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 x 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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$Ag^+/AgCl$ reference electrode. Modulation amplitude was 50 mV and drop time 1s.

Preparation of MT-containing, cytosolic extracts of marine organisms, collected at sites in Portugal and the United Kingdom, been described previously (2). Extracts were heated at 80 °C (10 min) to remove interfering proteins.

Quantification of MT, based on rabbit liver metallothionein MT-I (working standard 10 mg/l in distilled water), was accomplished by standard addition method.

RESULTS AND DISCUSSION

The basis for the determination of thiolic proteins (including MT), in which complexation with cobalt(II) ions plays a decisive role, lies in the linear relationship between the concentration of protein and the second of the two waves, designated 'A' and 'B', following the cobalt reduction wave (3, 4, 5). A further condition necessary is the presence of sulphydryl or dissulphidic groups in the protein molecule.

A typical polarographic scan of a sample of *Ruditapes (=Venerupis) decussatus*, showing the two separate protein waves following the cobalt reduction wave, is shown in fig. 1.

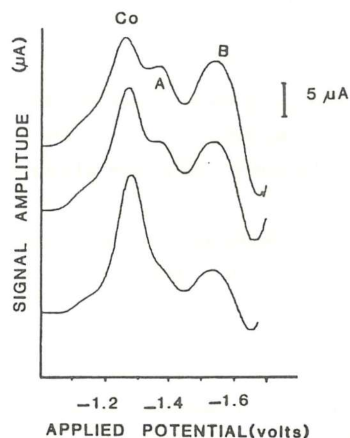

Fig. 1. Differential pulse polarograms of the Brdicka electrolyte containing 50, 100, 150 μl of cytosol of *R. decussatus*; obtained using static mercury drop electrode. Co, 'A' and 'B' denotes the reduction of the cobalt (Co) and the protein (A, B), respectively.

A MT calibration curve (fig. 2) generated at 20 °C, was linear over the range 5 μg/l to 300 μg/l ($r=0.9985$, $P< 0.001$); this is equivalent to 0.77 nM to 46.15 nM, based on an average molecular weight for MT of 6500 (6). Data points represent the means of three or more replicate determinations. The detection limit of 5 μg/l obtained in the present study (at 5 μA full scale) represents some improvement over that previously reported by Thompson & Cosson (5) and could be enhanced further by carrying out analysis at lower temperature.

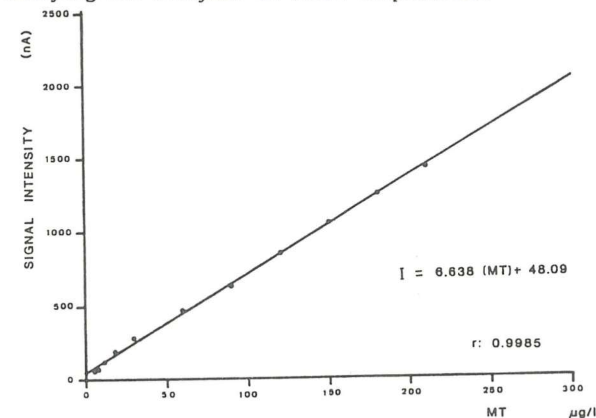

Fig. 2. Metallothionein (MT) calibration (Signal intensity, I, against concentration of Rabbit liver MT) using static mercury drop electrode, at room temperature. Linear regression equation and coefficient are indicated.

Sensitivity is clearly one of the advantages of this technique. Using the conditions described here, only a few microlitres of cytosol are needed for MT assay.

Levels of MT-like proteins in a variety of marine molluscs, collected at sites in Portugal and the United Kingdom, are shown in Table I. Values range from 1.69 - 21.3 mg/g (dry weight), depending on the species and tissue analysed.

Comparison of the pulse polarographic determination of MT with other commonly used methods (7) shows that its high specificity represents the most sensitive physicochemical method for direct quantification, currently available. In our view this method will be an important asset for clarifying the primary function of metallothionein-like proteins, and in determining responses in relation to heavy metal contamination in the marine environment.

TABLE I - Metallothionein levels in different marine invertebrates

Species	Size mm	Tissue	MT mg/g*	Site
Cerastoderma edule	30	Whole animal	4.55	Ria Formosa, Portugal
Donax vitatus	32	Whole animal	6.37	"
Littorina littorea	20	Digestive gland	11.93	Plymouth, U.K.
	20	Remaining tissues	2.55	"
Littorina saxatilis	13	Digestive gland	15.45	Minehead, U.K.
Mytilus edulis	58	Whole animal	2.43	Whitsand bay, U.K.
	70	Digestive gland	8.04	"
Nucella lapillus	26	Digestive gland	5.29	Plymouth, U.K.
	26	Remaining tissues	1.95	"
Patella vulgata	35	Digestive gland	21.30	"
	35	Remaining tissues	1.69	"
Ruditapes decussatus	20	Whole animal	4.29	Ria Formosa, Portugal
	37	Whole animal	6.34	"

* dry-weight

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