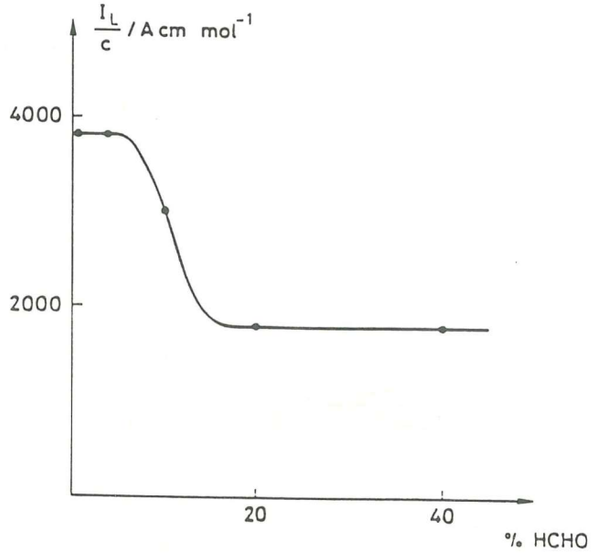

Figure 3. i_L/c as a function of % HCHO. Temperature 363K.

It can, thus, be concluded that, at high concentrations of formaldehyde the product is ethylene glycol and at low concentrations is methanol. These results confirm the necessity of using a high concentration of formaldehyde and demonstrate clearly the advantage of using an elevated temperature.

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ELECTROANALYTICAL DETERMINATION OF DIAZINON: DIFFERENTIAL PULSE POLAROGRAPHY AND ADSORPTIVE STRIPPING VOLTAMMETRY

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Diazinon, O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorotioate, is a non-systemic organophosphorated pesticide frequently used againts aphids and larvae.

Diazinon shows, a relatively long residual action and is effective as a control of soil, fruit, vegetable and rice insects, is also useful for the control of pest in household and on livestock.

The reported electroanalytical methods (1,2) for the determination of diazinon present a detection limits of 2.0 p.p.m., a high value for the determination of this pesticide in residues. In this work two methods are proposed for its determination by differential pulse polarography, DPP, (detection limit: 2.65 10^{-6} M) and by adsorptive stripping voltammetry, AdSV, (detection limit: 4.01 10^{-9} M).

Reagents and solutions. Stock solutions of pure diazinon were prepared by dissolving the compound in methanol. The solutions were stored in the dark to mininise the risk of decomposition. The supporting electrolytes were Britton-Robinson buffer 0.12 M and acetate buffer 0.10 M. Alls chemicals used were of analytical-reagent grade.

Apparatus. A Metrohm Polarecord E-506, equipped with an E-505 stand, was employed. In DC and DPP techniques, a three-electrode system was used, the working electrode being a dropping-mercury electrode (DME). The reference electrode was a saturated calomel electrode and the counter electrode was a platinum wire. Highly purified nitrogen was passed through the solution to remove dissolved oxigen. For the adsorptive stripping voltammetry study the working electrode was Metrohm EA-290 hanging mercury electrode.

Polarographic study. In aqueous-methanolic medium and with Britton-Robinson buffer as supporting electrolyte, the pesticide shows a reduction wave (DC) or peak (DPP) whose currents are function on the percentage of methanol (MeOH) and the time elapsed between preparation and measure of the solutions. At a MeOH percentage of 40 % (v/v) the polarographic current is stable for a minimum of 60 minutes.

The half-wave and peak potentials shift towards more negative values as the pH increases:

$$E_{1/2} = -(0.710 \pm 0.010) - (0.105 \pm 0.003)\ pH \qquad (r = 0.9987,\ n = 7)$$

$$E_p = -(0.705 \pm 0.019) - (0.116 \pm 0.004)\ pH \qquad (r = 0.9986,\ n = 7)$$

The variations of the limiting and peak currents with the pH show in the figure 1. At pH values besides 6.0 the wave is ill-defined and shows a minimum, which difficults its measure. In both polarographic techniques a decreasing of the currents as the pH increasing was observed. To carry out the electroanalytical determination of diazinon a medium HOAc 0.06 M/ NaOAc 0.04 M is recommended.

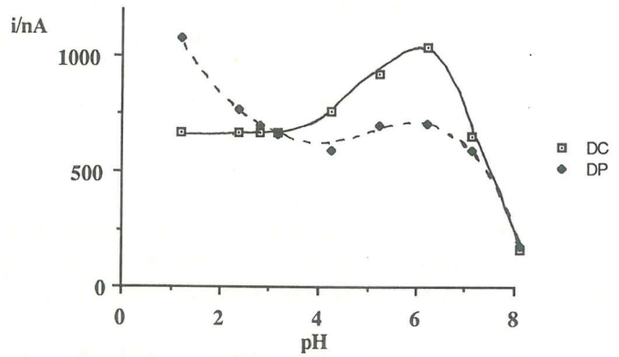

Figure 1. Limiting and peak currents of solutions of diazinon 2.70 10^{-4} M.

The influence of temperature (coefficient = 1.5 % $^{o}C^{-1}$) and the drop time on the wave seem to indicate a no diffusion controlled process. Furthermore, a adsorption pre-wave. which increases with the drop time and the concentration of pesticide, was observed.

It is possible determine diazinon by differential pulse polarography in 40 % (v/v) methanol-water medium with HOAc 0.06 M/NaOAc 0.04 M as supporting electrolyte, pulse amplitude 50 mV and scan rate of 4 mVs^{-1}.

In this conditions the results obtained fitting the following equation:

$$i_p\ (nA) = (2.18 \pm 0.02)\ 10^6\ [M] + (6 \pm 1) \qquad (r = 0.9999,\ n = 13)$$

with a linear response between 1.24 10^{-6} and 9.33 10^{-4} M, a detection limit (3s/m criterion) of 2.65 10^{-6} M (0.81 p.p.m.), with a precision of 2.90 %.

Adsorptive stripping voltammetry. In order to propose a more sensitive method for the determination of diazinon a study by adsorptive stripping voltammetry was carried out using a hanging mercury drop electrode (HMDE).

Spontaneous adsorption of diazinon on the surface of the dropping mercury electrode was observed by conventional and differential pulse polarography. This adsorption can be used as an effective pre-concentration step prior to voltammetric measurement; in this way, highly sensitive determination of the compound can be achieved by adsorptive stripping voltammetry.

In the procedure, after the preconcentration and rest steps, the adsorbate is measured in the redissolution process by means of its, DPP, faradaic signal. The electrode must be pretrated by setting it at -1.2 V potential for 2 min and before doing a new experience four drops of mercury should be discarded in order to avoid electrode memory effects. From the variation of the measured signal with the different studied variables (see e. g. Figure 2) the following working condictions are recommended: preconcentration at -0.4 V for 100 s, stirring the solution at 1400 rev min^{-1}, rest time of 20 s and a measure redissolution process scaning the potential between -0.8 and -1.2 V using the DPP technique.

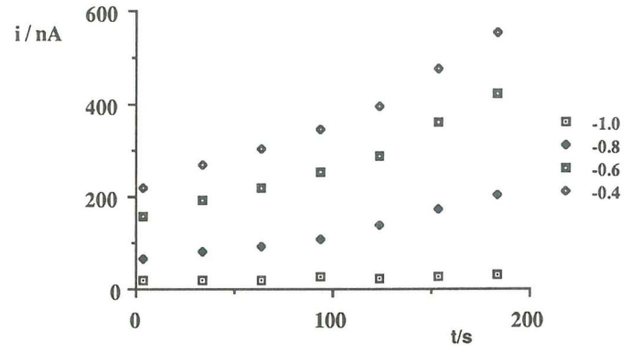

Figure 2. Variation of redissolution current with the preconcentration potential and accumulation time. [Diazinon] = 6.86 10^{-5} M.

In this conditions it is posible determine diazinon by adsorptive stripping voltammetry. The calibration data fitting the following equation:

$$i_p\,(nA) = (5.83 \pm 0.03)\ 10^6\ [M] + (0.5 \pm 0.1) \qquad (r = 0.9996,\ n = 26)$$

with a linear response between 1.16 10^{-8} and 2.93 10^{-4} M, a detection limit (3s/m criterion) of 4.01 10^{-9} M (1.3 p.p.b.), with a precision of 2.08 %.

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QUANTIFICATION OF METALLOTHIONEINS IN MARINE INVERTEBRATES USING DIFFERENTIAL PULSE POLAROGRAPHY

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INTRODUCTION

Metallothioneins (MT) are cysteine rich (~30%), low molecular weight proteins which form a complex with heavy metals such as cadmium, copper, mercury and zinc. Functions attributed to MT include detoxification, storage and regulation of metals. Their induction may signify exposure to excessive concentrations of metal ions in cells. Consequently, the potential value of these relatively specific biochemical indicators of metal contamination would seem to be obvious. To date however, the full value of MT as a monitoring tool has rarely been demonstrated, partly due to difficulties in determining protein concentrations.

The object of the present study was to design and evaluate a sensitive protocol for quantifying MT in a variety of marine invertebrates, using differential pulse polarography.

MATERIAL AND METHODS

A differential pulse polarographic assay for MT was accomplished using a PARC Model 174A analyser, a PARC/EG&G Model 303 static mercury drop electrode (SMDE) and a flat-bed X-Y recorder. Capillary electrodes were cleaned in acid and silanized.

The Brdicka supporting electrolyte was prepared according to and Imber & Thompson (1) and contained 1.0 M NH_4Cl, 1.0 M NH_4OH and 2.0 mM of $[Co(NH_3)_6]Cl_3$. The electrolyte was prepared weekly and stored at 4 °C when not in use.

Triton X-100 (SIGMA) (2.5×10^{-2} % (v/v)) was used to suppress secondary maxima and minima and to eliminate baseline noise.

Ten milliliters of electrolyte were dispensed directly to the cell, together with 100 µl of Triton X-100 and 25 - 250 µl aliquots of standard/sample. The cell was then purged for 2 minutes with purified N_2 prior to analysis. Scanning was from -1.4 V to -1.6 V at 2mV/s using a

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