The analytical possibilities of the first peak are adventageous and have been investigated using as supporting electrolyte a Britton-Robinson buffer (pH=8.0). In this medium norfloxacin yields only a well defined peak at -1.4 V (vs S.C.E.). The influence of the drop time, the pulse amplitude, temperature and concentration of norfloxacin in the polarographic response were studied. The polarographic process is irreversible and there in an adsorption process on the electrode surface.

Analytical applications. The analytical usefulness of polarography for the determination of norfloxacin was studied. Calibration show a good linearity between limiting current (DC) or peak (DP) and norfloxacin concentration in the following ranges:

$il(nA) = 3.8 + 9.7\ 10^5\ C(M)$, (r=0.9998) range $1.05\ 10^{-5}$ M to $3.93\ 10^{-4}$ M

$ip(nA) = -0.35 + 3.9\ 10^6\ C(M)$, (r=0.9998) range $1.94\ 10^{-6}$ M to $2.43\ 10^{-5}$ M

For the norfloxacin concentrations higher than $2.43\ 10^{-5}$ M, and when the DP technique is used, the graphs calibration is not linear. Detection limits of 0.9 µg/ml (DC) and 0.05µg/ml (DP) for both techniques were found. its relative standar deviations were ±1.6 % and ±0.8 % respectively. The polarographic method proposed was applied to direct determination of norfloxacin in pharmaceutical preparations and good results were obtained(Table1)

Table 1

Determination of norfloxacin in Tablets

Technique	norfloxacin mg/tablet	Content according to manufacturer's laboratory
DC	449 ± 25	400 mg/tablet
DP	375 ±21	

REFERENCES

1.-BURNIE, J.; Drugs of Today, 20, 391-5 (1984)

DESIGN AND DEVELOPMENT OF A FLOW THROUGH GLUCOSE REACTOR

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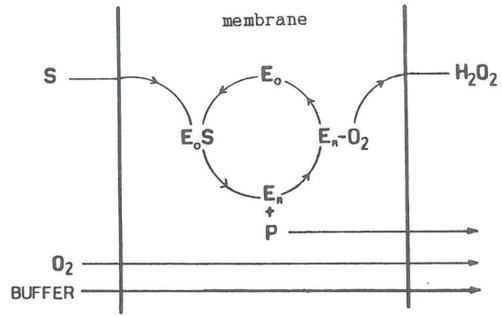
A flow-through mini-reactor containing immobilised glucose oxidase (GOD) was designed, developed and evaluated. The immobilisation of glucose oxidase directly on an electrode surface caused very high losses in activity [1]; immobilisation on a nylon support was much more satisfactory. A mini-reactor was developed, containing a nylon membrane with covalently immobilised GOD, and coupled to the inlet of a wall-jet cell electrochemical detector [2], where the hydrogen peroxide produced by the enzymatic reaction was detected.

The mechanism of reaction of GOD with glucose is of the double displacement type (ping-pong) [3]



where S - substrate; P - product, and E_O and E_R are oxidized and reduced forms of the enzyme respectively.

The active sites of the enzyme are the flavin molecules and the reaction can be summarized as follows:

- in the membrane

$$\text{Glucose} + \text{FAD} \rightarrow \text{Gluconolactone} + \text{FADH}_2$$

$$\text{FADH}_2 + \text{O}_2 \rightarrow \text{FAD} + \text{H}_2\text{O}_2$$

- at the platinum wall-jet electrode (E = 0.65V vs Ag/AgCl)

$$\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2e + 2\text{H}^+$$

We assume enzyme kinetics following the Michaelis-Menten theory [4] and that, since $i_L \alpha [H_2O_2]$, consequently $i_L \alpha$ [Glucose].

The enzyme GOD was covalently bound to discs of nylon type 66 material, using procedures corresponding to the following immobilisation sequence

nylon-G-*BSA*-G-HMDA-G-GOD

where G is glutaraldehyde, HMDA is hexamethylenediamine and *BSA* bovine serum albumin denatured by heat.

The enzyme glucose oxidase used was from *Aspergillus niger* (Type VII, E.C. 1.1.3.4, Activity 353,000 $u.g^{-1}$ with O_2 saturated reaction) from Sigma Chemical Company.

All other chemicals were commercially supplied and were analytical grade. Experiments were carried out in 0.1M acetate buffer at pH = 5.1 and were done at room temperature (t= 19-22°C). Glucose standard solution was prepared by dissolving α,D-glucose in acetate buffer, and left for 24h at room temperature before use in order to allow equilibration of the isomers.

The flow injection analysis was done according to the scheme of Fig.1 using a Valco (GCW - HC) titanium injector valve with a 15µl loop.

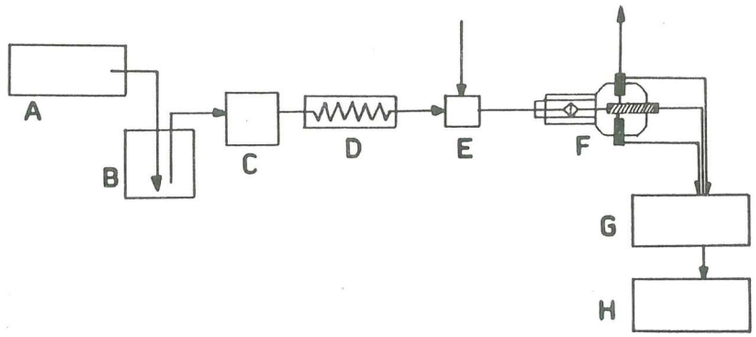


Fig.1. Flow injection analysis set up: A-oxygen cylinder: B-buffer solution; C-peristaltic pump; D-damping system; E-injector point; F-mini-reactor/wall-jet cell; G-potentiostat; H-XYt recorder.

The parameters of the mini-reactor were set by analysing the response to the injection of a standard peroxide solution under different flow rates and with different nozzle/electrode distances in the wall-jet cell.

Results for the flow injection of standard glucose solutions using the nylon membrane with immobilised GOD showed that there is maximum activity for flow rates of V_f = 1.0 $cm^3.min^{-1}$ (≃0.0167 $cm^3 \cdot s^{-1}$).

The flow-through glucose reactor was tested for determinations of glucose in human blood serum samples, after calibration with glucose standards in a region that includes the concentration of glucose in normal individuals. Fig.2 shows the results obtained.

The values of glucose concentration in the serum were verified using the bio-spectrophotometric (Sigma recommended assay procedure) and colorimetric (Nelson test - oxidation with ammonium phosphomolybdate) methods.

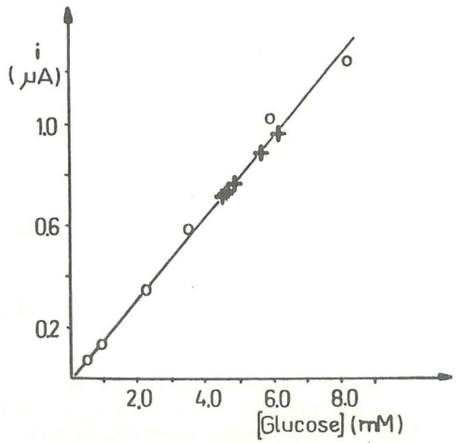


Fig.2. Calibration graph with glucose (O) and analysis of blood serum samples (X) with the bioelectrochemical sensor.

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.

References

1. Biosensors, ed. A. Turner, I. Karube and G.S. Wilson, Oxford Science Publications, 1987.
2. W.J. Albery and C.M.A. Brett, J.Electroanal.Chem., 1983,148,201-220
3. M.K. Weibel and H.J. Bricht, J.Biol.Chem., 1971,246,2734.
4. A. Lehninger, Biochemistry, Worth Publishers Inc., NY, 1975.

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

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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: