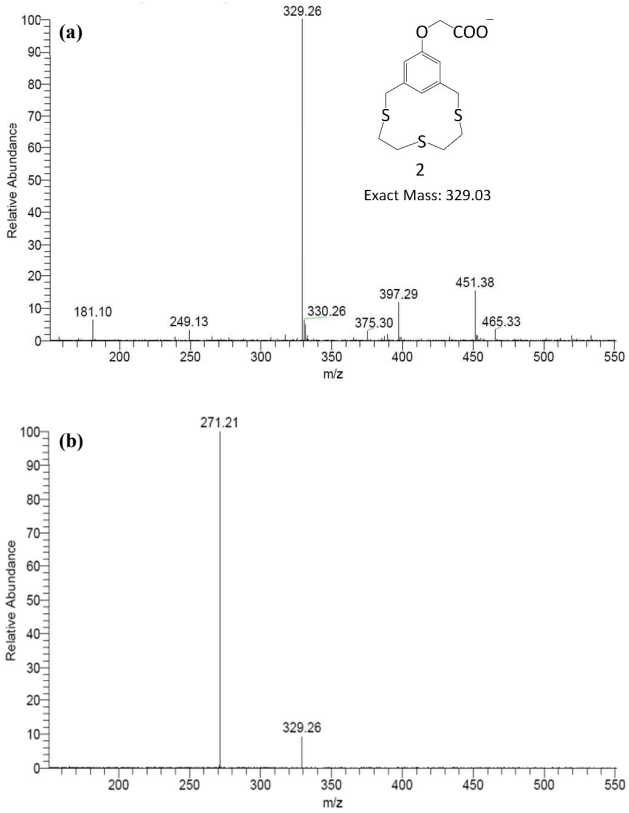


Fig. 3 Mass spectra of macrocycle **2** present in the reaction mixture (a) ESI(-)-MS (b) ESI(-)-MSMS

2.2. Interferences Studies

Studies on possible interferences of the reactive and non-reactive species in the detection of CEES have been previously established.[20a] The selectivity of SM over other CWAs such as Sarin, Cyclosarin, Soman, Tabun, Vx and bis(2-chloroethyl)ether (BCEE) are demonstrated here in organic and aqueous medium. Following similar reactions as mentioned earlier in this paper, 2.0 equivalents of these interfering agents were allowed to react with **1** (0.2 mM) in methanol containing 3.0 equivalents of K_2CO_3, followed by the treatment with **SQ** (15.0 µM). Interestingly, we observed the disappearance of the

colour with these agents (Fig. 5). Therefore, the use of slight excess of interfering agents did not have any effect on chemical sensing. Because under these conditions, most of the potential interferents are decomposed (except BCEE) and is not available to react with **1.** BCEE, being not reactive did not react with **1**. In these cases unreacted **1** bleaches **SQ**, hence no response was found with the interferents.

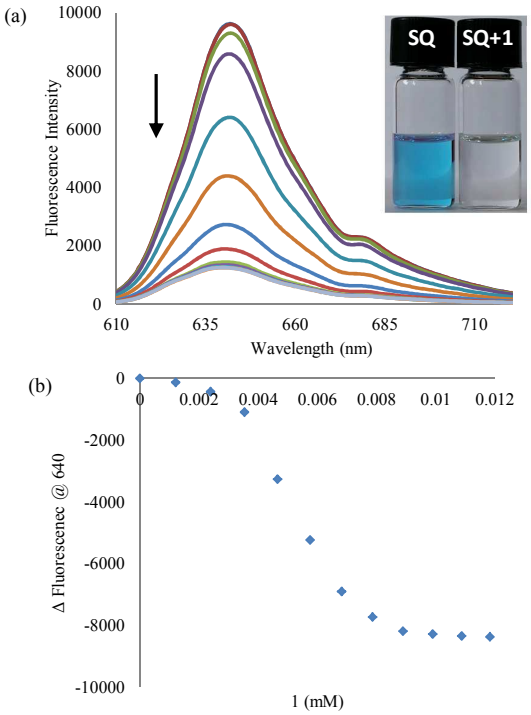


Fig. 4 (a) Fluorescence intensity of **SQ** in $CHCl_3$ at 0.3 µM at 640 nm in the presence of increasing amounts of **1** (0.1 mM) in MeOH (Excitation wavelength at 625 nm) Inset: Colorimetric response of **SQ** in $CHCl_3$ at 15.0 µM with **1**, From left to right: **SQ** only and **SQ** + **1** (42 µM). (b) Isotherm showing decrease in fluorescence intensity of **SQ** with the addition of **1**.

Fig. 5 Chromogenic response of **SQ** (15.0 µM) with SM (59 µM with **1** conc. at 42 µM) and other intereferent (59 µM) (from left to right) sarin, soman, cyclosarin, Tabun, Vx, and BCEE.

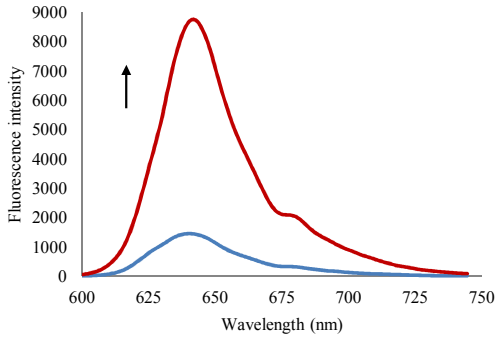


Fig. 6 Fluorescence data of **SQ** (0.3 µM) in the presence and the absence of SM (55 µM) with **1** conc. at (40 µM).

2.3. Reactivities of thiols

In an attempt to compare the thiols reactivities towards SM, **1** along with 1,3-benzenedimethanethiol (BDMT) and benzyl mercaptan (BM) were allowed to react with SM, separately under similar experimental conditions. We observed that **1** reacts faster in comparison with BDMT and BM. Even among the dithiols **1** and BDMT, **1** reacts at faster rate than BDMT. The difference in the reactivity of dithiols (**1** and BDMT) over monothiol (BM) towards a bialkylating SM can be attributed to the formation of macrocyclic compound 2,5,8-trithia[9]-m-cyclophane. It is evident that SM in the presence of an ionizing solvent shows greater electrophilicity enhancement due to the formation of three-membered cationic sulphonium heterocycles.[23] Therefore, it can be inferred that the presence of carboxylate group in 1 could be posing greater degree of ionization in the medium thus enhancing the reactivity towards SM over BDMT. Another aspect which renders the use of BDNT in place of **1** lies in its poor water solubility of both staring material and macrocyclic product. Fast rate of reaction with SM and good solubility in both organic and water medium suggested us to use **1** for our present study.

2.4. Detection in real-time scenario

Once deployed, SM can remain active from several hours to few weeks depending upon the environment conditions.[24] It has been found persistent in soil and and water for decades[25] that lead to the lethal accidents.[26] Therefore, it becomes imperative to tune our protocol in order to determine the presence of SM in water, on sufaces, in soil and in the vapour phase. Our first focus was on the detection in water. An advantage of using **1** is that it is soluble in both organic and water medium.[20] A SM solution in water was allowed to react with **1** (0.2 mM) in water in the presence of 3.0 equivalents of K_2CO_3 at 80 ^oC for one minute. This reaction mixture was then treated with **SQ** (15 µM) in a mixed solution of $CHCl_3$:acetonitrile (4:96). The solution was not bleached, thus showing the presence of SM. A control experiment (absence of SM in water) caused bleaching of **SQ**, as shown in Fig. 7a. Similar selectivity for SM over other CW agents, their mimics and other reactive electrophiles (in excess) was also tested using aqueous solution of **1** and **SQ** in mixed solution of acetonitrile:$CHCl_3$ (4:96 ratio). These possible interferents did not respond ot the sensing protocol.

In order to determine the presence of SM in soil, a spiked soil sample was directly treated with **1** (0.2 mM) in methanol containing 3.0 equivalents of K_2CO_3 at 80 ^oC. After centrifugation of the reaction mixture, the solution was allowed to react with **SQ** dye (15 μM). The persistence of a blue colour of the dye indicates the presence of the agent in soil. Additionally, an unspiked soil sample was also treated with **1** (0.2 mM), which bleached the dye immediately, showing its absence in soil (Fig. 7b). To complete the list of possible matrices, we next sought to investigate the detection of SM in the vapour phase with the intention of developing test strips and devices. **1** (200 μL, 0.2 mM) in methanol containing 3.0 equivalents of K_2CO_3 was sprayed equally on two TLC plates; one was heated (80 ^oC) and exposed to SM vapour for 5 minutes. This plate along with an unexposed SM plate was then treated with a drop of **SQ** dye (30 μM). The unexposed TLC plate shows the disappearance of blue colour while the exposed one shows the persistence of colour (Fig. 7c). Hence a simple and user-friendly test-strip assay can be created for the detection of **SM** in vapour phase.

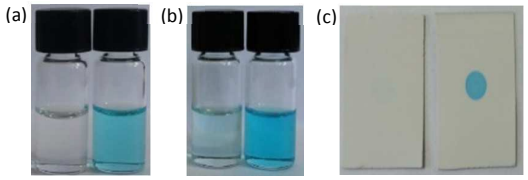


Fig. 7. (a) Chromogenic response of **SQ** (15.0 μM) when no SM is present in the soil sample (left vial) and when SM is present in the soil sample (right vial). (b) chromogenic response of **SQ** (15.0 μM) in $CHCl_3$:acetonitrile (4:96) in water, Left vial: response in the absence of SM, Right vial: response in the presence of SM. (c) Detection of gaseous SM with **SQ** dye adsorbed on TLC plate: Left: unexposed with SM, Right: exposed with SM).

2.5. Sensitivity

SM is the deadliest when used in a large excess. The relative toxicities of SM by inhalation and through skin per individual were found to be 1500 (LC_{50} mg.min/m^3) and 100 (LD_{50} in mg) respectively. However, the minimum quantity required to cause blisters on the skin is 0.2 mg. The lower detection limit of our protocol for SM was determined to be 40 μM and 18 μM by the visual and fluorescence methods, respectively which are far below the level of toxicity to cause any health hazards.

3. Conclusion

In summary, we have demonstrated the chromo-fluorogenic detection of sulfur mustard (SM). The detection of SM has proven to be quite selective and discriminative as no interferences was observed from other CWAs and reactive and non-reactive agents in organic and aqueous medium. The developed protocol was also proven to be highly sensitive for the agents as LOD was much lower than lethal doses. Successful extension of the protocols on detection of the agent in analytical settings such as in spiked water and soil samples, contaminated surfaces, and in gaseous phase will be useful in real-time monitoring. Further research work is directed towards customizing, miniaturizing and further simplifying the technique, in order to develop a chemosensor kit and portable devices for onsite and offsite deployment.

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