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Chromogenic and fluorogenic reagents for chemical warfare nerve agents' detection

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The ease of production, the extreme toxicity of organophosphorus-containing nerve agents, and their facile use in terrorism attacks underscores the need to develop accurate systems to detect these chemicals. Among different technologies we review here recent advances in the design of chromo-fluorogenic methods for the specific detection of nerve agents. Optical sensing (especially colorimetric detection) requires usually low-cost and widely used instrumentation and offers the possibility of so-called "naked eye detection". Recent reported examples suggest that the application of chromo-fluorogenic supramolecular concepts for the chromogenic or fluorogenic sensing of nerve agents might be an area of increasing interest that would allow developing systems able to overcome some of the limitations shown by classical analytical methods.

1 Introduction

The current rise in international concern over criminal terrorist attacks using Chemical Warfare Agents (CWAs) has brought about the need for reliable and affordable detection of toxic gases. According to the Organization for the Prohibition of Chemical Weapons and the Chemical Weapons Conventions,

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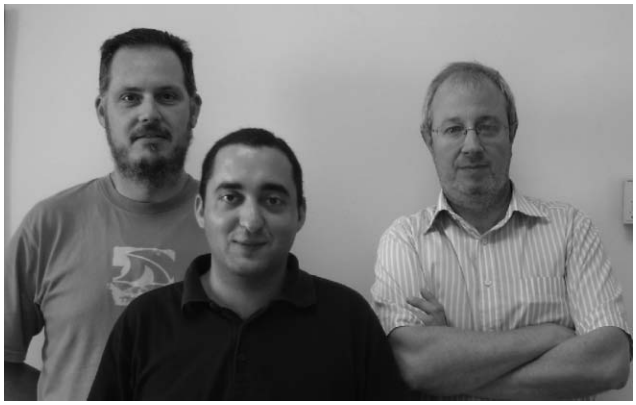

Santiago Royo Calvo (middle) graduated in chemistry at the University of Valencia in 2005 and is currently carrying out PhD studies in the Martínez-Máñez group. Ramón Martínez-Máñez (right) received his degree in chemistry from the University of Valencia in 1986 and carried out PhD studies in Valencia (Spain) under the supervision of Professor P. Lahuerta. He carried out postdoctoral studies in Cambridge (UK) with Professor E. C. Constable. His research interest covers many aspects of supramolecular chemistry and materials chemistry for guest recognition and sensing. Félix Sancenón (left) graduated in chemistry in Valencia in 1991 and carried out research PhD studies with Professor R. Martínez-Máñez. A Marie Curie grant enabled him to join in 2004 Professor L. Fabbrizzi's group in Pavia. His research interest focuses on chromo-fluorogenic chemosensors and reactands.

Ana M. Costero (left), born in Guadalajara (Spain) in 1953, studied chemistry at the University of Zaragoza and received her PhD in 1982 in Valencia (Spain). After a postdoctoral appointment in Pittsburg (USA) in the Rebek group, she began her independent research career. Her research interest includes design, synthesis and study of ligands as chemistry sensors in specific recognition of cations, anions and other neutral species. Salvador Gil (middle) and Margarita Parra (right) were born in Valencia, Spain in 1958. After receiving their PhD from the University of Valencia in 1988 and 1986, respectively, they joined the Fleming (Cambridge, UK) and Ley groups (Imperial College, UK) for their postdoctorate appointments. They returned to the University of Valencia and were incorporated to the Costero group.

substances are considered chemical weapons if they, through a "chemical effect on living processes, may cause death, temporary loss of performance, or permanent injury to people or animals". A chemical warfare agent must be highly toxic, yet not so toxic so as to make it too difficult to handle. The substance must be capable of being stored for long periods in containers without degradation and without corroding the packaging material. It must be relatively resistant to water, air and heat so that it does not lose effect when dispersed.[1] Chemical warfare agents are classified in several groups; *i.e.* nerve agents, asphyxiant/blood agents, vesicant agents, choking/pulmonary agents, lachrymatory agents, incapacitating agents and cytotoxic proteins.[2] All of these nerve agents are among the most dangerous of the chemical warfare species (see Table 1).[3]

Nerve agents are a family of highly toxic phosphoric acid esters, structurally related to the larger family of organophosphate compounds. In fact, development of nerve agents was a by-product of insecticide research and development. It was not until the early 1930s that German chemists observed that organo-phosphorus compounds could be poisonous and the first production of G-agents was developed during the Second World War: Tabun (GA), Sarin (GB), Soman (GD) and Cyclosarin (GF). Immediately after the war, further research resulted in discovery of new types of nerve agents; *i.e.* V-agents (VG, VM, VX, VE) that are approximately ten-fold more poisonous than Sarin.

Deadly nerve agents have rapid and severe effects on human and animal health, either as a gas, aerosol or liquid. They enter the body through inhalation or though the skin. Poisoning may also occur through consumption of liquids or foods contaminated with these agents. The effects of the nerve agents are mainly due to their ability to inhibit the action of acetylcholinesterase, a critical central nervous system enzyme.[4] Given that the normal function of this enzyme is to hydrolyse acetylcholine wherever it is released, its inhibition results in the excessive concentration and accumulation of acetylcholine at various sites of action such as endings of the parasympathetic nerves, ciliary body, bronchial tree, gastrointestinal tract, bladder and blood vessels, the cardiac muscle and endings of sympathetic nerves. The sequence of symptoms varies with the route of exposure. While respiratory symptoms are generally the first to appear after inhalation of the nerve agent vapour, gastrointestinal symptoms are usually the first after ingestion. Inhibition of acetylcholinesterase is a progressive process and the degree of inhibition depends not only on the concentration of nerve agent but also on the time of exposure.

Table 1 Toxicity of the most important nerve agents to man[a]

Nerve agent	LCt_{50} Inhalation mg min/m^3	LD_{50} Skin mg/individual
Tabun	200	4000
Sarin	100	1700
Soman	100	300
V-agents	50	10

[a] Data taken from ref. 3.

The main difference between G-agents (tabun, sarin, soman and cyclosarin) and V-agents is related with the non-persistence of the former whereas the later are persistent. The term persistent agent generally refers to agents that are not rapidly hydrolysed and are not very volatile so that are able to persist in an area for a long period of time. Related compounds, diisopropylfluorophosphate (DFP) and diethylchlorophosphate (DCP), have been typically used as model compounds for the design of indicators (see Fig. 1). However it has to be taken into account that DFP and DCP are not viable nerve agents because they are readily hydrolysed (poorly persistent).

The most important chemical reactions of nerve agents take place directly at the phosphorus atom. The P–X bond is easily broken by nucleophilic reagents, such as water or hydroxyl ions. In aqueous solution at neutral pH the nerve agents decompose slowly, whereas the reaction is greatly accelerated after the addition of base. The resulting product is a non-toxic phosphoric acid.[5]

The ease of production and extreme toxicity of organophosphorus-containing nerve agents underscores the need to detect these odourless and colourless chemicals. As a consequence, intense research efforts have been directed to develop sensitive and selective systems for the detection of these compounds.[6] A variety of detection methods for CWAs have been developed including surface acoustic wave (SAW) devices,[7] enzymatic assays,[8] electrochemistry,[9] interferometry[10] and gas chromatography-mass spectrometry.[11] However, all the methods presented at least one of the following limitations; low responses, lack of specificity, limited selectivity, low sensitivity, operational complexity, non-portability, difficulties in real-time monitoring and false positive readings.

An alternative to those classical methods is the design of colorimetric or fluorimetric chemosensors or reagents. In fact one of the most convenient and simplest means of chemical

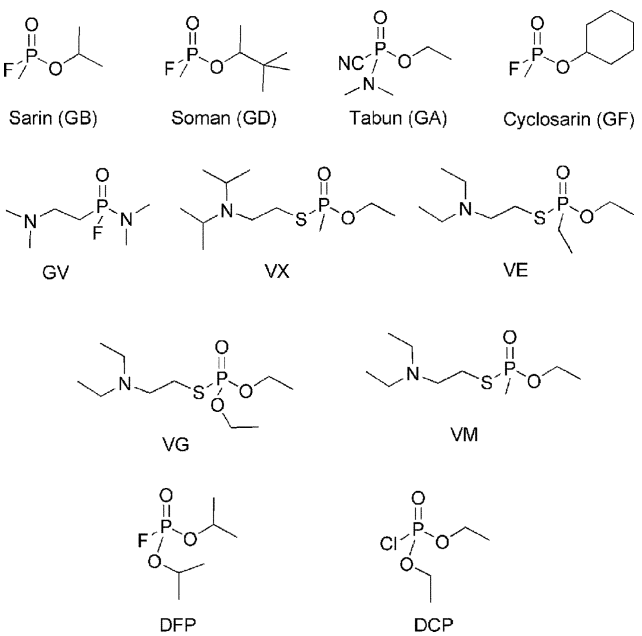

Fig. 1 Chemical structures of G and V nerve agents and the simulants DFP and DCP.

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detection is the generation of an optical event, *i.e.* changes in absorption or emission bands in the presence of target analytes. Optical outputs have been extensively used in recent years for the development of chemosensors for ion and neutral molecule recognition and sensing based on supramolecular concepts.[12] Additionally, optical detection (especially colorimetric detection) require only usually low-cost and widely used instrumentation and offers the possibility of so-called "naked eye detection" for rapid semiquantitative determinations. Several methods are actually used for the colorimetric detection of nerve agents (such as M8, M9, *etc* test strips used by the US armed forces) in liquids and vapours based on the use of different simple dyes and enzymatic assays coupled with pH-induced colour changes. Although these are systems that show certain limitations such as false positives, they are standard methods against which new colorimetric systems would be judged. Additionally to these systems, recent reported examples strongly suggest that the application of chromo-fluorogenic supramolecular concepts for the visual detection of nerve agents will be an area of increasing interest and would allow developing systems able to overcome some of the limitations shown by classical analytical methods. Stimulated by these concepts, we show in this short review, a comprehensive overview of the strategies and protocols developed for the preparation of chromo-fluorogenic reagents for nerve agent detection and their less toxic simulants.

2 Chromo-fluorogenic nerve agent detection

As far as we know, colorimetric probes for the detection of nerve agents were first described by Schönemann in 1944.[13] The method was further studied by Gehauf *et al.* and was based in the oxidation of certain amines to give coloured products in the presence of several organophosphorus compounds.[14] Addition of organophosphorus compounds to aqueous solutions of sodium perborate at pH 10 containing an aromatic amine (benzidine, *o*-toluidine, *o*-dianisidine, *o*-phenylenediamine, 4-amino-2-acetamido-*N*-diethylaniline, 4-amino-2′-ethoxydiphenylamine-2-sulfonic acid, 4,4′-diamino-*sym*-diphenyl urea and 4,4′-diaminostilbene) induced the formation of yellow to orange coloured dyes. The mechanism was based in the formation of a peracid derived from the organophosphorus compound that further induced the oxidation of the amine. The test was used to detect small amounts of Sarin, Tabun, DPF and other related compounds. In 1957 the same authors developed a similar assay for the detection of nerve agents (Sarin, Soman and Tabun) by means of fluorescence measurements.[15] A mixture of indole and sodium perborate (1 : 2) in water–acetone (1 : 1, v/v) mixtures with the pH fixed at 9.5 presented negligible fluorescence with a pale yellow colour. Upon addition of increasing quantities of Sarin a brilliant green fluorescence appeared (emission band centred between 460–490 nm) due to the oxidation of the indole to indoxyl. The emission intensity was directly related with the amount of the added Sarin indicating that this method can be used for the detection and estimation of small amounts of nerve agents.

Moreover, these are rare studies and the development of chromo-fluorogenic sensors and reagents for nerve agent detection have been very scarce. Only, recently the possibility of visual detection of nerve agents has gained attention mainly fuelled by the possible deliberate use of chemical warfare agents by terrorist organizations. The chemosensors and reagents described below are conceptually more sophisticated that the former probes reported nearly forty years ago and are somehow inspired in concepts recently applied to the development of chromo-fluorogenic chemosensors for target guests.

2.1 PET-based fluorescent sensors

Photo-induced electron transfer (PET) processes have been extensively used in the progress of fluorescent chemosensors for cation and anion sensing and recently the same principles have been applied to the detection of nerve agents and nerve agent simulants. The ease of emission intensity modulation by the functionalization of a suitable fluorophore with certain binding sites has resulted in the synthesis of myriads of receptors during the last ten years. A scheme of how PET processes can be used for the fluorogenic detection of target guests is shown in Fig. 2. Fluorescence in a molecule is observed when an excited electron placed in the LUMO orbital goes to the HOMO releasing the excess of energy in form of light. If the energy of another "external" orbital (in Fig. 2 from another part of the molecule) lies between the energy gap of the HOMO and LUMO of the fluorophore a PET process from the "external" orbital to the photo-excited fluorophore can take place inducing a quenching process through a non-radiative path. When coordination of a target guest induces the removal of the energy level between the HOMO and LUMO of the fluorophore, the emission intensity increases resulting in the corresponding detection of the guest.

PET processes have been successfully employed in the design of fluorescent chemosensors for DFP, DCP and certain selected phosphate esters. The chemical structures of these chemosensors were characterized by the presence of a rigid scaffold functionalized with two subunits. One of the subunits possesses a nucleophile which is highly reactive towards phosphorus substrates (a hydroxyl moiety) and the other is a tertiary amine with an appended fluorophore through methylene spacers (see Fig. 3). As a consequence of this design, the emission of the fluorophore is quenched *via* a PET process from the lone pair of the tertiary amine to the photo-excited fluorophore. Upon addition of the target phosphonate an acylation reaction with the primary alcohol takes place. This acylation induces a rapid intramolecular N-alkylation that leads to the formation of a quaternary ammonium salt.

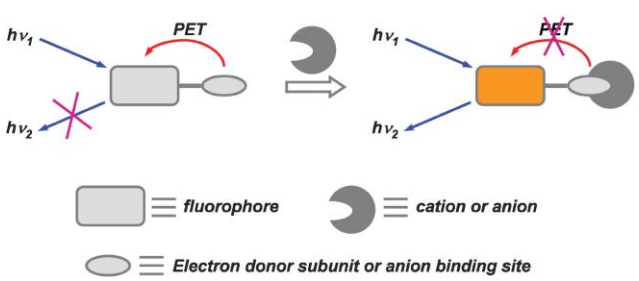

Fig. 2 Scheme of the use of PET processes for the fluorogenic detection of target guests.

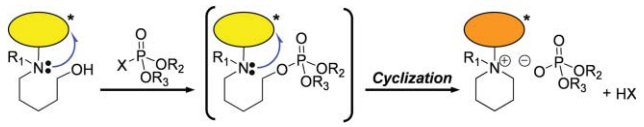

Fig. 3 General scheme of PET indicator molecules for the fluorogenic detection of nerve agents.

This quaternization induced the inhibition of the PET and the restoration of the full emission of the appended fluorophore.

The first example of a reactand based on this approach was developed by Pilato and co-workers[16] using a platinum 1,2-enedithiolate complex with an appended alcohol (molecule **1** in Fig. 4). The reaction between **1** and phosphate, thiophosphate and phosphinate esters (10^{-1}–10^{-6} mol dm^{-3}) in dichloromethane induced the formation of a highly fluorescent **cyc1** species with emission bands from a ILCT (intraligand charge-transfer) excited state centred at 605 and 710 nm (excitation at 450 nm). Complex **1** was also immobilized in cellulose acetate/triethylcitrate (triethylcitrate acts as a plasticizer) thick films (RTV-108 and RTV-118) and its ability to serve as gas sensor screened. For these thick films the time required for minimum detection of phosphate and for complete conversion of **1** to **cyc1** drops with the increase of plasticizer content. The best results were obtained with films that present a plasticizer concentration of 50% and for which the generation of **cyc1** was found to be linear with phosphate exposure time.

In a similar approach, Zhang and Swager developed molecules **2–4** based in flexible weakly conjugated chromophores, that upon phosphorylation and cyclization, resulted in an increase of the emission intensity (Fig. 5).[17] The response of **2–4** to the simulants DCP and DFP was quite different. Thus, **2** based on a thienylpyridyl scaffold reacts with DCP and DFP to form the corresponding phosphorylated species but these were unable to undergo cyclization to give the highly fluorescent **cyc2** derivative that was only obtained by forcing reaction conditions ($SOCl_2$/KI, $AgClO_4$). Reactand **3** bearing a

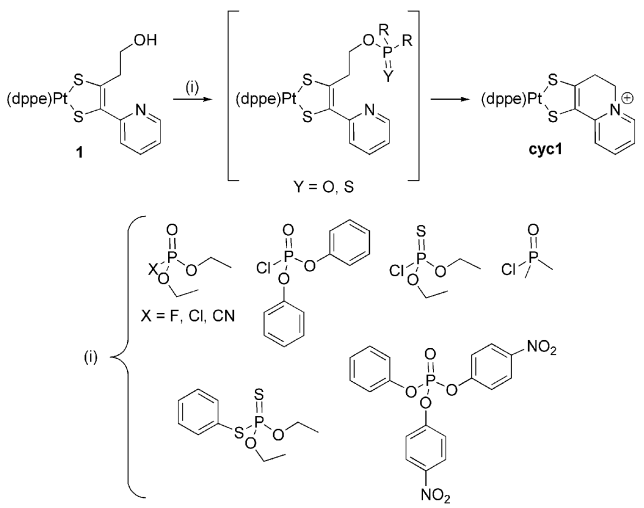

Fig. 4 Chemical structure of a platinum 1,2-enedithiolate complex with an appended alcohol used for the fluorogenic detection of phosphate, thiophosphate and phosphinate esters.

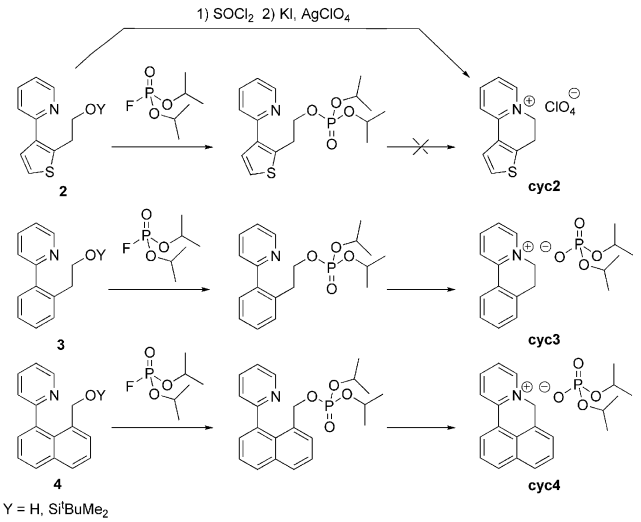

Fig. 5 Chemical structures and reactivity of indicator molecules **2–4**.

phenyl ring instead of a thienyl one, showed a more appropriate sensing behaviour, *i.e.* the emission intensity of dichloromethane solutions of **3** became highly fluorescent (5000-fold enhancement in the emission) due to the formation of the cyclization product **cyc3** upon reaction with one equivalent of DCP or DFP. This very remarkable emission enhancement was ascribed to the elimination of the free rotation between the two rings in **3** upon cyclization. The only drawback in using **3** as fluorogenic reagents lies in the fact that the kinetics of the cyclization reaction was too slow. In order to enhance the reaction kinetics the indicator molecule **4** was synthesized and tested. This reactand contains a very rigid naphthalene subunit with a restricted conformation that favours cyclization. Another point of interest is that **4** in its native state presents a relatively strong emission that allows for ratiometric detection. **4** was deposited in cellulose acetate thin films and its response towards DFP and DCP tested. Upon addition of such films to warfare agents simulants, the emission band of the free molecule centred at 378 nm was shifted gradually (about 5 min to complete the shift) to 438 nm, assigned to **cyc4**. The kinetics of the cyclization reaction with **4** was 17 times faster than that presented by **3**.

Dale and Rebek developed a family of fluorescent sensors (see Fig. 6) for DCP also based on the PET mechanism outlined in Fig. 3.[18] Compounds **5–8** are derived from Kemp's triacid (as a rigid scaffold) functionalized with a primary alcohol located in close proximity to a tertiary amine bearing a pyrene fluorophore connected through methylene spacers (ranging from one to four methylene units). The fluorescence

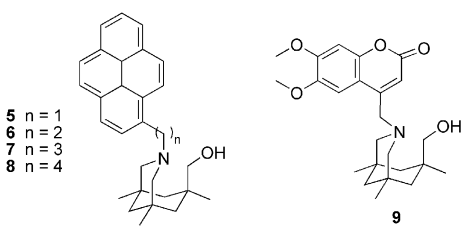

Fig. 6 Chemical structure of compounds **5–9**.

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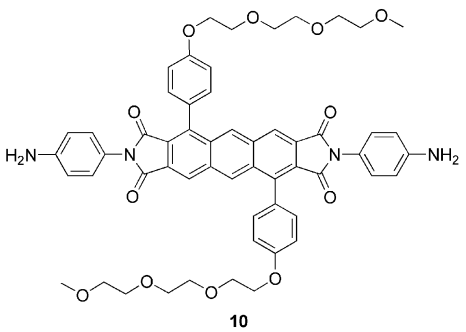
of methanol solutions of **5–8** (λ_{exc} = 340 nm) was quenched due to a PET process from the lone pair of the amine to the photo-excited pyrene fluorophore. Exposure of **5** to DCP in methanol triggers phosphorylation of the primary alcohol followed quickly by an intramolecular substitution reaction (see the general scheme in Fig. 3). The final quaternized ammonium salt produced by this cyclization reaction no longer possesses a lone pair of electrons, and a 22-fold emission enhancement was observed. The quenching efficiency is reduced in indicators **6–8** due to the presence of additional methylene spacers and, as a result, the enhancements in the emission intensity observed for **6–8** are less significant; for example only a 1.1-fold enhancement for **8** was found. The sensor design is modular and the authors also synthesized reactand **9** bearing a 6,7-dimethoxycoumarin fluorophore. Again a 20-fold increase in the emission intensity was found upon addition of DCP to methanol solutions, due to phosphorylation, followed by formation of the corresponding quaternary ammonium salt.

Another approach for the development of PET-based molecular indicators for detection of nerve agents consists of using phosphorylation reactions of an amine that is integrated into the structure of a fluorophore. Using this principle, the highly substituted anthracene bisimide **10** was synthesized (see Fig. 7) and used as a fluorescent sensor for acetyl chloride, thionyl chloride and several toxic chemicals such as dichlorothiophosphate (DCTP), methylphosphonic dichloride (MPDC), dimethylphosphinic chloride (DMPC) and dimethyl methylphosphonate (DMMP).[19] DMF solutions of **10** showed the typical absorption band of the anthracene. Upon excitation at 425 nm no emission was observed due to an intramolecular PET process from the amine moieties to the photo-excited anthracene. Reaction of **10** with acetyl chloride and thionyl chloride (these acid halides mimic the behaviour of organophosphonate-based nerve gases) gave rise to significant enhancements in the emission intensity (band centred at 490 nm) that was ascribed to the suppression of the PET process upon reaction of the amine with the acid halides and formation of the corresponding amide. Additionally, compound **10** dispersed on a silica support showed the same sensing behaviour towards acid halides. The presence of DCTP, MPDC and DMPC in DMF solutions of **10** also induced an enhancement in the emission intensity, whereas addition of DMMP (a less reactive Sarin simulant) induced negligible changes in the emission intensity.



10

Fig. 7 Chemical structure of compound **10**.

The preparation of hybrid materials for sensing nerve agents is another interesting approach. Recently polymer microbeads with fluoresceinamine (**11**) were used for this purpose.[20] Aqueous solutions at pH 7.5 of **11** are not fluorescent due to a PET process from the nitrogen lone pair to the photo-excited fluorophore. As in the above case, reaction of **11** with DCP induced the phosphorylation of the aromatic amine, suppression of the PET process, and an enhancement of the emission intensity (Fig. 8). The fluorescence of the phosphorylated molecule was pH-dependent and in the interval 7.5–11 the fluorescence is higher than at acidic pH. Fluorescent microbeads were prepared by adsorbing **11** onto carboxylate-functionalized polymer microbeads coated with poly(2-vinylpyridine). When the microbeads are subjected to DCP vapour, the conversion of fluoresceinamine into a phosphoramide causes a rapid (within seconds) intense fluorescence enhancement identical to that observed in solution. Additionally, the poly(2-vinylpyridine) provides a high density of proton-accepting pyridine nitrogen sites that neutralize the HCl released during the reaction, thereby maintaining the high fluorescence even after vapour exposure. No significant response is observed when the microbeads are subjected to other nerve agent simulants (DMMP, DIMP), a mustard gas simulant (methyl salicylate) and volatile organics (ethanol, heptane and toluene). The size, sensitivity and rapid response of **11** make it suitable for nerve agent vapour detection and inclusion into microbead sensor arrays.

2.2 Oximate-containing sensors

One important limitation of the PET-based sensors described in the previous section is related with the usually slow rates of the phosphorylation reactions. In order to avoid this problem other authors have used highly nucleophilic moieties in combination with a colorimetric system as an alternative strategy to prepare sensors for the detection of warfare agent simulants. Oximates (anions derived from the correspondent oximes) and hydrazones are the highly nucleophilic moieties employed in the development of these colorimetric indicators. The oximate and hydrazone moieties are known as ''supernucleophiles'' in which an atom containing an unshared electron pair (nitrogen or oxygen atom) is adjacent to a nucleophilic centre. If these ''supernucleophiles'' are implemented into an organic scaffold with absorption bands centred in the visible region, the reaction with the phosphorus centres from the nerve agents might induce changes in these bands leading to consequent colorimetric recognition of these deadly gases.

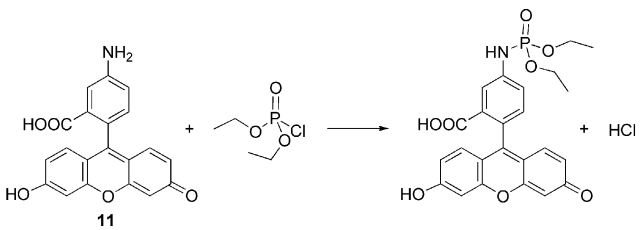


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Fig. 8 Chemical structure of compound **11** and its reactivity with the stimulant DCP.

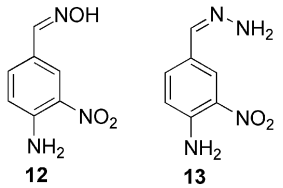

Fig. 9 Chemical structures of **12** and **13**.

Following this approach, molecules **12** and **13** (Fig. 9) bearing oxime and hydrazone moieties in a chromogenic nitrophenyl scaffold, were synthesized and tested against the chemical warfare nerve agent simulants DCP and DFP.[21] Solutions of **12** (3×10^{-4} mol dm^{-3}) in DMSO–NaOH (1 : 1) showed a visible band centred at 461 nm (assigned to n–π* transition) that was gradually shifted to 410 nm upon addition of DCP. In the presence of DFP a hypsochromic shift to 413 nm was observed. These shifts in the visible band of **12** were ascribed to the formation of the corresponding oximate–DCP or oximate–DFP derivative. These oximate complexes were unstable and suffered dehydration with time to give 4-amino-3-nitrobenzonitrile. The reactivity of **12** in the presence of DCP is shown in Fig. 10.

To discard the possibility of reaction between the OH^- group and the simulants in the basic DMSO–OH^- medium the authors repeated the same experiment but using the superbase P_4-*t*-Bu phosphazene. The same results were found, confirming that the changes in the visible band were due to the formation of the oximate supernucleophile and subsequent phosphorylation. In clear contrast, **13** failed to form the hydrazone–DFP adduct in basic conditions.

The same approach has been recently employed in the synthesis of the fluorescent reactands **14** and **15** consisting of hydroxylamine and coumarin moieties (Fig. 11).[22] DMSO

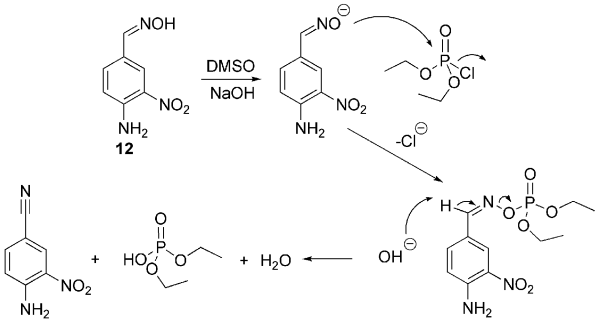

Fig. 10 The reactivity of **12** in the presence of DCP in DMSO–NaOH medium.

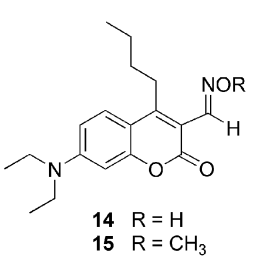

Fig. 11 Chemical structures of **14** and **15**.

solutions of **14** showed a broad band centred at 409 nm. Upon addition of 2 equivalents of the P_4-*t*-Bu phosphazene base, a new band centred at 443 nm appeared, that was ascribed to an oximate anion formed by deprotonation of **14**. Gradual addition of DFP induced a bathochromic shift to 409 nm. As above, these changes in the UV-Vis spectrum were assigned to the phosphorylation of the oximate anion upon addition of DFP. This was confirmed since addition of P_4-*t*-Bu base and DFP to DMSO solutions of **15** (without the possibility of deprotonation) induced negligible changes in the UV-visible spectra. **14** also acts as a fluorogenic reagent. Thus the oximate anion obtained upon addition of the P_4-*t*-Bu base to solutions of **14** is weakly fluorescent (excitation at 410 nm and emission at 450 nm) due to quenching the fluorescence through a PET mechanism induced by the high-energy lone pair of the oximate anion. Upon addition of DFP and subsequent phosphorylation, the energies of the oximate orbital were lowered, resulting in a turn on of the fluorescence due to PET inhibition.

Recent works in the field of oximate bases included several studies related with their basicity and reactivity towards organophosphorus compounds. The relevant pK_a values of a large set of oximate bases and the second-order rate constants (k^{Ox}) of these oximates with two model organophosphorus compounds, bis(4-nitrophenyl)phenylphosphonate (BNPPP) and bis(4-nitrophenyl)methylphosphonate (BNPMP), and three toxic compounds (Sarin, Soman and DFP) in water as well as in 70 : 30 (v/v) DMSO–water mixtures have been determined by Terrier *et al.* by means of potentiometric procedures.[23] A most relevant conclusion was that the reactivity of oximates showed a clear tendency to saturation upon increasing of their basicity, and the nucleophilic character of the oximate decreases regularly upon increasing the pK_a in the series. The authors suggested that the existence of a strong requirement for desolvation of the oximates prior to nucleophilic attack (which becomes more difficult with the increase in basicity) is the explanation of this saturation.

2.3 Molecularly imprinted polymers

Another approach used in the development of optical sensors for nerve agents relies on the use of molecularly imprinted polymers (MIPs). Molecularly imprinted polymers are synthetic materials that can mimic the functions of biological receptors but with fewer stability constraints. Generally MIP sensors for nerve agents incorporate a luminescent lanthanide (commonly europium) complex acting as a signal transducer. The narrow excitation and emission peaks of lanthanide spectra provide for highly selective and sensitive analysis. Detection of the analyte is based upon changes that occur in the spectrum when the nerve agent is coordinated to the Eu^{3+} metal centre.

Based in the MIP approach a polymer sensor for detection of pinacolyl methyl phosphonate (PMP, the hydrolysis product of Soman) in water has been prepared recently.[24] The lanthanide complex $Eu(DVMB)_3(PMP)(NO_3)_2$ (DVMB = divinylmethyl benzoate) was synthesized and used to prepare the MIP. Upon excitation at 465.8 nm (with an argon laser) of aqueous solutions of the complex $Eu(DVMB)_3(PMP)(NO_3)_2$

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two emission bands at 610 and 613 nm were observed. The band at 610 nm was assigned to the addition of PMP to the Eu^{3+} centre whereas the band at 613 nm was due to the emission of Eu^{3+}. In further studies the authors coated the distal end of an optic fibre with the imprinted polymer and the PMP content of water solutions at pH 13.0 was measured through coordination-induced enhancement of the Eu^{3+} emission. The response of the sensor to increasing concentrations of PMP exhibits an enhancement in the emission intensity of the two bands centred at 610 and 613 nm. This increase in intensity was assigned to a binding of PMP with the Eu^{3+} and subsequent exclusion of water from the coordination shell of the cation. Several common pesticides and herbicides were tested as interferents and none of them induced the appearance of the band centred at 610 nm but induced several degrees of emission enhancement in the 612–620 nm interval.

More recently, a new MIP was prepared with vinyl benzoate as matrix monomer, divinyl benzene as cross-linking reagent, $Eu(NO_3)_2$ as a source of Eu^{3+} and the corresponding nerve agent.[25] After the MIP preparation the nerve agent was removed by washing with 1 M HNO_3. By use of this procedure MIPs for Sarin, Soman and VX have been prepared. Several optic fibres were coated with the MIP and then used for the detection of the corresponding nerve agent in aqueous solutions at pH 9.5. For all the sensors an increase in the nerve agent content induced an enhancement in the lanthanide emission (excitation at 468 nm leads to a broad emission band between 610 and 630 nm) indicative of binding with the lanthanide centre and subsequent exclusion of water molecules.

In a recent work MIPs based on a dithiobenzoate substituted tris(β-diketonate) europium(III) complex prepared by RAFT (reversible addition fragmentation chain transfer) polymerization was used as a luminescent sensor for PMP (Fig. 12).[26] For this purpose the molecule **16** was synthesized and its complex with PMP prepared. Polymers were synthesized with the **16**–PMP complex and an ethylene glycol dimethylmethacrylate/methyl methacrylate matrix. The release of PMP (Soxhlet extraction with isopropanol) resulted in the polymeric sensory material. Upon addition of increasing quantities of PMP to the sensory polymer in aqueous solutions an enhancement in the luminescence band centred at 612 nm was observed. As in the above cases, this enhancement was assigned to the binding of PMP with the Eu^{3+} centres. Good sensitivity (detection limits in the low ppb range) and selectivity were obtained with the use of these luminescent MIPs.

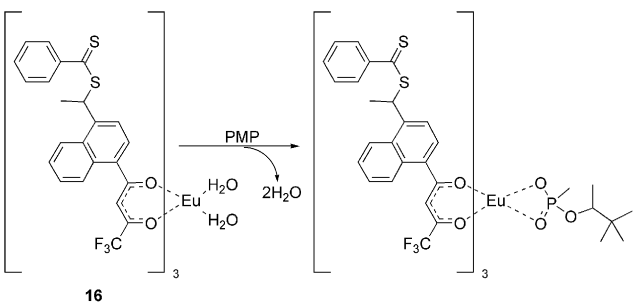

Fig. 12 Chemical structure of **16** and its PMP complex.

2.4 Miscellaneous

The 2,6-bis(1′-methylbenzimidazolyl)pyridine based molecular indicators **17** and **18** have been used in displacement-like assays with La^{3+}, Eu^{3+} and Zn^{2+} for the fluorogenic detection of triethylphosphate and tri-*o*-tolylphosphate (Fig. 13).[27] Solutions of **17** and **18** in $CHCl_3$–CH_3CN (9 : 1 v/v) are highly fluorescent, showing emission bands at 420 and 375 nm, respectively. Addition of La^{3+} cation to solutions of **17** and **18** induced bathochromic shifts in the emission spectra (73 and 38 nm for **17** and **18**, respectively) coupled with quenching of the emission intensity, ascribed to the formation of [**17**·La^{3+}] and [**18**·La^{3+}] complexes. Addition of triethylphosphate to solutions of both complexes induced an instantaneous blue shift of the emission band and an increase in the emission intensity, indicating the release of the indicators from the complex, as phosphate binds to La^{3+} cation. Moreover, addition of tri-*o*-tolylphosphate to solutions of [**17**·La^{3+}] yields only a very small response, even at high concentrations of the aromatic phosphate, indicating an excellent selectivity for detection of alkyl phosphates over bulky aromatic phosphates. In order to explore the influence of different metal ions in the sensory response of the complexes, solutions of [**17**·Eu^{3+}] and [**17**·Zn^{2+}] were also prepared and titrated with triethylphosphate. The behaviour obtained with [**17**·Eu^{3+}] was quite similar than that presented by [**17**·La^{3+}]. In clear contrast, complex [**17**·Zn^{2+}] showed no increase of free-ligand fluorescence even in the presence of large amounts of triethylphosphate. This absence of response was attributed to the fact that Zn^{2+} binds **17** more strongly than La^{3+} and Eu^{3+}, disabling the displacement reaction. The same behaviour was observed for complexes [**18**·Eu^{3+}] and [**18**·Zn^{2+}], namely a response to triethylphosphate with [**18**·Eu^{3+}] and no response with the [**18**·Zn^{2+}] complex. In order to facilitate the application of these complexes in practical devices, complex [**18**·Eu^{3+}] was adsorbed onto hydrophobic silica particles resulting in a solid material that changes its fluorescence (from pink to blue) upon exposure to triethylphosphate vapours.

Another interesting approach for the design of chromofluorogenic probes for nerve agent detection comes from the use of gold nanoparticles as signalling subunits coupled with an enzymatic assay. The sensing cocktail consists of the acetylcholine esterase enzyme (AChE), $HAuCl_4$, gold nanoparticle seeds and the enzyme substrate acetylthiocholine (see Fig. 14).[28] In this system, with a concentration of the enzyme fixed at 0.13 units mL^{-1}, the plasmon absorbance band of the nanoparticles increases in intensity, becomes broader, and was blue shifted upon addition of increasing quantities of acetylthiocholine. TEM analyses revealed that as the

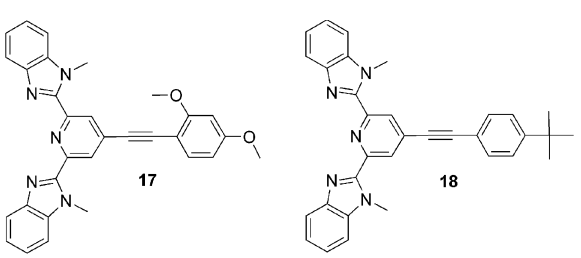

Fig. 13 Chemical structures of **17** and **18**.

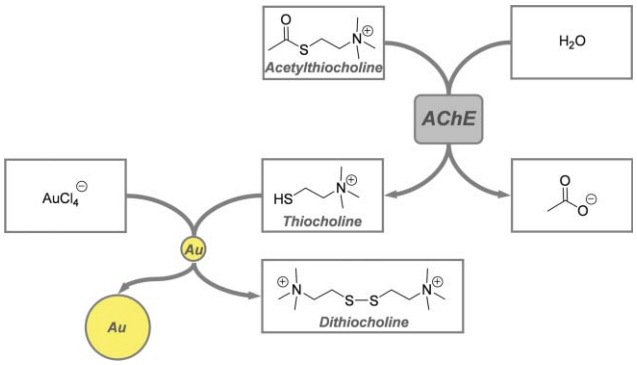

Fig. 14 Detection system based on the use of gold nanoparticles.

concentration of acetylthiocholine increases larger particles were formed (reaching a diameter of 300–500 nm). This can be explained as follows: (i) upon addition of increasing quantities of acetylthiocholine the hydrolysis to thiocholine is enhanced, (ii) thiocholine acted as a reducing agent for $AuCl_4^-$ to metallic gold that is deposited in the nanoparticle seeds, (iii) as the amount of reductant increased, larger gold particles with increased nanoclustering on the particle surface were formed, giving rise to enhanced plasmon absorbances. This catalytic enlargement of gold nanoparticle seeds was used to detect two mimics of nerve agents, namely 1,5-bis(4-allyldimethylammoniumphenyl)pentane-3-one dibromide (**19**) and diethyl-*p*-nitrophenyl phosphate (paraoxon, **20**) (Fig. 15). Both compounds induced the phosphorylation of the active site of acetylcholine esterase with the subsequent deactivation of the enzyme. As the concentration of the mimic compound increased, the absorbance of the gold nanoparticles decreases, implying that the biocatalytic growth of the gold nanoparticles is inhibited. A surface immobilized assay was constructed by binding the gold nanoparticle seeds to glass plates. Upon addition of acetylcholine esterase and acetylthiocholine, the surface turns blue (λ = 570 nm), ascribable to the plasmon band of gold nanoparticles. Addition of the nerve agent mimic induced the decrease of the plasmon band and the colour of the glass slide changed to pink.

3 New trends and future perspectives

Nerve agents are classified as chemical weapons of mass destruction by the United Nations according to UN Resolution 687. Their production and stockpiling was outlawed by the Chemical Weapon Convention of 1993, yet nerve agents have been used in the Iran–Iraq war and more recently in the Gulf War. Additionally, one of the most widely publicised uses of nerve agents was the 1995 terrorist attack in which members of Aum Shinrikyo delivered Sarin against passengers on the Tokyo subway; twelve citizens died and thousands who touched the liquid or inhaled its vapours were seriously injured. Among promising new technologies, the use of chromo-fluorogenic reagents able to detect nerve agents to the naked eye is especially appealing. In fact this research area has grown in interest and there have been an increasing number of publications in the last few years. However, and despite the development of certain chromo-fluorogenic detection systems of varying sensitivity and specificity, the design of methods for detection of nerve agents showing very low false alarm rates is still a challenge. In fact, many of the reported detection systems we have reviewed above, and others based on different technologies, display signalling events to simulants and also to other less toxic phosphorus derivatives. However, accuracy and low false readings are especially significant when civilians are concerned because false positives can trigger panic reactions. Hence more selective and specific sensors capable of very accurate and thorough analysis and profiling of nerve agents should be developed. Future work in this area will surely focus on present shortcomings as well as in the development of affordable and reliable systems able to be employed in a wide range of different situations.

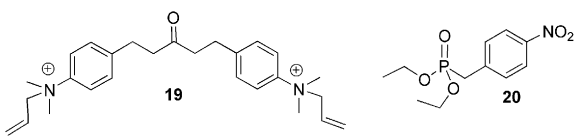

Fig. 15 Chemical structure of the simulants **19** and **20**.

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