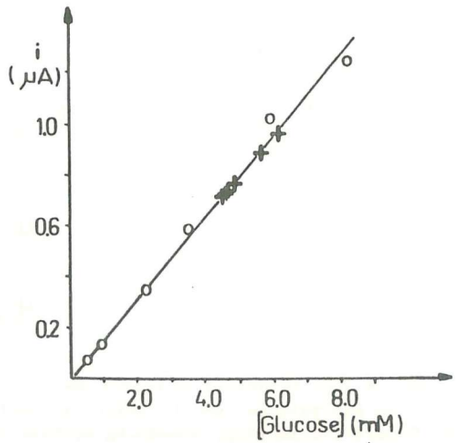

Fig.2. Calibration graph with glucose (O) and analysis of blood serum samples (X) with the bioelectrochemical sensor.

In conclusion, this glucose sensor, mini-reactor plus wall-jet cell electrochemical detector, showed a good detection limit, good linearity and a very short response time. The sensor will measure glucose in the range 10μmol.dm^{-3} to 15mmol.dm^{-3} and the immobilised enzyme has a lifetime of around two months.

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AMPEROMETRIC DETERMINATION OF PARAOXON IN THE PRESENCE OF p-NITROPHENOL BY FLOW INJECTION ANALYSIS

RODRIGUEZ GONZALO, E.; CARABIAS MARTINEZ, R.; HERNANDEZ MENDEZ, J.
Department of Analytical Chemistry, Bromatology and Food Sciences. Faculty of Chemistry. University of Salamanca. Salamanca. Spain.

SUMMARY

In the present work, the alkaline hydrolysis of Paraoxon in flowing streams is studied, and a method is proposed for the determination of Paraoxon in flow systems by amperometric detection of its hydrolysis product, p-nitrophenol, on a glassy carbon electrode. With this method, Paraoxon can be determined in the presence of Parathion owing to the different hydrolysis rates in alkaline media exibited by these pesticides.

INTRODUCTION

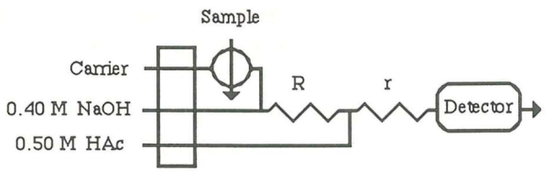
Paraoxon (diethyl p-nitrophenyl phosphate) and Parathion (diethyl p-nitrophenyl phosphorothionate) are organophosphorus pesticides with extremely high toxicity for mammalian. Both of them are hydrolyzed in alkaline media to yield p-nitrophenol, among other species (1).

The determination of mixtures of Paraoxon and Parathion is of great interest owing the importance of its control in environmental analyses (2-4). This communication reports on the determination of Paraoxon in the presence of Parathion in a flow injection system with amperometric detection. The method is based on the measurement of the oxidation signal of the p-nitrophenol produced in the alkaline hydrolysis of Paraoxon. The oxidation process has been chosen because the use of reduction process requires the removal of oxygen which is notoriously difficult in flow-through configurations.

EXPERIMENTAL

Methanolic solutions of Paraoxon and Parathion were prepared from 99% pure commercial products (Riedel-De Haen AG, Seelze-Hannover). All chemicals were of analytical-reagent grade.

Alkaline hydrolysis of Paraoxon was carried out inside the flow system using a three channel manifold with two confluence points, as shown in the figure:



The sample, an hydroalcoholic solution of Paraoxon, was introduced into a carrier stream and hydrolyzed by mixing with a 0.40 M sodium hydroxide solution in the reactor (R). The resulting solution was neutralized with a 0.50 M acetic acid solution before reaching the detector. All solutions, as well as the carrier, were 20% (v/v) MeOH/H_2O.

A *wall-jet* cell (Metrohm E-656) with a glassy carbon working electrode, a silver-silver chloride reference electrode and a gold counter electrode was used for electrochemical detection. The detector was coupled to a Metrohm E-611 potenciostat and E-586 Labograph recorder. Amperometric measurements of p-nitrophenol oxidation process were carried out at 1200 mV (vs Ag/AgCl).

RESULTS AND DISCUSSION

Electrode pretreatment and applied potential

It is well-known that phenolic compounds exhibit electrode deactivation by a mechanism of coupling of phenoxy radicals to form a polymer film. Accordingly, electrode pretreatment is necessary on the prevention of electrode passivation. Furthermore, glassy carbon electrodes are shown to exhibit a substantial improvement in their stability following a simple preanodization procedure (5).

Electrode was preanodized for 30 min at different potentials between 1100 and 1500 mV and the decreases in the signal after 16 repetitive injections were measured. From the obtained results, the most effective pretreatment was a hand polishing with alumina followed by a preanodization step at 1300 mV (vs Ag/AgCl) for 30 min while the blank solution was flowing.

The hydrodynamic voltammogram obtained with the described flow system shows a plateau at potentials higher than 1100 mV and so the applied potential for detection of p-nitrophenol formed in Paraoxon hydrolysis was 1.200 V (vs Ag/AgCl).

Experimental variables

In order to obtain maximum sensitivity a systematic study was performed to optimize the different variables (shape and reactor length, injection volume and total flow rate) affecting the determination of Paraoxon. The results were just as predictable for a system with chemical reaction in the flowing stream.

The optimum values found were as follows: knotted reactor (R) of 3 m length, injected volume 157 µl and total flow rate 3.5 ml min^{-1}.

Chemical variables as sodium hydroxide and acetic acid concentrations were 0.50 M and 0.40 M, respectively, in all experiences.

Influence of Paraoxon concentration

Under the above conditions, the relationship between intensity and concentration of Paraoxon injected was linear up to 9.75 10^{-5} M, fitting the equation $i(nA) = 2.95 + 1.97\ 10^{6}\ C(M)$ with $r = 0.9991$. The detection limit and the relative standard deviation were 7.0 10^{-7} M and 2.8 % (for 2.18 10^{-5} M Paraoxon) respectively.

Determination of Paraoxon in the presence of Parathion

Owing to the low solubility of Parathion in water, determination was carried out in 40% (v/v) MeOH/H_2O medium under the optimum experimental conditions reported for Paraoxon.

Table 1. Determination of Paraoxon in the presence of Parathion

Paraoxon/M	Parathion/Paraoxon	Recovery/%
1.09 10^{-4}	0.01	96.2
1.09 10^{-4}	0.05	97.4
8.70 10^{-5}	0.51	104.1
1.09 10^{-4}	1.09	104.9
1.02 10^{-4}	1.64	95.2
9.72 10^{-5}	2.19	104.7
8.70 10^{-5}	2.54	113.3

CONCLUSIONS

Hydrolysis reaction inside flow system avoids sample handling and control of hydrolysis time unlike other methods published (6). Futhermore, the use of oxidative detection mode avoids the need to remove dissolved oxygen.

With the proposed method it is possible to determine Paraoxon in the presence of Parathion, with an error lower than 5% for Parathion/Paraoxon ratios below two.

ACKNOWLEDGEMENT

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DEVELOPMENT OF FLOW INJECTION AMPEROMETRIC MONITORS FOR THE DETERMINATION OF NITRATE AND OTHER SPECIES

Arnold G. Fogg, S. Paul Scullion and Tony E. Edmonds

Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire, UK

Our studies of applications of flow injection analysis with amperometric detection suitable for use in monitoring situations have been carried out mainly using a laboratory built wall-jet detector which holds only a glassy carbon working electrode.[1] This is used partly immersed in a suitable electrolyte in conjunction with conventional saturated calomel reference and platinum foil counter electrodes. Phosphate can be determined by injecting samples into a carrier stream of an acidic molybdate reagent.[2] The 12-molybdo-phosphate formed is determined by reduction at the glassy carbon electrode held at +300 mV vs SCE. For determinations in inexpensive and plentiful samples, such as a hydroponic fluid, the reverse flow injection method (rFIA), in which acidic molybdate reagent is injected into a sample stream, can be used more appropriately.[3]

The shapes of signals obtained in rFIA would be expected to be different from those obtained in normal FIA.[4] In nFIA reaction occurs initially at the two extremities of the sample bolus but after

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