Novel chemiluminescent detection of chemical warfare simulant

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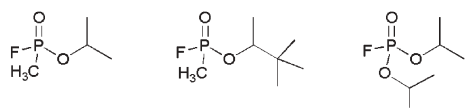
A glow assay technology for the detection of a chemical warfare simulant is presented, which is based on modulating the peroxyoxalate chemiluminescence pathway by way of utilising an oximate super nucleophile that gives an "off–on" glow response.

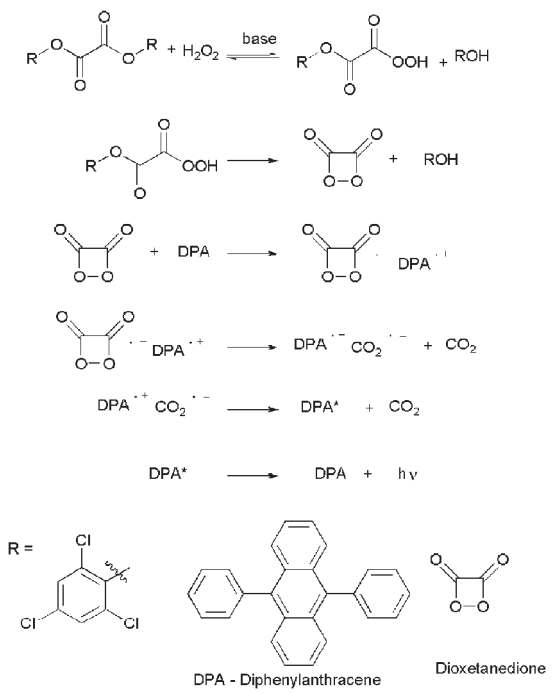
The increasing incidence of terrorism is a worldwide concern. The phosphoryl fluoride class of chemical nerve agents includes the lethal substances known as sarin (isopropyl methyl phosphonofluoridate) and soman (pinacolyl methyl phosphonofluoridate), often referred to as GB and GD respectively. These chemicals are potent inhibitors of acetylcholine esterase, and are often referred to as "nerve gases".[1] Diisopropyl fluorophosphonate (DFP) is a less toxic simulant that is used as a GB/GD mimic. With the possible use of these dangerous chemicals in either warfare or terrorist actions, there is a need to develop efficient methods for their detection[2] and decontamination.[3] Recently Swager developed a sensitive, fluorescent chemosensor for the detection of nerve agents.[4] He showed that upon intramolecular cyclization reaction of nerve agents, he could transform a flexible chromophore into a rigid delocalized system causing "off–on" response in the micromolar concentration range. There have been many other methods developed for the detection of these species, including colorimetry,[5] surface acoustic wave devices (SAW),[6] enzymatic assays,[7] and interferometry.[8]



Our group recently reported a colorimetric detection method based on a chromogenic indicator containing a super nucleophilic moiety.[9] Upon phosphorylation with DFP a hypsochromic shift was observed. In another approach, our group found that the fluorescent signal of a coumarin oxime can be modulated upon phosphorylation giving rise to a fluorescent "off–on" signal.[10] In this paper we describe a proof of principle study, whereby the detection method is achieved by peroxyoxalate chemiluminescence [POCL], a novel method of detecting the nerve gas analog DFP.

Chemiluminescence has become a powerful and versatile analytical tool for sensitive and selective detection of a wide variety of chemical species.[11] POCL has been used for the determination of a number of species, such as hydrogen peroxide, and many fluorescent compounds, in particular, polycyclic aromatic hydrocarbons.[12] Peroxyoxalate chemiluminescence was first observed by Chandross from the reaction between oxalyl chloride and hydrogen peroxide in the presence of 9,10-diphenylanthracene, giving rise to an intense blue chemiluminescence.[13] The general reaction mechanism was studied by McCapra and is shown in Scheme 1.[14] Hydrogen peroxide reacts with the oxalate to form a peroxyoxalate intermediate, which then undergoes an intramolecular cyclization with the displacement of the second phenolic leaving group. The four-membered ring, dioxetanedione (a dimer of carbon dioxide), formed in this process has been widely cited as the key intermediate in the chemiluminescence mechanism.[15] The essential feature of this Chemically Initiated Electron Exchange Luminescence (CIEEL) mechanism is the electron transfer from the fluorophore to the dioxetanedione. This produces a radical cation–radical anion pair. The dioxetanedionyl radical anion fragments to carbon dioxide and the carbon dioxide radical anion, which is a better reducing agent than the dioxetanedionyl radical anion. Back electron transfer to the fluorophore radical cation from the carbon dioxide anion produces the singlet excited state of the fluorophore, the relaxation of which subsequently yields the observed fluorescence emission.

Our strategy was to take advantage of the CIEEL mechanism to develop a glow assay technology to detect the presence or absence



Scheme 1 CIEEL mechanism.

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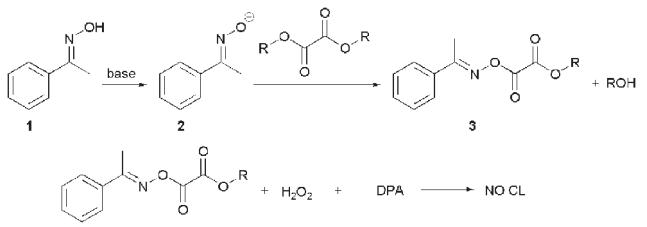
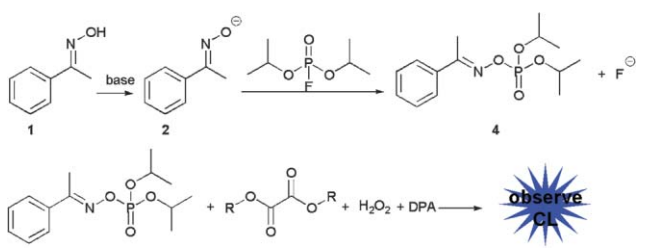


Scheme 2 In the absence of analyte no chemiluminescence.

of phosphoryl fluoride type nerve gases. Our design involves modifying the CIEEL pathway in order to modulate the response of the DPA mediated light production upon binding of a specified analyte. We envisioned creating a competing reaction that scavenges the oxalate ester in the absence of an analyte such as sarin gas, thus not allowing the light producing reaction to continue. When sarin is present this scavenger is bound and the oxalate ester is able to react with H_2O_2 in order to ultimately produce light.

It is well known that the oximate moiety is a highly nucleophilic center, known as a super nucleophile. It is capable of reacting with the phosphorus(V) center of the chemical warfare agent (CWA) simulants.[9] A simple oxime **1** was synthesized by reacting acetophenone with hydroxylamine hydrochloride and sodium hydroxide. The oxime was successfully converted to an oximate **2** by deprotonation with the super base P_4-*t*-Bu phosphazene.[9] As shown in Scheme 2, in the absence of analyte, the oximate super nucleophile should react with trichlorophenyl oxalate (TCPO) ester forming oximate–oxalate adduct **3**. In this manner the oxalate ester cannot participate in CIEEL and there should be no signal. Alternatively, in the presence of the nerve gas analog DFP an oximate–DFP adduct **4** should be formed. The oxalate ester would now be available to participate in the chemiluminescent pathway (Scheme 3). In this manner we sought to turn "on" the glow signal indicating the presence of analyte.

In order to observe whether the relevant species are reacting properly, the various reactions were monitored with proton, carbon, phosphorus, and fluorine NMR spectroscopy. NMR experiments were carried out in NMR tubes in deuterated acetonitrile as all solution studies were carried out in acetonitrile. The disappearance of the sharp oxime proton signal of **1** at δ 9.4 upon addition of P_4 base confirmed the formation of oximate **2** by ^{1}H NMR spectroscopy. In addition, there was a clear difference in the chemical shift of the aromatic region of the oxime and oximate anion. The oximate–oxalate adduct **3** showed a distinct change in chemical shift in the aromatic region, confirming its formation. A similar change was observed for the oximate–DFP adduct **4**. Further, the formation of oximate–DFP adduct **4** was evident



Scheme 3 Observe CL in the presence of nerve gas analog.

from the ^{19}F NMR spectroscopy where the doublet at δ −77.08 and δ −79.65 in DFP was replaced by a singlet at δ −149.69 in the oximate–DFP adduct.

All solution studies were carried out in acetonitrile as it gave high CL intensity. A calibration curve was developed by using varying equivalents of DFP to demonstrate the change of CL intensity with the number of equivalents of DFP. For the calibration curve, and for the solution studies, we first prepared a series of 7.4×10^{-2} M oxime solutions in five vials. These solutions were titrated with 1 equivalent of P_4 base. These five solutions were then treated with 0, 0.25, 0.5, 0.75, 1 equivalents of DFP and allowed to react for 2 minutes. Each mixture was then treated with 1 equivalent of TCPO ester for five minutes, followed by diphenylanthracene fluorophore, and hydrogen peroxide (Fig. 1). The chemiluminescent intensity of each of these solutions was immediately measured in a Glowmax 20/20 Luminometer. The experiment was repeated two times. The chemiluminescent intensity increased with increased number of equivalents of DFP in good agreement with the proposed mechanism.

The same experiment was carried out in 10 times more diluted samples, and the results are shown in Fig. 2. According to these findings, when the DFP concentration is as low as 1.5×10^{-3} mol dm^{-3}, it can be easily detected by this methodology.

The presence of DFP can also be conveniently detected with the naked eye in this approach, as seen in Fig. 3. The bright blue chemiluminescent signal was turned "on" immediately in the

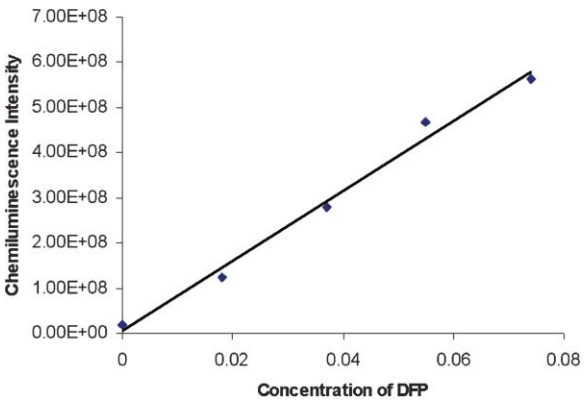


Fig. 1 Chemiluminescence intensity correlates with concentration of DFP. DFP concentration at maximum CL intensity is.0.074 M.

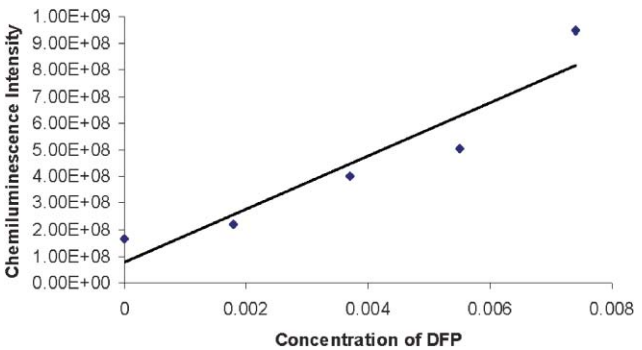


Fig. 2 Chemiluminescent intensity with increased number of equivalents of DFP in 10 times more diluted samples. DFP concentration at 1 equivalent of DFP is 7.4×10^{-3} M.

Fig. 3 The "naked eye" detection of nerve gas analog at 0.074 M concentration of DFP. A high concentration of DFP is used solely to show the glow.

solution when the DFP was added, whereas a solution which did not contain DFP did not give any detectable chemiluminescent signal by eye. As this reaction does not require an additional light source, it has an added advantage over the colorimetric or fluorimetric methods which require an ultraviolet or visible light source to illuminate the sample.

In conclusion, we have presented here a proof of principle study of how to detect CWAs by modulating the chemiluminescent pathway. It was successfully shown that oximate super nucleophile perturbed the CIEEL pathway by reacting with peroxyoxalate, one of the key components responsible for the production of light. The chemiluminescent signal is "on" by way of forming oximate–DFP adduct, indicating the presence of analyte. The dye used in this approach is not limited to DPA, which gives a blue emission; but rather the use of any other chemiluminescent fluorophore enables further versatility of the system. Specifically, bis-phenylethynyl anthracene is another well known fluorophore that could be used in place of DPA, and bis(carbopentyloxy-3,5,6-trichlorophenyl) oxalate is another alternative to TCPO. Currently, work is in progress on lowering the detection limits to approaching ppm in air, and testing for false positives with other electrophiles, for ultimate practical applications.

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