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Detection and discrimination of organophosphorus pesticides in water by using a colorimetric probe array

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ABSTRACT

Detection and discrimination of several organophosphorus pesticides in water using a colorimetric probe array containing twelve dyes has been achieved. A clear discrimination for malathion, leptophos, dichlorvos, dibrom and diazinon was observed. The array was used to determine the concentration of diazinon in orange leaves.

Keywords:

Colorimetric array, organophosphorus pesticides, PLS prediction, PCA analysis

1. Introduction

Organophosphorus pesticides (OPs) constitute nowadays the most widely used class of available pesticides. They are currently employed to protect plants from disease and insect damage in agriculture, but also in home gardens and in veterinary practice. OPs are considered safer than their parent organohalide pesticides due to their faster degradation via microbial or environmental processes [1-3]. Chemically, OPs can be classified in three main groups, namely organophosphates, which contain a P=O bond (oxon pesticides), organothiophosphates, in which the oxygen has been replaced by a sulfur atom, (P=S, thions), and organophosphonates which are closely related to nerve agents such as Sarin, Soman or Tabun.

OPs are not only highly toxic to insects but also to human beings. In fact they are one of the most common causes of poisoning of humans across the world via intoxication through inhalation, ingestion or skin absorption [4-6]. The toxicity of OPs towards insects and mammals is due to their inhibition of the acetylcholinesterase enzyme by phosphorylation. This results in an accumulation of the acetylcholine neurotransmitter in the synaptic junctions causing muscle contraction, convulsions, respiratory depression, and even death by asphyxiation. The very large amounts of OPs used in

fields and gardens cause their accumulation in soils, fruits, vegetables and water, increasing the risk of exposure to humans. Due to this environmental pollution and risks for human health, detection of pesticides is an issue of high interest [7-11].

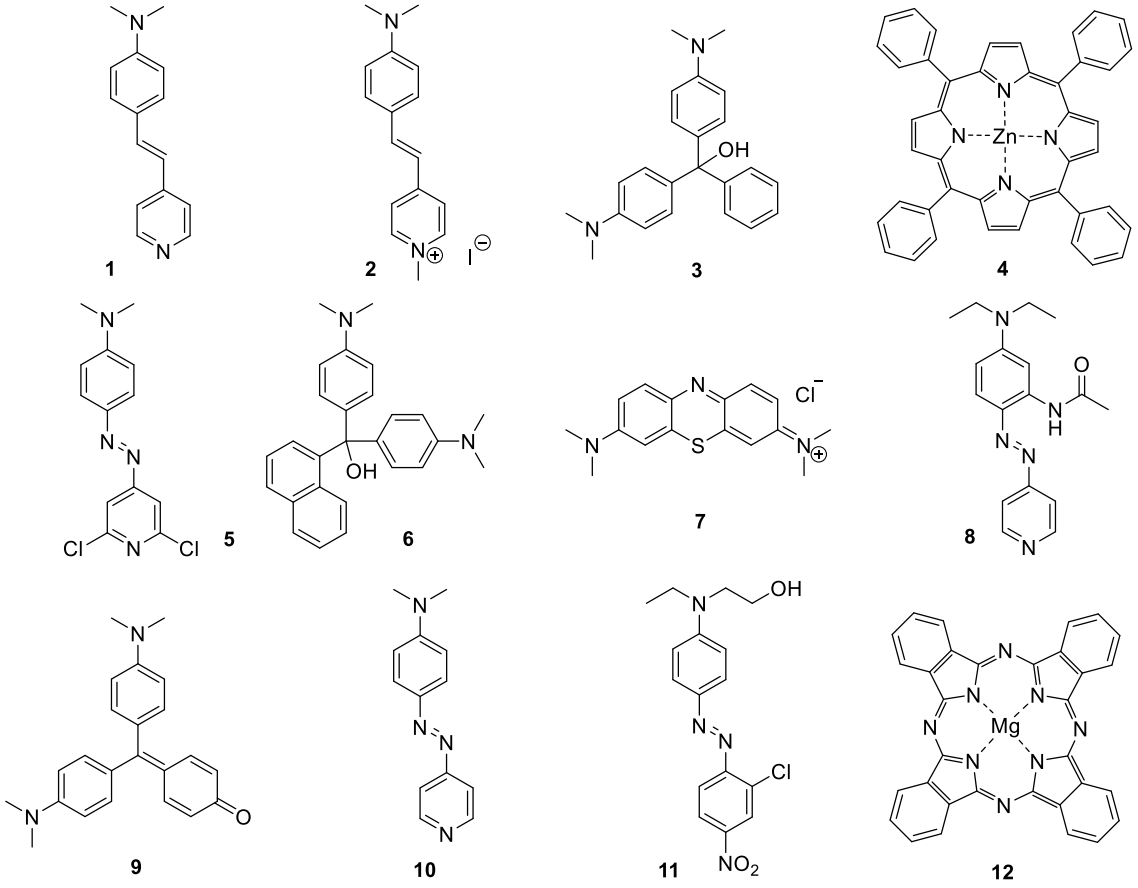
Analytical procedures based on biosensing assays **[**12-15**]** or instrumental techniques such as electrochemical methods, mass, FTIR or NMR spectroscopy **[**16-21**]** have been classically used to detect these compounds. However most of these methods are complex, time-consuming, and non-portable. As an alternative the potential optical sensing of OPs using chromo- or fluorogenic probes **[**22-24**]** is particularly appealing because these methods regularly use widely available instrumentation, and in the case of chromogenic sensors colour modulations can be measured using low-cost systems, or in some cases they can be easily detected by the naked eye. In fact, there are few technologies as inexpensive as visual imaging.

Moreover, in the field of chromo-fluorogenic probes, the design of array-based systems (also known as optoelectronic noses) is becoming increasingly popular due to their capability of multianalyte sensing and versatility and the possibility to be applied in complex systems **[**25-27**].** In fact, optoelectronic noses, based in relatively simple arrays of dyes have been applied for the detection of different volatile compounds (VOCs) including odorants, volatile amines **[**28-31**]**, or drugs **[**32, 33**]** via simple colour modulations.

Based on the above issues, and following the general interest of us and others in developing colorimetric probes for toxic organophosphonate derivatives [28-31] 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 pesticides in water.

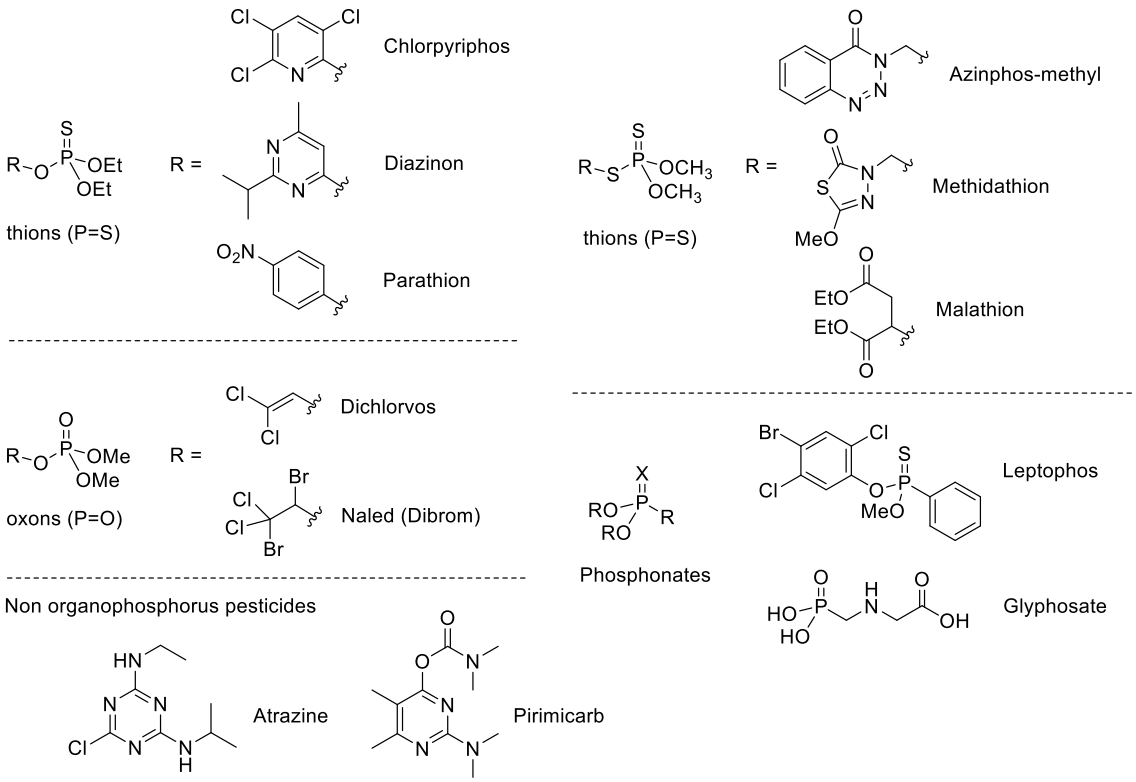
2. Results and discussion

Optoelectronic noses are usually based on the use of chromo-fluorogenic probes displaying a large cross-reactivity and use a full range of different intermolecular interactions. In our case, inspired by our own experience in the field and in previously reported optoelectronic noses, a total of 12 dyes were selected (see Scheme 1) including push-pull chromophores containing reactive sites such as pyridine, alcohol and amine groups (able to be phosphorylated) and compounds capable of coordinate with the studied pesticides or with their possible hydrolysis products.



Scheme 1. The 12 dyes used in the chromogenic array

The array was prepared by placing 2 µL of the corresponding dye solution in a 5x5 cm silica gel plate (see Supporting Information for details) and the response of the colorimetric array was tested in the presence of 12 analytes including thions (chlorpyriphos, diazinon, parathion, azinphos-methyl, methidathion and malathion), oxons (dichlorvos, dibrom), phosphonates (leptophos, glyphosate) and two non-organophosphorus pesticides (atrazine and pirimicarb). The chemical structures of these studied pesticides are shown in Scheme 2.



Scheme 2. Chemical structures of the ten organophosphorus and two non-organophosphorus pesticides used in this study

In a typical sensing experiment, a drop (2 μL) of a solution containing the corresponding pesticide (in H_2O:MeOH 95:5 v/v) was deposited on each probe of the colorimetric array, and then the array was dried in air for 5 min. The sensing array in the absence of the tested pesticides was used as control, which showed minor colour variations. Five completely independent experiments for each pesticide were performed with the aim of checking the reproducibility of the chromogenic array response. A scanner was used to obtain pictures of the array (see Supplementary data) and, from the photographs, Lab coordinates were measured using image processing software (Photoshop). Difference maps were obtained from the absolute values of the differences of Lab coordinates for each dye before and after reaction with the corresponding pesticide.

Colour differences were analysed using principal component analysis (PCA), which is a powerful linear unsupervised pattern recognition procedure and a simple suitable method to project data onto a two-dimensional plane. 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 (PCs). PCs are ordered so that PC1 displays the largest amount of variance, followed by the next largest, PC2, and so forth. In our case (see Fig. 1) the first principal component contained 58.60% of the variance of the data. The first two components represented 72.86% of total variance, whereas

seven PCs were needed to account for 95% of variance. A clear clustering of the data was found for the pesticides malathion, leptophos, dichlorvos, dibrom and diazinon and this was not confused with the presence of other pesticides (see Supplementary data for details).

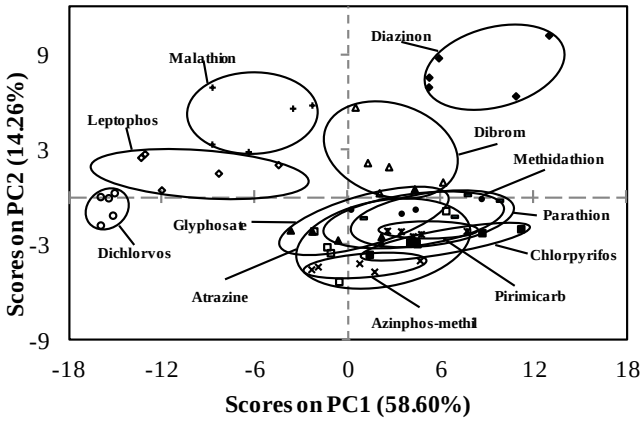


Fig. 1. PCA score plot of PC1 and PC2 for pesticides in Scheme 2 (5 each) and the trial clustering

Colour data were also analysed using hierarchical cluster analysis (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. 2). HCA also shows a clear clustering for malathion, leptophos, dichlorvos, dibrom and diazinon. Moreover, other tested samples were gathered in a larger cluster. In particular a cluster (without clear discrimination between individual samples) was observed for parathion, chlorpyrifos, pirimicarb, glyphosate, azinphos-methyl, atrazine and methidathion (see Fig. 2 and Supplementary data for details).

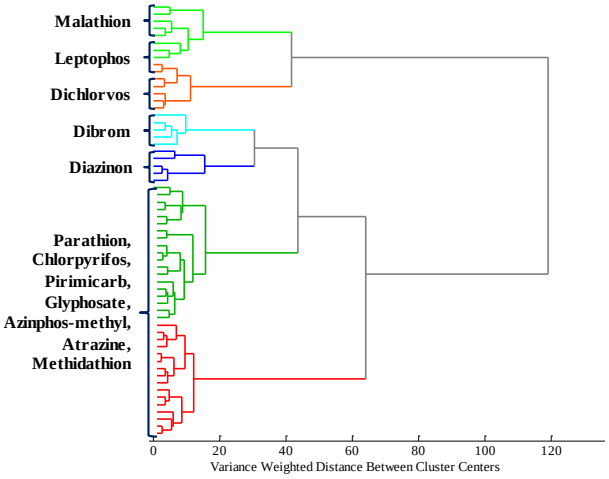


Fig. 2. HCA dendrogram showing the Euclidean distances between the trials

Moreover, in order to know the influence of the concentration on the chromogenic array response, a partial least squares regression (PLS) model for prediction of the diazinon concentration in aqueous solutions, also containing clorpyrifos and parathion as potential interferents, was created using the chromogenic array. A total of sixteen aqueous solutions containing different concentration levels (i.e. 10^{-5}, 10^{-4} and 10^{-3} mol dm^{-3}) of pesticides on each sample were established (see Experimental Section for details). Fig. 3 shows the PLS graph in which the measured vs. the predicted diazinon concentrations values (as logarithm of the concentration) are plotted. In the plot, measured values represent the real diazinon concentrations, while the predicted values are calculated according to the PLS algorithm using the Lab colour coordinates from the chromogenic array. Ideally, the predicted values should lie along the diagonal line, indicating in this case that the predicted and actual values are the same. The PLS prediction model for diazinon concentrations shows a good agreement between the measured and predicted values suggesting that the array could sense diazinon even in mixtures with other pesticides.

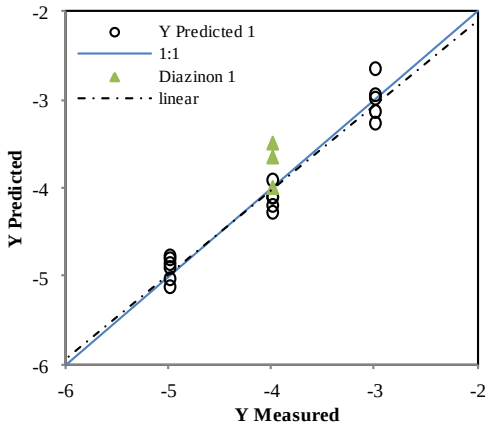


Fig. 3. Plot score of prediction model of diazinon for samples prepared with both the calibration set (o) and in orange leaves (validation set) (Δ)

Encouraged by these results we attempted to study the potential use of the chromogenic array for the detection of diazinon in a more realistic medium. In particular the optoelectronic nose was used to detect diazinon in leaves from orange trees which were previously spiked with this pesticide. In a typical experiment diazinon was extracted from the orange leaves with MeOH, the extract evaporated and the residue dissolved in H_2O:MeOH (9.5:0.5 v/v). Three replicates were performed separately. Then a drop of the corresponding solution was deposited on each probe on the optoelectronic array and the Lab coordinated measured following a similar procedure as described above. As it can be seen in Fig. 3 the prediction of the concentration of the diazinon in the final solution (10^{-4} mol dm^{-3}) fits well in the pattern found in the PLS prediction model described above (Fig. 3), suggesting that simple chromogenic arrays could be used for the detection of pesticides in real samples.

3. Conclusions

In summary, a 12-member colorimetric array has been used for the detection or classification of different pesticides. The array was based in the use of push-pull chromophores containing reactive sites (i.e. alcohol, amine, pyridine active groups) capable to react with certain pesticides and derivatives able of coordinate with the studied pesticides or with their possible hydrolysis products. The chromogenic array was able to discriminate between different pesticides, especially a clear classification was observed for malathion, leptophos, dichlorvos, dibrom and diazinon. Moreover the chromogenic array was able to detect and predict concentration levels of diazinon in leaves from orange trees. We believe that this approach may become important for the design of simple chromogenic arrays, moreover the combination of simple colour changes using optoelectronic noses combined with smartphones and intelligent home appliances offer additional interesting in-field application opportunities in this field.

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Appendix A. Supplementary data.

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Biographies

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Ana M. Costero 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. She is a member of Centre of Molecular Recognition and Technological Development (IDM) of UV. Her research interest includes design, synthesis and study of ligands as chemistry sensors in specific recognition of cations, anions and neutral species.

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Ramón Martínez-Mañez graduated in Chemistry from the University of Valencia in 1986, received his Ph.D. in 1990 from the same university. After a postdoctoral period at Cambridge (UK), he joined the department of chemistry at UPV. He became a full professor in 2002. His main areas of interest are in the field of chromo-fluorogenic and electrochemical sensors and molecular probes for anions, cations and neutral chemical species. He is also interested in the design of controlled delivery systems.