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Discrimination of nerve gases mimics and other organophosphorous derivatives in gas phase using a colorimetric probe array†

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A colorimetric array for the chromogenic discrimination of organophosphorous derivatives in gas phase has been developed.

Nerve agents, used as chemical warfare weapons, are some of the most toxic known chemical agents. They are hazardous as liquid and vapour and can cause death within minutes after exposure.[1] Nerve agents are organophosphonate derivatives and show certain similarities with organophosphates used as pesticides. Nerve agents are highly toxic to mammals due to their capacity to interfere with the action of the nervous system through the inhibition of acetylcholinesterase, resulting in acetylcholine accumulation in the synaptic junctions hindering muscles relaxation.[2] Moreover apart from nerve gases, many organophosphates and organophosphonates act as "nerve agents" (*i.e.* are inhibitors of the acetylcolinesterase enzyme) and they are one of the most common causes of poisoning worldwide *via* intoxication through inhalation, ingestion and dermal absorption.

Some analytical procedures based on biosensing assays and physical techniques have been used to detect these compounds.[3] However, they show certain limitations in their use typically involving difficult portability and low selectivity. Among the techniques that develop easy handling of disposable systems, the use of chromogenic chemosensors is one of the most promising since they are versatile, printable and colour modulations can be easily measured using image capturing systems, or allow the detection of colour changes by the naked eye.[4] In fact few technologies are as inexpensive as visual imaging. Although some chromogenic indicators have been described for the detection of nerve gases they are generally based on a single compound and in some cases present restrictions such as lack of specificity.[5] In fact, the presence (or absence) of certain colour/fluorescence changes is not necessarily an indication of the presence (or absence) of nerve gases and in some cases chromo-fluorogenic systems show false positives or false negatives. In fact more exact correlations seem to be necessary in order to use chromogenic systems for safety applications. Moreover, the development of rapid methods for the individual signalling of a certain nerve agent and the unequivocal distinction from other organophosphates or phosphonates may prove important.[6] For instance, even though emergency response protocols are similar for all "nerve gases", their different toxicity and the experimental evidence that some antidotes are ineffective for some of them, indicate the importance of distinguishing certain compounds within this family of toxic chemicals.[7]

A suitable approach to avoid some of these drawbacks when using single analyte indicators is the possibility of designing optoelectronic noses, as potent and versatile systems to be applied in complex systems.[8] In fact, optoelectronic noses, built by an array of dyes capable of offering information through colour changes, have been applied for the detection of different volatile compounds (VOCs)[9] including odorants,[10] volatile amines,[11] *etc.* Based on the above issues, and following our general interest in developing colorimetric probes in this field[12] we report herein a prospective study of the use of an array of chromogenic indicators which we have applied to the detection and differentiation of several organo-phosphates, phosphonates and nerve agent simulants in gas phase.

Optoelectronic noses usually focus on chemical sensors with a large cross-reactivity and rely on a full range of intermolecular interactions. In our case, inspired by previously reported optoelectronic noses, and based on our own experience a total of 16 dyes (see Scheme 1) were chosen. These include push–pull chromophores containing active sites such as alcohol, amine, pyridine groups capable of potentially reacting with the organophosphorous compounds shown in Scheme 2 (*i.e.* dyes 1, 2, 3, 5, 6, 7, 9, 10, 11, 13, and 14), and some dye derivatives capable of inducing colour modulations in the presence of their possible hydrolysis products such as fluoride, cyanide, phosphate and protons (*i.e.* dyes 1, 4, 8, 9, 12, 13, 15 and 16).

The array was prepared by placing 2 μL of the corresponding dye solution (of *ca.* 10^{-4} and 10^{-2} M depending on

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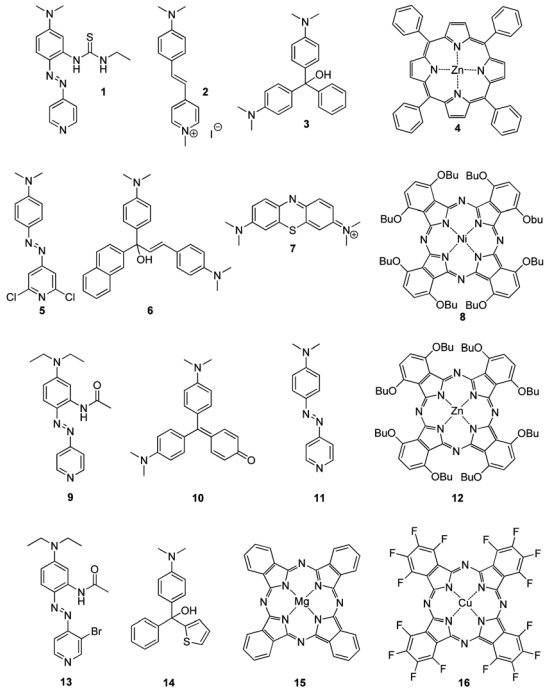
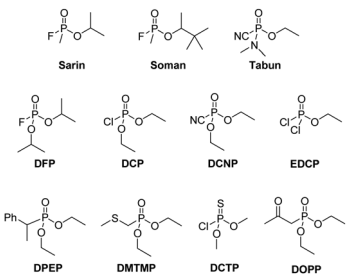


Scheme 1 The 16 dyes used in the chromogenic array.



Scheme 2 Organophosphorous derivatives used in this study (diisopropylfluorophosphate (DFP), diethylchlorophosphate (DCP), diethylcyanophosphonate (DCNP), ethyldichlorophosphate (EDCP), diethyl(1-phenylethyl)phosphonate (DPEP), diethyl(methylthiomethyl)phosphonate (DMTMP), dimethylchlorothiophosphate (DCTP), diethyl-(2-oxopropyl)phosphonate (DOPP)) and the nerve agents Sarin, Soman and Tabun.

the compound) in a suitable support (silica gel and aluminium oxide plates or polyurethane and PVC films were tested). The best results were obtained using silica gel plates (see ESI†). As model compounds for nerve gases we have used DFP, DCP and DCNP which are derivatives which display a similar reactivity to that of nerve agents such as Tabun, Sarin and Soman, although they lack their acute toxicity. The response of these colorimetric arrays was tested in the presence of 9 analytes including nerve agent simulants DFP, DCP and DCNP, phosphates (EDCP and DCTP), phosphonates (DOPP, DMTMP and DPEP) and HCl (see Scheme 2).

In a typical experiment the corresponding saturated analyte vapour at 25 °C (see ESI†), together with the chromogenic array were placed in a closed box for 30 minutes. The experiments were carried out in air with a relative humidity of 60–65%. Three completely independent experiments for each analyte were carried out to check the reproducibility of the array response. The same sensing array in the absence of the tested gases was employed as a control, which showed minor colour variations throughout the experiments (see ESI†). A scanner was used to obtain pictures of the array. Moreover RGB coordinates were measured from the photographs using image processing software (Photoshop). Difference maps were obtained by the difference of red, green and blue (RGB) coordinates of each sensing dye and the initial values were measured in the control. The subtraction of the two images resulted in a difference vector of $3N$ dimensions where N is the total number of compounds. Each dimension ranges from −255 to 255; *i.e.* an RGB value of (0, 0, 0) is black, whereas a value of (255, 255, 255) is white. To facilitate visualisation only, the colour palette of the difference map was enhanced by expanding the colour range from 0–100 to 0–255; any RGB change of <4 was treated as background noise and ignored, while changes of >100 were mapped to be 255. The difference vector is conveniently visualised in Fig. 3 as a map of the absolute values of the colour changes. At a glance, Fig. 1 clearly shows the existence of characteristic colour fingerprints for the studied analytes.

Colour differences were also analysed using PCA, which is a powerful linear unsupervised pattern recognition procedure, that was used here as a simple method to project data onto a two-dimensional plane. Typically, PCA decomposes the primary data matrix by projecting the multi-dimensional data set onto a new coordinate base formed by the orthogonal directions with data maximum variance. The eigenvectors of the data matrix are called principal components and are not intercorrelated. The principal components (PCs) are ordered so that PC1 displays the largest amount of variance, followed by the next largest, PC2, and so forth. The first principal component contained only 51.64% of the variance of the data. The first two components represented 63.29% of total variance, whereas ten PCs were needed to account for 95% of variance. This large number of independent dimensions is in agreement with the use of chemical sensing systems that employ several forms of interactions between dyes and the organo-phosphate and phosphonate derivatives. Moreover this increasing dimensionality also helped to discriminate among highly related samples (very similar compounds). Fig. 2 shows the resulting PCA for the 9 sampling organo-phosphate and phosphonate derivatives (three replicates) when using all the dyes (16 × 3 coordinates per dye). Here the PCs score plot uses two PCs (PC1 and PC3) representing the 60.86% of variance.

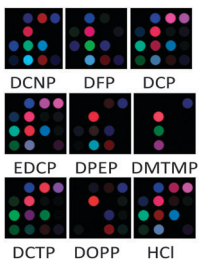


Fig. 1 Colour difference maps. Maps are generated from the absolute values of the differences of the red, green and blue values for each dye before and after equilibration with the corresponding analytes as saturated vapours at 25 °C.

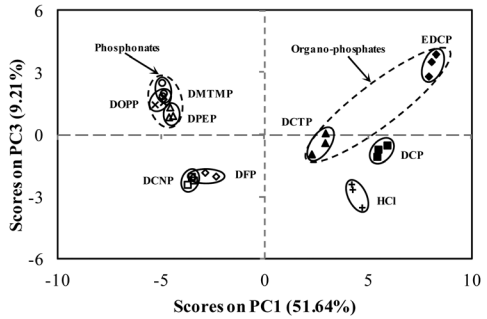


Fig. 2 PCA score plot of PC1 and PC3 for some products in Scheme 2 and HCl (3 each) and the trial clustering.

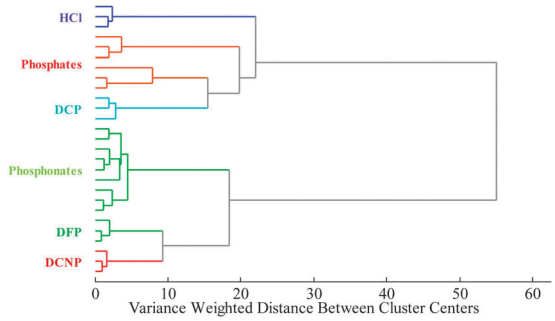


Fig. 3 HCA dendrogram showing the Euclidean distances between the trials.

A clustering of the data was found. A clear discrimination of the nerve agent simulants DCNP, DFP and DCP was observed and this was not confused with the presence of acidic vapours (HCl). Moreover other tested products were gathered in larger clusters which allowed us to classify the samples into organo-phosphates (EDCP and DCTP) and phosphonates (DOPP, DMTMP and DPEP). This good classification should be ascribed to a combination of the selectivity of the sensors, while still keeping a remarkable degree of cross-reactivity finally resulting in a good sample discrimination.

Colour data were also analysed using HCA, which is an unsupervised method of multivariate analysis that considers the complete dimensionality of the data. HCA classifies the samples by measuring the interpoint distances (Euclidean distance) between all samples in the *N*-dimensional space. In this case to define a cluster we used Ward' s (minimum variance) method. HCA provides a graphic diagram in the form of a dendrogram (see Fig. 3). HCA shows a clear clustering for all the nerve agent simulants, phosphates, phosphonates and HCl. Possible interferents such as NO_x or CO_2 were considered but they showed no response (see ESI†). The influence of the temperature (0° and 55 °C) and the humidity (saturated H_2O atmosphere) after 24 h on the array was evaluated but no colour changes were detected (see ESI†). Finally, in order to know the influence of concentration on the array response, a PLS model for prediction of the DFP concentration was created using RGB colour coordinates from the chromogenic array. Concentrations of DFP of 108, 54, 44, 39 and 24 ppm (v/v) were tested. In this study a good agreement between the measured and predicted values was found suggesting that the array could display sensing of this hazard at concentrations of some few ppm (see ESI† for details).

In summary, a 16-member colorimetric probe array by embedding chromogenic chemosensors in a silica gel plate has been used for the classification of different phosphorous-containing gases. The array was based on the use of push–pull chromophores containing reactive sites (*i.e.* alcohol, amine, and pyridine active groups) capable of reacting with "nerve agents", and reactants capable of inducing colour modulation in the presence of possible hydrolysis products. The system allowed classifying the nerve agent simulants DFP, DCP and DCNP and was also capable of discriminating between other organo-phosphate, phosphonate derivatives and acidic vapours. This approach may become important for the rational design of minimal-size high-resolution arrays for the detection of target phosphorous-containing toxic volatile derivatives. As far as we know this is the first example in which discrimination between different organo-phosphorous-containing derivatives has been achieved using a chromogenic array. The paradigm might be general and easily adapted to the simple colorimetric detection of other toxic vapours.

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