

# Development of Batch Injection Analysis for Electrochemical Measurements of Trace Metal Ions in Ecotoxicological Test Media

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Batch injection analysis with square-wave anodic stripping voltammetry has been developed as a powerful and rapid technique for obtaining data on the concentration of the fraction of labile metal ions present in media used in ecotoxicological tests. Microlitre samples of solution, without pretreatment, are injected directly over a detector electrode, the surface of which is protected by a thin Nafion polymer coating against irreversible adsorption by organic components. Examples are given showing the effect of adding vitamins and organic extract, singly and together, to the ASTM medium employed for tests using *Daphnia magna* and with lead and cadmium test ions. Such a methodology can be extended to other electroactive species present in these and similar media. Copyright © 2000 John Wiley & Sons, Ltd.

## INTRODUCTION

Traces of heavy metal ions, resulting principally from untreated or only partially treated industrial effluents, are an important pollution and toxicity problem that affects the whole surrounding ecosystem. In natural waters, metal ions occur as various chemical species. These include free hydrated metal ions, soluble inorganic complexes, soluble organic complexes, metallic species in the form of colloid dispersions and those adsorbed on the surface of other colloidal particles or on inert suspended material.<sup>1</sup>

The toxicity of lead, cadmium and other metals is referred to as being dependent on the free ion activity and not on the total metal concentration.<sup>2</sup> Nevertheless, concerning organisms in fresh water, the activity of the free ion is insufficient to explain the biological response.<sup>3</sup> In algae, for example, which exhibit a net negative charge, there is competition for the site of metal ion binding by protons and by the non-toxic cations that give rise to water hardness, Ca<sup>2+</sup> and Mg<sup>2+</sup>,<sup>2</sup> which can reduce the toxicity of other metal ions.

The influence of sublethal concentrations of lead and cadmium on the growth and fertility of *Daphnia magna*, a model organism used in ecotoxicological tests, has been determined in chronic tests with ASTM 'hard water' medium. Vitamins are added to the medium as well as an organic extract, to supply micronutrients, at the beginning of the test.<sup>4</sup> The alga *Selenastrum capricornutum* is also added as food for *Daphnia magna*.

The objective of this work was to develop a method for the rapid measurement of the amount of labile metal ion in the media used for the tests with *Daphnia magna*. This is only possible using electrochemical methodology. Other requirements include the necessity of no sample pretreatment (so as not to alter the complexation equilibria), low analyte volume and furnishing a rapid response at the required submicromolar concentration levels. The technique chosen was square-wave anodic stripping voltammetry in combination with a special recently developed injection technique known as batch injection analysis (BIA),<sup>5</sup> in which as-collected analyte samples of typical volume 50 µl are injected directly over a detector electrode immersed in electrolyte solution. A full description of the system and its evaluation have been given elsewhere.<sup>5–7</sup> Despite the low analyte volumes, special polymer coating protection of the electrode surface to avoid fouling from components of the organic matrices is necessary, which was achieved by using a coating of the cation-exchange polymer Nafion.<sup>8</sup> This is a perfluoropolymer with negatively charged sulphonate groups at the ends of the chains, thus discriminating against species without positive charge. In order to achieve good results at the values of applied potential required, it was necessary to use a mercury thin-film electrode on a glassy carbon substrate, this being covered with a Nafion film, as described in the Experimental section. This type of electrode coating has also been used to good effect with samples of effluent.<sup>9</sup>

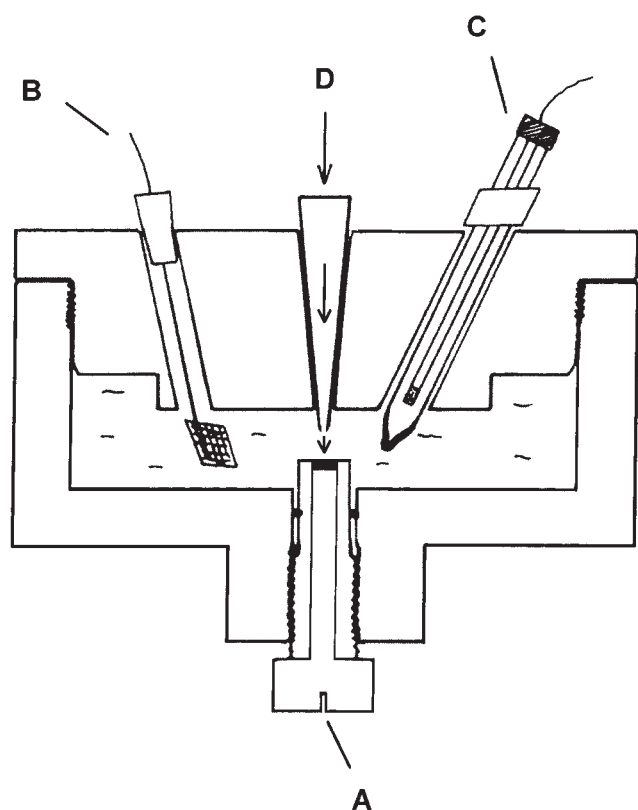
Low detection limits, down to 5 nmol l<sup>-1</sup>, can be reached by combining BIA with square-wave anodic stripping voltammetry (SWASV); see Ref. 7 for details of the procedure. Square-wave anodic stripping voltammetry is a preconcentration technique consisting of two steps: preconcentration and determination. In the preconcentration step the species of interest is accumulated on the electrode over a period of time by metal

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ion reduction to the zero oxidation state with a sufficiently negative applied potential. This is done during injection and for 30 s following injection in order to maximize the fraction of species in the zone of the electrode surface that is deposited. In the determination step oxidation of the accumulated species is forced to occur by applying a rapid square-wave voltammetric positive-going scan. Current peaks are observed, the height of which is proportional to the analyte concentration, their position on the potential scale identifying the metal ion. Advantages of this square-wave scan relative to other possible types of potential scan are that oxygen does not have to be removed from the solutions and that the system response is faster. The whole BIA-SWASV experiment can be carried out in a time interval of <1 min.

## EXPERIMENTAL

The electrochemical cell employed for BIA<sup>5</sup> is shown in Fig. 1. It is constructed of Perspex and contains ca. 40 cm<sup>3</sup> of 0.1 M KNO<sub>3</sub>-5 mM HNO<sub>3</sub> electrolyte. The detector electrode is of glassy carbon, diameter 5 mm, and the three-electrode arrangement also includes a platinum gauze counter-electrode and a saturated calomel electrode (SCE) as reference. The tip of a micropipette, internal diameter 0.47 mm, is fixed directly over the centre of the detector electrode at a



**Figure 1.** The batch injection analysis cell constructed of Perspex, diameter ca. 12 cm, capacity ca. 40 cm<sup>3</sup> of electrolyte: (A) detector disc electrode (glassy carbon) in Kel-F sheath; (B) Pt gauze auxiliary electrode; (C) saturated calomel reference electrode; (D) micropipette tip through which solution is injected.

**Table 1.** Chemical composition, pH and hardness of ASTM medium

Chemical compound (analytical grade)	Quantity (mg l <sup>-1</sup> )
NaHCO <sub>3</sub>	192
MgSO <sub>4</sub> ·7H <sub>2</sub> O	246
KCl	8
CaSO <sub>4</sub> ·2H <sub>2</sub> O	120
pH: 7.6–8.0	
Hardness (mg l <sup>-1</sup> CaCO <sub>3</sub> ): 160–180	

distance of ca. 2–3 mm, and injections are done from a programmable, motorized, electronic micropipette (EDP Plus ED-100, Rainin Instrument Co. Inc.). This permits three injection flow rates and injection volumes of 10–100 µl. Unless stated otherwise, in this work volumes of 50 µl of analyte were injected at the lowest of the three possible flow rates of 24.5 µl s<sup>-1</sup>.

Nafion films on the glassy carbon electrode were prepared in the following way.<sup>8,9</sup> A 5-µl aliquot of a 0.25% solution of Nafion in ethanol (diluted from a 5 wt.% Nafion solution in alcohol and water, Sigma) was applied over the electrode surface, followed immediately by 3 µl of *N, N'*-dimethylformamide casting solvent. The solvents were evaporated in a cool air stream while rotating the electrode slowly at 50 rpm for ca. 30 s, followed by curing at a temperature of ca. 70°C in a warm air stream for another 1 min. This led to films of thickness of ca. 1 µm.

Mercury films were prepared by electrodeposition through the Nafion coating within the BIA cell. For this purpose, a volume of 10 µl of a 0.10 M solution of Hg<sup>2+</sup> in 0.1 M KNO<sub>3</sub>-5 mM HNO<sub>3</sub> was injected directly over the electrode at an applied potential of -1.0 V, this potential being applied for a total of 60 s.

The BIA-SWASV experiments, as well as the preparation of mercury thin films, were carried out using a computer-controlled CV-50W voltammetric analyser (Bioanalytical Systems). In SWASV experiments, the square-wave parameters were: frequency 100 Hz, potential increment 2 mV and square-wave amplitude 25 mV. Metal ions were deposited by injection of 50 µl sample at 24.5 µl s<sup>-1</sup> dispensation rate using an applied potential of -1.0 V vs SCE for 30 s, with preconcentration continuing after the end of injection for a total of 30 s to maximize electrolysis efficiency.<sup>6</sup>

The composition of the ASTM medium is shown in Table 1. The composition and final concentration of vitamins after addition to ASTM medium are given in Table 2. The organic extract added to the ASTM

**Table 2.** Vitamins added to ASTM culture medium: final concentrations

Vitamin		Quantity (mg l <sup>-1</sup> )
Thiamine HCl	B1	0.075
Cyanocobalamine	B12	0.001
Biotin	H	0.0008

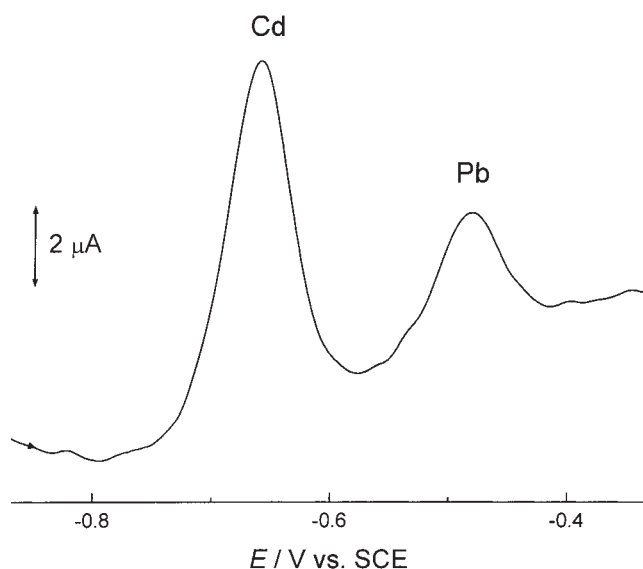
medium ( $4 \text{ ml l}^{-1}$ ) is a filtered solution of the powder extract of the marine alga *Ascophyllum nodosum* 'Marinure 25', the average composition of which is shown in Table 3.<sup>10</sup> The concentration of the extract solution is adjusted until the absorbance of a solution diluted by a factor of 10 is 0.62 for a 1 cm path length at a wavelength of 440 nm.

## RESULTS AND DISCUSSION

Results will be presented from BIA-SWASV experiments for measurement of trace metal ions in order to show how the technique developed can be applied usefully to culture media employed in ecotoxicological tests. The combination of a mercury thin-film electrode, which has a large overpotential for the evolution of hydrogen, and a  $1 \mu\text{m}$  thickness Nafion coating allows the cations to pass through while excluding neutral and anionic species.

A typical voltammetric trace for analysis of cadmium and lead ions is shown in Fig. 2. This illustrates that mixtures of both ions can be determined simultaneously because the potentials for redissolution are well separated at  $-0.66 \text{ V}$  for cadmium and  $-0.48 \text{ V}$  vs SCE for lead. Preconcentration was carried out by deposition at an applied potential of  $-1.0 \text{ V}$  vs SCE. Calibration curves can be constructed from standards before analysing the unknown sample, or spiking the solution using the standard addition method can be employed. The detection limits of the order of  $5 \text{ nmol l}^{-1}$  obtainable with this injection technique<sup>8</sup> correspond to  $0.56 \mu\text{g l}^{-1} \text{ Cd}^{2+}$  and  $1.04 \mu\text{g l}^{-1} \text{ Pb}^{2+}$ .

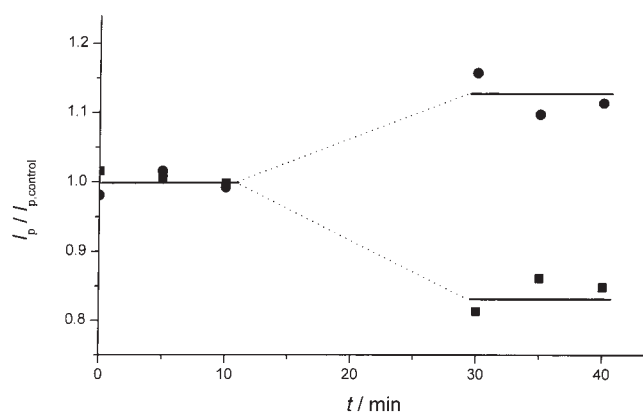
The exclusion properties of Nafion films can be particularly useful for situations in which the sample matrix is complex, as is the case with culture media. This can lead to strong adsorption, even when the sample volume is of the order of microlitres. Thus, the results below are designed to show the potentialities of this technique for the direct and rapid analysis of



**Figure 2.** Typical voltammetric trace for BIA-SWASV following injection of  $50 \mu\text{l}$  at  $24.5 \mu\text{l s}^{-1}$  of a solution containing  $10^{-7} \text{ M Cd}^{2+}$  and  $10^{-7} \text{ M Pb}^{2+}$  into  $0.1 \text{ M KNO}_3$ - $5 \text{ mM HNO}_3$  electrolyte. Deposition time  $30 \text{ s}$ . Square-wave parameters: amplitude  $25 \text{ mV}$ , frequency  $50 \text{ Hz}$  and scan increment  $2 \text{ mV}$ .

culture media, through showing the influence of the various components on the voltammetric response due to the labile fraction of metal ion in the injected sample.

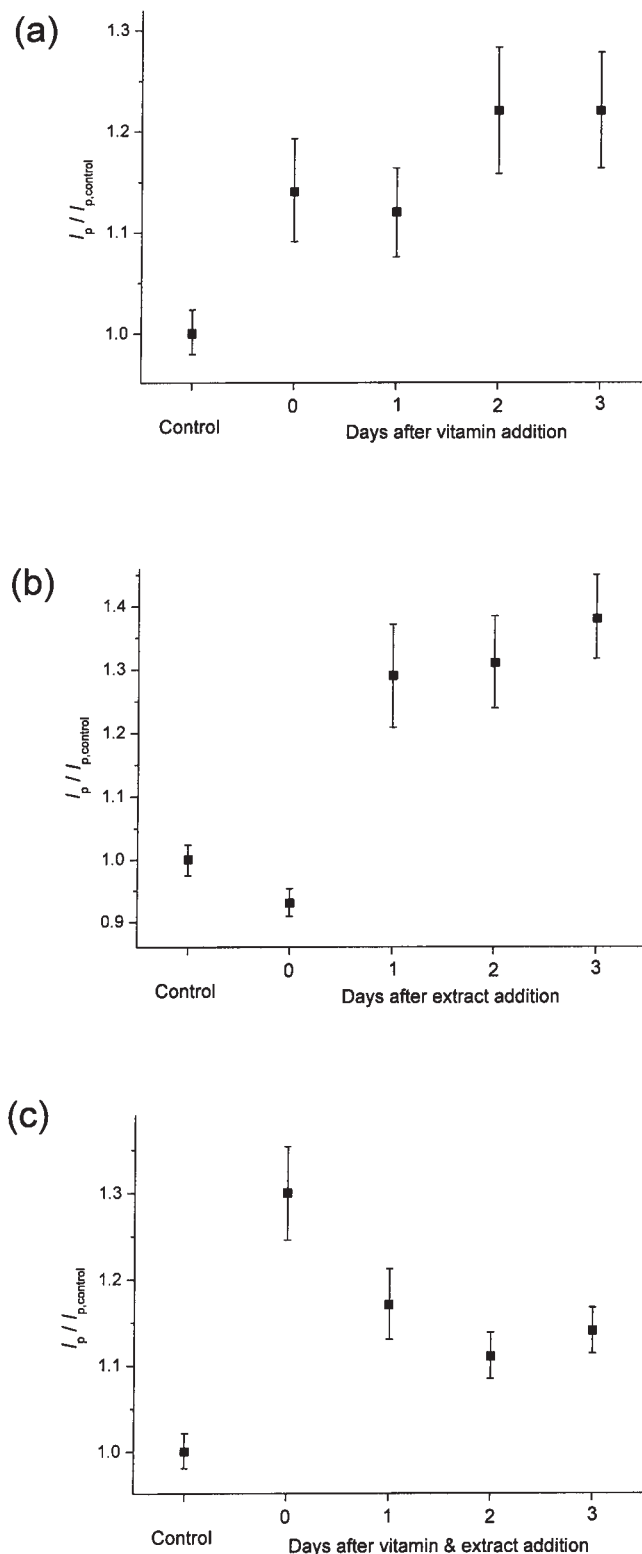
In Fig. 3 are shown the influence of vitamins and of organic extract added to ASTM medium on the stripping peak currents of lead ions. These experiments were conducted at lead concentration levels of  $150 \mu\text{g l}^{-1}$ , similar to those used in chronic tests. Three injections of sample were performed at ca.  $5 \text{ min}$  intervals. After addition of the additive, another three determinations were made, usually beginning  $30 \text{ min}$  after commencement of the series of tests. Between sample injections, injections of blank electrolyte served to clean the Nafion surface of adsorbed matter and the response, which otherwise decreases with time (not shown), returns to the initial level. It was found necessary, in general, to make six blank injection to be completely certain that the electrode surface is clean.



**Figure 3.** Influence of vitamins and organic extract on the normalized BIA-SWASV stripping peak currents,  $I_p$ , of  $\text{Pb}^{2+}$  ( $150 \mu\text{g l}^{-1}$ ) in ASTM medium. Six injections of sample, three before (control) and three after addition of (●) vitamins and (■) organic extract. Other conditions as in Fig. 2.

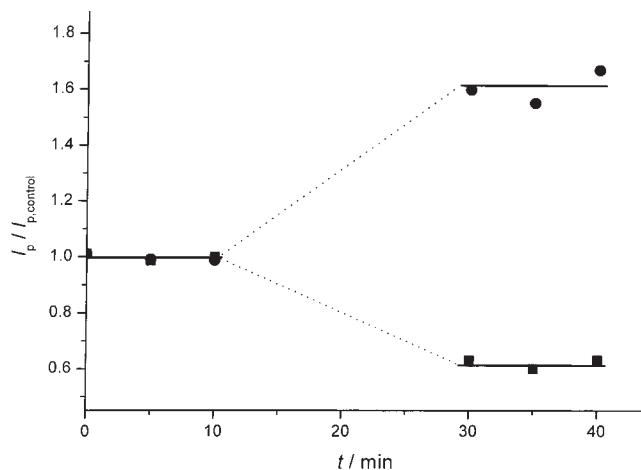
**Table 3.** Average composition of the powder additive 'Marinure 25', extract of *Ascophyllum nodosum* (Pann Britannica Industries Ltd., Waltham Abbey, UK)

Component	Quantity (%)	Component	Quantity ( $\text{mg l}^{-1}$ )
Dry residue	92-95	Aluminium	5.01
Organic	50-55	Boron	82
Inorganic	40-45	Cobalt	1.6
		Copper	5.0
Nitrogen	1.4	Iron	3000
Phosphorus	0.05	Iodine	1800
Potassium	2.5	Manganese	12.0
Calcium	1.2	Nickel	5.0
Magnesium	0.8	Vanadium	0.7
Sulphur	3.7	Zinc	100.0
Chlorine	4.0	Citochinines and other growth hormones	160-260



**Figure 4.** Influence of solution composition on the stripping peak currents of  $\text{Pb}^{2+}$  ( $150 \mu\text{g l}^{-1}$ ) in ASTM medium over a period of 3 days after addition of additives: (a) vitamins, (b) organic extract, (c) vitamins plus organic extract. Control is without additives.

As can be seen, the effect of addition of vitamins is to increase the peak currents obtained whereas that of organic extract is to decrease them. A decrease signifies blocking of the pores in the Nafion coating, whereas an increase suggests the formation of a weak



**Figure 5.** Influence of vitamins and organic extract on the normalized BIA-SWASV stripping peak currents,  $I_p$ , of  $\text{Cd}^{2+}$  ( $5 \mu\text{g l}^{-1}$ ) in ASTM medium. Six injections of sample, three before (control) and three after addition of (●) vitamins and (■) organic extract. Other conditions as in Fig. 2.

complex that aids in concentrating the metal ion in the vicinity of the electrode surface under the influence of the applied potential before it is transported through as the free cation to the mercury below. Thus, this BIA technique can also be used as an indirect probe of the formation of such weak complexes. In this context, the fact that samples are not pretreated before injection, which could alter the natural chemical equilibria, is extremely useful.

Important complementary information can be obtained from examination of the time evolution of the medium composition. Figure 4 shows how the concentration of labile cation varies over a period of 3 days in medium containing vitamins, organic extract or the two together, relative to ASTM medium without additives. With regard to vitamins (Fig. 4a), there is an increase in stripping peak current that increases further up to 2 days after vitamin addition. This can be explained through the formation of weakly bound complexes with the lead ions, most of which are formed rapidly and have a tendency to be adsorbed on the Nafion surface. The applied potential in the preconcentration step is sufficiently negative to remove the lead ions from the complexes that migrate through the Nafion coating to the mercury film underneath. The result is an enhancement of the fraction of injected lead ions reaching the mercury electrode during the preconcentration step, and thence a higher stripping peak current.

In relation to organic extract (Fig. 4b), there is a decrease in stripping peak current immediately after its addition, as seen in Fig. 3; however, after 24 h the signal has increased and remains relatively stable over the following days. This suggests that there has been some initial electrode blocking and then the slow formation of weak complexes between lead ions and components of the organic extract, in a similar way as for the vitamins.

When both vitamins and organic extract are added together (Fig. 4c), there is an initial large increase of stripping signal, which becomes less after 1 day. If there is competition between the various cation binding

sites, mentioned above, the effect being to concentrate the metal ions near the electrode surface, then it is probable that the complex that is formed faster will have most effect in the initial period, i.e. with vitamins. After this, competition between binding sites deriving from the two types of additive will occur and so there is a lesser increased stripping peak current relative to the value in the absence of additives.

Some results obtained for cadmium ions are shown in Fig. 5. Because these ions are much more toxic than lead ions, a concentration of  $5 \mu\text{g l}^{-1}$  was employed, similar to that in chronic tests with *Daphnia magna*.<sup>11</sup> It can be seen that there is an analogous influence of vitamin and organic extract addition, as in the case of lead ions, suggesting that the interaction between the cation and the organic components is similar.

A full explanation of the complexation phenomena will require more detailed experiments with varying concentrations of vitamins and organic extract. It has been demonstrated that the BIA technique is an excellent tool for such a purpose.

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## CONCLUSIONS

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It has been shown that batch injection analysis with square-wave anodic stripping voltammetry is a convenient and practical way to measure the labile fraction of metal ions in ecotoxicological test media, without the addition of any electrolyte to the samples, which could alter the chemical equilibria. Such complex solution matrices can be analyzed owing to the small volume injected and the protection of the electrode surface with a Nafion film. The time evolution of formation of complexes can be monitored with minimum alteration of solution composition. Applications to determinations during the course of ecotoxicological tests are anticipated.

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