FULL PAPER
THE ANALYST
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Determination of mercury(II) by invertase enzyme inhibition coupled with batch injection analysis

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Received 22nd March 2002, Accepted 20th June 2002
First published as an Advance Article on the web 16th July 2002

The determination of mercury(II) ions at the trace level by inhibition of the invertase enzyme-catalysed hydrolysis of sucrose into glucose and fructose coupled to electrochemical batch injection analysis was investigated using two approaches. In the first, the glucose produced was detected by injection of 100 μl samples into the batch injection cell containing a platinum electrode modified by immobilised glucose oxidase. In the second, the glucose and fructose present in injected samples were oxidised directly at a copper-modified glassy carbon electrode. The experimental parameters were optimised and the degree of enzyme inhibition by mercury(II) ions under both conditions was measured. Mercury concentrations in the ng ml^{-1} range were determined by these two techniques with low sample and reagent consumption. Comparison is made between the two methods and perspectives as a screening test for field application are indicated.

Introduction

The determination of traces of heavy metals in biological material, natural waters, soil and air has become very important since the environment is especially vulnerable to this type of pollutant.[1] Heavy metals are accumulated and stored in living organisms. Among them, the influence of mercury on the environment is particularly serious owing to its strong toxicity and the high level of its use in industrial processes. The toxicity of mercury and its compounds is attributed to their harmful effect on the central nervous system, disturbing haem synthesis and causing neuropsychiatric disorders.[2]

Heavy metals are well known to inhibit the activity of enzymes and application of this phenomenon to the determination of hazardous toxic elements offers several advantages.[3–5] First, the detectors used in the method of enzyme inhibition can be very sensitive because the reduction of enzyme activity by single inhibitor molecules can be large owing to an amplification effect. The effect of heavy metals seems to involve the thiol groups of proteins.[6] Moreover, enzymes are often specific to the inhibitor, such as mercury ions.[7,8] In this way, the inhibition of enzymatic activity by mercury may offer a good choice as a simple, selective and sensitive screen test, by performing inhibition tests with the enzyme invertase dissolved in mercury ion-containing solution.

The determination of mercury is usually performed with techniques such as cold vapour atomic fluorescence spectrometry (CVAFS),[9] cold vapour atomic absorption spectrometry (CVAAS),[10] inductively coupled plasma atomic emission spectrometry (ICP-AES)[11] and inductively coupled plasma mass spectrometry (ICP-MS),[12] which require sample pretreatment, expensive instrumentation and skilled operators. These spectroscopic techniques, commonly used for trace measurement of mercury in the laboratory, are not suitable for the task of on-site testing and monitoring. It is highly desirable to be able to carry out these measurements in the field. Recently, a flow injection system with a gold-plated piezoelectric quartz crystal[13] was successfully used as a rapid laboratory screening method for total trace mercury monitoring in natural waters at sub-micrograms per litre levels.

The portable nature and excellent sensitivity of electrochemical techniques make them very attractive for field monitoring of trace metals,[14] especially using anodic stripping voltammetry. They are sensitive to the labile fraction of ionic mercury. The best electroanalytical performance for measuring low concentrations of mercury ions has been achieved with gold[15] and gold-plated[16] electrodes. The main disadvantages of gold electrodes are the well-known structural changes of the gold surface, caused by amalgam formation, and the time-consuming and complex electrochemical pre-treatments that are needed to achieve reproducibility. Various types of carbon electrode have also been employed. Of particular note is the addition of thiocyanate ions to the samples using a glassy carbon rotating disk electrode which led to a detection limit of 50 fmol l^{-1},[17] and the use of boron-doped diamond to minimise the contribution of background currents.[18]

The development of an electrochemical biosensor based on invertase and glucose oxidase enzymes has been reported[19] with the enzyme invertase dissolved in mercury ion-containing solution. Invertase forms the ideal enzyme for studies of inhibition owing to its low cost, good stability and high specific activity. Glucose, produced through catalysis of sucrose by the soluble enzyme invertase, reacts with immobilised glucose oxidase (GOx) and produces hydrogen peroxide. The activity of invertase in the presence of mercury decreases, causing a lowering of glucose production, which is monitored by the glucose sensor and correlated with the concentration of mercury in solution.

In another approach, an electrochemical sensor with a copper-based chemically modified electrode (CME) was described.[20] Oxidation processes at copper-based electrodes in strongly alkaline solution have received considerable attention because of their application to the detection of carbohydrates and related compounds in liquid chromatography and capillary electrophoresis.[21–24] Copper electrodes have been used at constant potential without any electrode fouling.[20,25] A glassy

 DOI: 10.1039/b202887e

carbon electrode was employed on which $CuCl_2$ was deposited, leading to adsorbed Cu(II).[25] The attractiveness of this approach stems from the fact that carbohydrates are not generally electroactive at the carbon electrodes commonly used for detection in flow systems.

The measurement of electroactive species using the batch injection analysis (BIA) technique[26–29] is particularly attractive for monitoring environmental pollution problems. A volume of 10–100 μl of sample is injected directly over the centre of an electrode immersed in electrolyte held at an appropriate applied potential. At common dispension rates of 25–75 μl s^{-1}, it takes 1.3–4 s to dispense 100 μl. Measurement of the current response for oxidation or reduction, which reaches a maximum value during the injection and then tails off afterwards, is directly related to the concentration of species. This technique has been applied to various types of electroactive species, including trace heavy metal ions at the nanomolar level by square-wave anodic stripping voltammetry.[30] In this case, the injected ions are preconcentrated on a mercury thin-film electrode surface by applying a potential in order to reduce the metal ion to the metal itself during injection and during an extra 30 s following injection. Furthermore, by protecting the electrode surface with ion-exchange polymers, it is possible to make measurements in complex matrices, such as effluents and nutrient solutions, without electrode blocking and without the necessity for sample pre-treatment.[30–32]

In this work, mercury ions were measured by invertase enzyme inhibition using the BIA technique at two different types of modified electrode: platinum modified with glucose oxidase and glassy carbon modified with copper. The effect of mercury ions on the reduction of invertase activity was studied and the various parameters optimised. The linear range and the detection limit were also determined. Additionally, interference studies and analysis of real samples analysis were carried out.

Experimental

Apparatus and equipment

For experiments with GOx-modified platinum electrodes, amperometric measurements were made with an Autolab PGSTAT30 potentiostat (Ecochemie, The Netherlands) controlled by GPES 4.8 software. A large open wall-jet cell modified for batch injection analysis was employed, as described previously.[29] The cell, of volume 40 cm^3, was filled with inert electrolyte. The working electrode was a 3 mm diameter platinum disc, previously polished with alumina down to 0.05 μm particle size and rinsed thoroughly with distilled water, the reference electrode was a saturated calomel electrode (SCE) and the auxiliary electrode was platinum gauze. Samples were injected from a Rainin EDP-Plus 100 motorised programmable electronic micropipette, which permits injection of volumes between 10 and 100 μl, at calibrated flow rates of 24.5, 47.6 and 75.3 μl s^{-1}. The tip internal diameter was 0.47 mm and the end of the tip was placed 2–3 mm above the centre of the working electrode.

For experiments involving Cu-modified electrodes, amperometric measurements were made with a CV27 voltammograph (Bioanalytical Systems). The working electrode was a 3 mm diameter glassy carbon disc, polished with alumina down to 0.05 μm particle size and rinsed thoroughly with distilled water, the reference electrode was an Ag/AgCl (3 M KCl) electrode and the auxiliary electrode was a stainless-steel rod.

Reagents

The lyophilised enzymes glucose oxidase (from *Aspergillus niger*, 40.30 units mg^{-1}), invertase (from baker's yeast, 500 units mg^{-1}) and glutaraldehyde (25%) were obtained from Sigma. All other reagents were of analytical-reagent grade and solutions were prepared with Milli-Q-purified water (conductivity < 0.1 μS cm). Phosphate buffer (Na_2HPO_4–KH_2PO_4), ionic strength 0.1, pH 6.0 or sodium hydroxide solution were used as electrolytes. A stock standard solution of 1.0 M sucrose was prepared weekly in 0.01 M NaOH to avoid any spontaneous hydrolysis.

Enzyme immobilisation

Enzyme immobilisation was carried out, at room temperature, by mixing 10 μl of the appropriate buffer containing glucose oxidase (60 units of enzyme) and bovine serum albumin (35 mg per 100 μl) with 5 μl of 2.5% glutaraldehyde. The mixture was gently mixed for several seconds, then 7 μl were placed on the polished platinum electrode and allowed to dry in air at room temperature. The resulting electrode was yellow in colour. After 15 min the electrode was washed with a large volume of phosphate buffer. Enzyme-modified electrodes prepared in this way remained active for more than 1 month, when stored at 4 °C.

Copper-based electrode

The modified glassy carbon electrode was prepared by first placing the electrode in 0.1 M NaOH solution for about 10 min. The electrode was then rinsed thoroughly with water and immersed in 0.05 M $CuSO_4$ solution for 15 min. After removal and rinsing with distilled water, the electrode was ready for use.

Procedure at the GOx-modified platinum electrode

All measurements were performed at a constant applied potential of +0.65 V in order to oxidise the hydrogen peroxide produced by the enzyme reaction. A volume of 100 μl of sample solution containing invertase and sucrose, following a chosen incubation time, was injected on to the platinum electrode in the batch injection cell, which was filled with phosphate buffer. The reaction during incubation is

$$\text{sucrose} + H_2O \xrightarrow{\text{invertase}} \text{D-glucose} + \text{D-fructose}$$

and after injection in the BIA cell

$$\text{D-glucose} + O_2 \xrightarrow{\text{GOx}} \text{gluconic acid} + H_2O_2$$

at the platinum surface. The H_2O_2 produced is oxidised and the current is a linear function of the concentration of sucrose and invertase present in solution.

Experiments on mercury inhibition were conducted in two steps. In the first, a solution was prepared containing 1 μg ml^{-1} invertase and 20 mM sucrose. After a 20 min reaction time, a sample was injected into the BIA cell. The glucose produced by the enzymatic reaction was measured as the corresponding peak current for H_2O_2 oxidation. The current due to the slow, spontaneous hydrolysis of sucrose to glucose and fructose (blank) was also measured in an independent injection experiment and subtracted from the value of the peak current for H_2O_2 oxidation. The resulting peak current is designated I_1.

In the second step, a solution containing the same concentration of invertase as before was spiked with mercury ions and incubated for 10 min. Sucrose was then added and after a 20 min reaction time a sample was injected as above and the peak current, after blank subtraction, was designated I_2. This current is smaller than I_1 as a consequence of the reduction in the enzyme activity after its inhibition by mercury ions.

The degree of inhibition can be calculated from the enzyme activity in the presence and in the absence of mercury ions according to

$$I\ (\%) = 100(I_1 - I_2)/I_1$$

The optimised procedure is shown in Scheme 1.

Procedure at the copper-modified glassy carbon electrode

At the Cu-modified electrode, all measurements were performed at a constant applied potential of +0.35 V *vs.* Ag/AgCl in 0.1 M NaOH alkaline solution. Experiments were conducted with and without invertase inhibition by mercury ions, in a similar way as was done using the GOx-modified electrode (see Scheme 1). A volume of 100 μl of sample solution, initially containing 4 μg ml^{-1} invertase and 20 mM sucrose, after a 45 min incubation time, was injected over the modified electrode. NaOH was added to the solution to stop the enzyme reaction just before injection. During incubation:

$$\text{sucrose} + H_2O \xrightarrow{\text{invertase}} \text{D-glucose} + \text{D-fructose}$$

The current is due to oxidation of fructose and glucose and is directly related to the concentration of sucrose and invertase present in solution. The currents without and with mercury inhibition, after blank subtraction, were I_3 and I_4, respectively.

Results and discussion

The main objective of this study was to take advantage of the electrochemical batch-injection analysis (BIA) capabilities for *in situ* rapid measurements of health hazardous compounds detected by the enzyme inhibition biosensor principle. Comparison was made between a GOx-modified platinum electrode and a Cu-modified electrode. In both cases, an amperometric technique involving the recording of BIA current transients at constant potential was used. In all experiments described, results are expressed as the maximum current obtained in the current peak for oxidation following sample injection into the BIA cell. An example of three successive current transients obtained following injection at fixed potential is shown in Fig. 1 in the absence and presence of mercury ions.

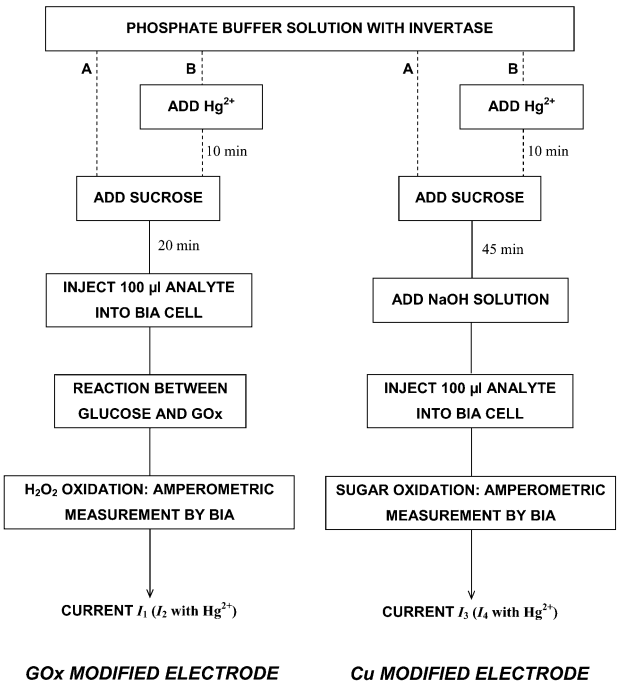
The activity of invertase in solution kept at 4 °C and at room temperature, 27 °C, over a period of 7 weeks was monitored by measurement of H_2O_2 oxidation using the procedure described in the Experimental section and remains the same even at room temperature during this time period. Such a high stability is important for future application in field measurements.

GOx-modified platinum electrode

A linear response was obtained for different concentrations of glucose, in the range 0.1–1.6 mmol l^{-1}, directly injected into the BIA cell on to the GOx-modified platinum electrode and this glucose sensor was found to be very sensitive for detection of low concentrations of glucose down to 0.01 mmol l^{-1}.

The extent of enzymatic reaction between 20 mM sucrose and invertase was studied for different reaction times before injection (Fig. 2). The enzymatic hydrolysis of sucrose by invertase demonstrates a good correlation between the time of enzymatic hydrolysis of sucrose into glucose and the peak current due to production of H_2O_2. From such experiments, a hydrolysis time of 20 min was chosen for subsequent experiments involving inhibition, because a sufficiently large oxidation peak could be obtained before and after adding mercury ion. At shorter times, the precision was not high enough and periods longer than 20 min would lead to unnecessarily long experiments.

The degree of inhibition of invertase by mercury ions was then investigated using the BIA method. The current peak for oxidation of H_2O_2 due to invertase inhibition (Fig. 3), shows the effect of mercuric ion concentration on invertase inhibition. The current peak for oxidation decreased when the concentration of



Scheme 1 Procedures at the GOx-modified platinum electrode and the Cu-modified glassy carbon electrode. The dashed lines represent alternatives without (A) and with (B) addition of mercury ions.

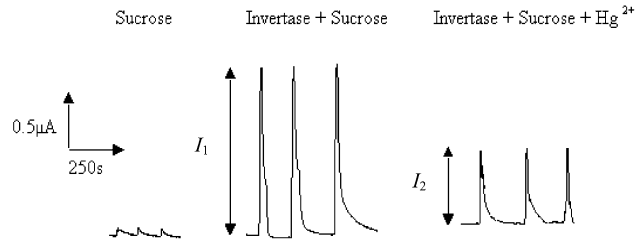


Fig. 1 Typical BIA sensor response to three successive injections of analyte sample on a platinum electrode with immobilised GOx in pH 6.0, 0.1 M phosphate buffer electrolyte with and without addition of 10 ng ml^{-1} Hg^{2+} (leads to 60% inhibition). Injection volume, 100 μl at 47.6 μl s^{-1} dispensing rate; 1 μg ml^{-1} invertase; 20 mM sucrose; applied potential, +0.65 V *vs.* SCE.

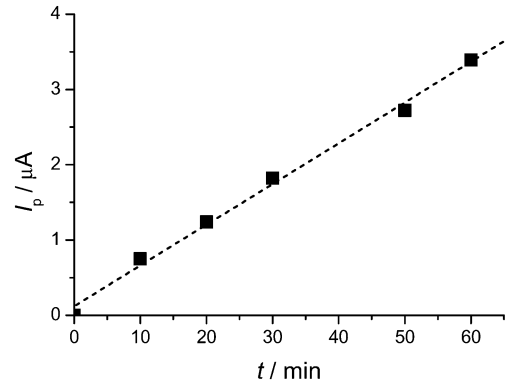


Fig. 2 Dependence of oxidation peak current for H_2O_2 oxidation with the glucose–BIA sensor on enzymatic hydrolysis time for 20 mM sucrose. Other conditions as in Fig. 1.

Hg^{2+} was increased in solution. Amine *et al.* reported an incubation time of 10 min to be necessary.[19] Under the experimental conditions described above, mercury could be determined in the 2.5–12 ng ml^{-1} range, which corresponds to 20–70% inhibition. The error of the slope is 3% calculated for three successive calibrations and 8% for three calibrations performed on three different days. An inhibition of 50% corresponds to 6.3 ± 0.5 ng ml^{-1}; the precision of the inhibition measurement was satisfactory in the range 20–70%, but the results became much less reproducible below 10% inhibition. For commercial biosensor devices developed for monitoring environmental contamination, the inhibition degree referring to the minimum detectable level of the pollutants is assumed to be more than 10%.[4] In this work, a value of 10% inhibition used as the detection limit corresponds to 1.0–1.6 ng ml^{-1}. It should be noted that compared with other, previous, methods for the determination of mercury based on the inhibition of different enzymes,[3–7,33,34] invertase is the most sensitive enzyme to mercury with the best detection limit achieved (1.0 ng ml^{-1}). A lower detection limit (0.2 ng ml^{-1}) was recently reported with glucose oxidase and the mediator 2-aminoethanethiol, but in that case Hg(II) reacts with thiol rather than with enzyme.[35]

Copper-modified glassy carbon electrode

At copper-modified glassy carbon electrodes, all three sugars can be directly oxidised, depending on the value of the applied potential, oxidation of sucrose occurring at a higher potential. Current transients resulting from analyte injection were measured for a sequence of applied potentials, first of a sample of the initial 20 mM sucrose solution, I_s, and second of a sample after reaction for 45 min of sucrose with 4 μg ml^{-1} invertase, I_t. The current I_t corresponds to the sugar mixture containing the sucrose that did not react plus the fructose and glucose from the enzyme reaction.

Table 1 shows the ratio, I_t/I_s, of BIA oxidation peak currents before addition of invertase, I_s, and after its addition and 45 min reaction, I_t. At less positive applied potentials, for example +0.22 V *vs.* Ag/AgCl shown in the table, the ratio cannot be determined, since there is no oxidation of sucrose and that of fructose and glucose is only just beginning to occur. For slightly more positive potentials, +0.35 V, the sucrose oxidation current is small and that due to glucose and fructose oxidation already large, leading to large values of the ratio of currents. As the potential is further increased, the current due to sucrose oxidation increases faster and the ratio falls. Since we are interested in the current due to glucose and fructose oxidation, a large ratio is the best and it was found corresponding to an applied potential of +0.35 V, when the sucrose contribution is not significant, as demonstrated from the calibration plot slopes given in Table 1.

Other studies at different electrolyte concentrations demonstrate that on increasing the NaOH concentration, the background current increases, so that a low concentration should be used whilst ensuring sufficient electrolyte conductivity.

Taking these facts into consideration, it was found that the best oxidation response could be obtained in 0.1 M NaOH at an applied potential of +0.35 V, additionally avoiding large background currents. These conditions were used for the mercury inhibition experiments.

Fig. 4 shows the influence of adding NaOH to the enzymatic hydrolysis solution. It is seen that the invertase enzyme reaction is stopped after a 10 min contact time, after which the solution can be analysed for glucose and fructose using the BIA cell with Cu-modified electrode. It is also seen in Fig. 4 that even after 48 h (2880 min) the response remains the same. The advantage of this methodology is, therefore, that whilst the measurement of the sugars is being performed by BIA, the enzymatic reaction has been completely stopped and there is no variation with time. Indeed, there can be circumstances where a large series of samples can be prepared and then analysed afterwards in the laboratory.

After optimisation of parameters, the variation with time of the amount of enzymatic hydrolysis of sucrose was studied. This experiment was done in order to choose the most appropriate time for mercury inhibition experiments. Fig. 5 shows a linear correlation between enzymatic hydrolysis time and the rate of glucose plus fructose oxidation in alkaline medium. Keeping in mind that a high percentage inhibition is often observed with low enzyme concentrations[19,34] and taking

Table 1 Variation with applied potential of the ratio of currents I_t/I_s and of the slopes of the calibration curves in 0.10 M NaOH solution at the Cu-modified glassy carbon electrode

	Applied potential/V			
Parameter	0.22	0.35	0.50	0.65
I_t/I_s[a]	—	28	12.5	3.7
Slope of calibration plot (nA mM^{-1})				
Sucrose	—	6.9	40	262
Fructose	1.6	81	163	319
Glucose	6.8	76	178	353

[a] I_t, current after reaction for 45 min of 20 mM sucrose with 4 μg ml^{-1} invertase, corresponding to the sugar mixture containing the sucrose that did not react plus the fructose and glucose from the enzyme reaction; I_s, current from the initial 20 mM sucrose solution.

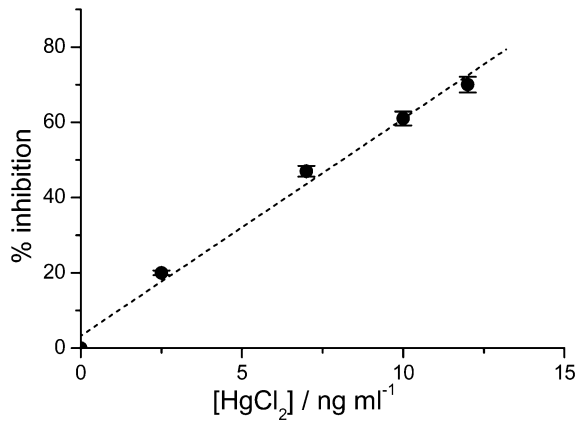


Fig. 3 Calibration curve for inhibition by Hg^{2+} after a 10 min incubation time at GOx-modified platinum electrode; error bars for five determinations are shown. Other conditions as in Fig. 1.

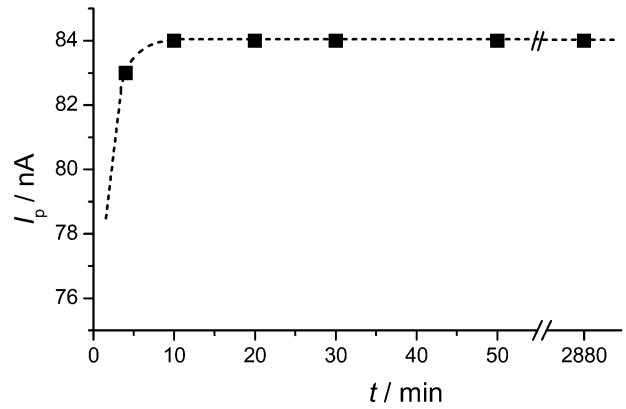


Fig. 4 Effect of addition of NaOH solution (final concentration 0.20 M) on enzymatic hydrolysis of sucrose, measured by the BIA response for glucose plus fructose oxidation resulting from analyte injection on to a copper-modified glassy carbon electrode in 0.1 M NaOH electrolyte solution. Injection volume, 100 μl at 47.6 μl s^{-1} dispensing rate; 4 μg ml^{-1} invertase; 20 mM sucrose; applied potential, +0.35 V *vs.* Ag/AgCl (3 M KCl).

into account the sensitivity of the electrode and the time of analysis, a compromise of 4 μg ml^{-1} invertase and a 45 min enzymatic hydrolysis time were chosen. An incubation time of 10 min for invertase with mercury ions reported in our previous work[19] as being necessary was adopted here also.

The degree of inhibition was then investigated. The oxidation current corresponding to glucose and fructose decreased, as expected, when the activity of invertase enzyme was reduced after adding mercury ions. The variation of percentage inhibition was successfully correlated with the concentration of mercury (Fig. 6). Linearity in the range 10–40 ng ml^{-1} $HgCl_2$, which corresponds to 15–61% inhibition, was obtained. The error of the slope is 4.8% for three successive calibrations and 8% for three calibrations each performed on different days. An inhibition value of 50% corresponds to 29.4 ± 2.4 ng ml^{-1} and 10% [used as the detection limit (see above)] was 3.0–4.0 ng ml^{-1}.

Comparison between the two methods

The determination of mercury ions by the enzyme inhibition method, at a GOx-modified platinum electrode, is seen to be more sensitive than direct oxidation of fructose and glucose at copper-modified electrodes. On the other hand, although the copper-modified electrode requires a longer hydrolysis time (Fig. 5), it has the advantage that the hydrolysis process can be stopped by adding sodium hydroxide solution and the samples pooled for later analysis by BIA. Hence there is no requirement for immediate analysis of the resulting solutions, unlike with the GOx-modified electrode. This results from the fact that sodium hydroxide solution cannot be used to stop the hydrolysis process because it will also inactivate GOx; the sample pH would then have to be readjusted before injection, increasing experimental complexity.

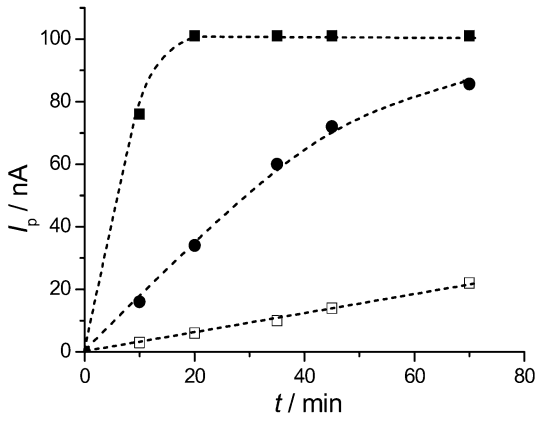


Fig. 5 Dependence of BIA oxidation peak current on enzyme hydrolysis time and invertase concentrations of (□) 1, (●) 4 and (■) 16 μg ml^{-1}. Other conditions as in Fig. 4.

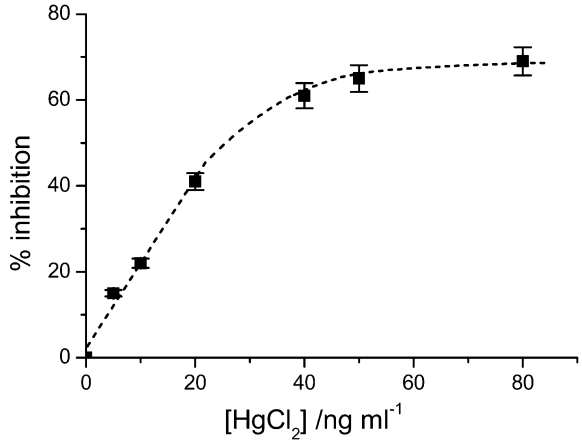


Fig. 6 Calibration curve for inhibition by Hg^{2+} after a 10 min incubation time at Cu-modified glassy carbon electrode; error bars for five determinations are shown. Other conditions as in Fig. 4.

The copper-modified electrode method is based on using a copper(II) film, which can be easily renewed in the field with copper sulfate solution, thus avoiding the use of glucose oxidase enzyme, which needs to be stored at 4 °C. On the other hand, the amount of invertase used in solution with the copper-modified electrode was four times larger than that using the GOx-modified platinum electrode and also the hydrolysis time was increased from 20 to 45 min, in order to increase the quantity of glucose and fructose formed. It is important that the response of blank solution (sucrose) should be negligible with respect to the response of glucose and fructose produced from enzymatic hydrolysis of sucrose. For that reason, the hydrolysis time and enzyme concentration were increased. Under the conditions described above, the response of sucrose is less than 5% using the GOx-modified electrode and less than 15% using the Cu-modified electrode with respect to the response of sucrose in the presence of invertase.

Nevertheless, when coupled to batch injection analysis, the procedure at the copper-modified electrode is faster and more time-efficient than that at the GOx-modified platinum electrode and should be preferred when this is of paramount concern.

Interference studies

The inhibition of invertase by several cations and molecules was also evaluated. It has been reported that heavy metals inhibit invertase only at high (μg ml^{-1}) concentrations.[19,36,37] Under our experimental conditions, it was found that 1000 ng ml^{-1} of Cu(II), Zn(II), Cd(II) or Pb(II) causes inhibitions of $< 10\%$. A concentration of 1 mmol l^{-1} causes 18% inhibition (phenol) and 16% inhibition (urea), whereas 0.1% surfactant (Tween 20) leads to 18% inhibition.

Analysis of real samples

The proposed GOx-based method was tested on real samples by determination of Hg^{2+} in three different waste waters before and after spiking with 5 ng ml^{-1} Hg^{2+}. The enzyme and sucrose concentrations, incubation time, reaction time and potential applied were the same as used previously. The samples were acidified at pH 2.0 to ensure that the mercury ions are labile and were filtered through membrane filters (0.45 μm pore size) before analysis. Any interference from other metal cations, that could be present in high concentration, was successfully avoided by adding 10^{-3} mol l^{-1} potassium sodium tartrate masking agent to the samples. Under these conditions, the recoveries obtained were 84–96%. The results of the analysis of these three different samples are given in Table 2.

Conclusions

The principle of combination of electrochemical batch injection analysis with enzyme biosensors has been successfully demonstrated. It presents great advantages for rapid *in situ* measurements, with particular application to the measurement of mercuric ion by enzyme inhibition in the ng ml^{-1} range. Compared with chemically modified electrodes which require preconcentration of mercury ions (similar to incubation time in the case of the enzymatic inhibition) at an applied potential as part of the anodic stripping voltammetry procedure, the enzyme-based procedure requires simpler equipment and can

Table 2 Determination of Hg^{2+} in waste water samples using the GOx-modified electrode

	Sample 1	Sample 2	Sample 3
Detected value in diluted sample/ng ml^{-1}[a]	3.3 ± 0.30	4.5 ± 0.13	5.0 ± 0.14
Detected value after 5 ng ml^{-1} spike/ng ml^{-1}	7.5 ± 0.7	9.0 ± 0.7	9.8 ± 0.8
Recovery (%)	84	90	96
Real value/ng ml^{-1}[a]	82.5 ± 8.0	112.5 ± 3.3	125 ± 3.5
Relative standard deviation (%)	9	3	3

[a] Real value is obtained by multiplying the detected value by the dilution factor (25). Each individual experiment was performed at least three times, then the results were averaged.

easily be coupled to BIA. Two approaches were investigated, one involving an immobilised GOx enzyme at platinum electrodes and the other involving direct oxidation of the glucose and fructose, produced by enzyme reaction, at a copper-modified glassy carbon electrode. Both are suitable for field application: whereas the former is more sensitive, the latter has advantages as a screening test.

A similar approach can be expected to be fruitful for other metal ions and enzyme inhibition schemes.

Acknowledgements

The authors acknowledge financial support from the Scientific and Technical Cooperation Agreement Portugal–Morocco (ICCTI–CNCPRST, 2000–01) and the Portuguese Foundation for Science and Technology–Project POCTI (co-financed by the European Community fund FEDER). This work was also supported by a grant from the International Council for Science (Sweden).

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