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Copper-modified gold electrode specific for monosaccharide detection Use in amperometric determination of phenylmercury based on invertase enzyme inhibition

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Abstract

The electrochemical oxidation of mono- and disaccharides at various copper-modified electrodes is reported: glassy carbon modified at open circuit or by electrochemical deposition of copper, gold modified by electrochemical deposition, and at bulk copper electrodes. A comparative study of these four electrodes was made by linear sweep voltammetry and amperometry. The maximum oxidation peak separation between disaccharides and monosaccharides is about 200 mV. After optimization, amperometric determination of monosaccharides was done at +0.30 versus Ag/AgCl in 0.15 M NaOH at the copper-modified gold electrode.

Using the developed method, the enzymatic activities of invertase and β-galactosidase were determined through their reaction with sucrose and lactose, respectively. Validation was carried out by a spectrophotometric method based on 3,5-dinitrosalicylic acid, and it was shown that the proposed electrochemical method is more sensitive.

The analytical utility of the copper-modified gold electrode was tested for the determination of organic mercury. Addition of phenylmercury standards to the invertase solution caused a decrease in the enzyme activity, and allowed the determination of phenylmercury in pharmaceutical samples. The concentration has been determined in the 10–55 ng ml^{-1} range.

Keywords: Copper; Electrodeposition; Phenylmercury; Invertase; Sugars

1. Introduction

In recent years, there has been extensive interest in chemically modified electrodes (CMEs) and in their application in fields such as charge storage and fuel cells, electrochromic devices, electrochemical sensors, etc. [1,2]. Modification with organic or inorganic polymers impregnated with dispersed metal particles has been investigated [3,4], as has electrodeposition of metals or their complexes on inert electrode surfaces [5]. The latter represents a simple and efficient procedure to obtain very thin and uniform films with a high degree of adherence and coverage. The film characteristics (thickness, porosity, chemical composition, etc.) can be easily modulated by the control of the electrodeposition process parameters such as potential limits and waveforms, time of electrolysis, and chemical composition of the electrolyzed solution [5].

The electrochemical detection of glucose as well as of other sugars has been investigated at a number of transition metal electrodes [6,7]. Noble metals such as Au and Pt suffer from self-poisoning effects, so cleaning and regeneration steps are needed to obtain stable amperometric responses [6–9]. For this reason, other transition metal-based electrodes have been proposed as electrode materials for the detection of carbohydrates in flow injection and liquid chromatographic analysis at fixed applied potential [10–12].

CMEs with surface-confined catalytic species show some advantages over metallic electrode substrates. Encapsulation of metallic particles into organic polymers or directly on an inert surface provides a good physical dispersion of the catalytic centers and leads to a highly active electrode

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surface suitable for efficient catalysis [13]. Recently CMEs based on Ni, Co, and Au deposited on glassy carbon have been successfully employed for the determination of sugars in anion-exchange chromatography [14–19].

The electrochemical oxidation of mono-and disaccharides in sodium hydroxide solution on an oxidized Cu-rotating disk electrode was reported by Torto et al. [20]. It was demonstrated that the number of electrons transferred by disaccharides is different from that transferred by monosaccharides, although the actual mechanism of carbohydrate oxidation is still not completely clarified. Baldwin has suggested an electrocatalytic mechanism for carbohydrate oxidation, which might be initiated by a Cu(III) surface species with formation of Cu chelates [21–24].

In previous work, we have studied monosaccharide (glucose) and disaccharide (sucrose) oxidation in alkaline media using a glassy carbon electrode modified with copper at open circuit, using the enzyme invertase to convert sucrose into glucose [25]. However, this type of electrode did not allow detection of sugars down to the micromolar concentration range. It was also used to explore the possibility of measuring concentrations of Hg(II) by inhibition of the invertase reaction. It has been previously shown that the total amount of phenylmercury, ethylmercury and methylmercury can be determined by invertase inhibition in the ng ml^{-1} range and that a large number of heavy metal ions do not interfere [26]; surfactants in solution at the 0.1% level lead to inhibition of 10–20% [25].

Organomercury compounds are a particular concern in terms of exposure, bioavailability and absorption [27,28]. Amongst more recently developed methods, apart from electrochemical ones, capillary zone electrophoresis after complexation can be used with UV detection [29] as well as high pressure liquid chromatography [30]. Enzyme inhibition represents another strategy for organomercury compounds that has been investigated, for example, with spectrophotometric [31] or thermometric [32] detection.

In this paper, the aim was to investigate whether the electrocatalytic activity of different copper-containing electrode materials could be exploited for the selective amperometric detection of monosaccharides. For this purpose, a bulk copper electrode, a glassy carbon electrode modified with copper at open circuit, and glassy carbon and gold electrodes modified by electrochemical deposition of copper microparticles, designated as GC/Cu and Au/Cu, were investigated as possible electrochemical sensors for the detection of mono- and disaccharides in alkaline media. Linear sweep voltammetry was used to elucidate the electrocatalytic properties of the modified electrodes. The best type of copper-modified electrode was investigated for measurement of enzyme activity and enzymatic determination of organic mercury in pharmaceutical compounds, based on the inhibition of sucrose hydrolysis by soluble invertase, via amperometric measurement of glucose and fructose concentration after a chosen incubation time.

2. Experimental

2.1. Reagents

Glucose, fructose, galactose, mannose, xylose, arabinose, sucrose, lactose, maltose, trehalose, cellobiose and potassium sodium tartrate were obtained from Farco Chemical Supplies Company. Sodium hydroxide was obtained from Eka Nobel. The 3,5-dinitrosalicylic acid (DNS) and phenylmercury acetate were from Sigma–Aldrich. Antiseptic pharmaceutical samples Sulfa-Bleu and Polyfra (Alcon), were purchased from pharmacies.

The lyophilized enzymes invertase and β-galactosidase were from baker's yeast, 960 and 9.0 units mg^{-1}. All other reagents were of analytical grade and solutions were made with distilled water. Phosphate buffer (Na_2HPO_4/NaH_2PO_4), ionic strength 0.1, pH 6.0, or sodium hydroxide solution were used as electrolytes. A stock solution of 1.0 M sucrose was prepared weekly in 0.01 M NaOH to avoid any spontaneous hydrolysis.

2.2. Instrumentation

Linear sweep voltammetry (LSV) and cyclic voltammetry (CV) measurements were made with an Autolab PGSTAT10 potentiostat (Ecochemie, The Netherlands) controlled by GPES 4.7 software. The gold, glassy carbon and copper working electrodes were respectively 2, 3 and 2 mm diameter.

Amperometric measurements were made with a CV27 voltammograph (Bioanalytical Systems, USA) connected to a X–Y recorder (Yokogawa, Japan).

The three electrode system consists of the working electrode, a stainless steel rod auxiliary electrode and an Ag/AgCl (3 M KCl) reference electrode.

2.3. Electrode preparation

The gold and glassy carbon electrode surfaces were prepared by polishing with 0.05 μm α-alumina powder on a microcloth using water as lubricant. Before each modification, copper particles were removed from the electrode surface by sonication for 5 min in hydrochloric acid (18%, w/w).

The electrodeposition of copper on gold and glassy carbon electrodes was carried out at −0.3 V versus Ag/AgCl in 50 mM $CuSO_4$. The mass of copper, m_{Cu}, deposited into the electrode is related to the charge consumed during the electrodeposition through

$$m_{Cu} = 63.5 n_{Cu} = \frac{63.5Q}{2F}$$

where n_{Cu} is the number of moles of copper of atomic weight 63.5, Q is the charge in coulombs, and $F = 96{,}485\ C\ mol^{-1}$ is the Faraday constant, with two electrons transferred per copper ion. A mass of 0.2 mg cm^{-2} was deposited, which

corresponds to a charge of 0.608 C cm^{-2}. Following deposition, the modified electrode was conditioned by cycling 10 times between −0.3 and +0.8 V at 100 mV s^{-1} in 0.2 M NaOH. Although some of the charge could be used to reduce dissolved oxygen, the concentration of oxygen in aqueous solutions of ∼10^{-4} M is too small to cause a significant error. It is expected that the microscopic surface roughness obtained by this deposition procedure is high.

For open circuit modification with copper, the glassy carbon electrode was immersed into an aqueous solution of 50 mM $CuSO_4$ for 15 min at open circuit. After removal and rinsing with distilled water, the electrode was ready for use.

2.4. Electrochemical procedures

Linear scan experiments were performed in the potential range 0.0–1.0 V in 0.2 M NaOH electrolyte solution at a sweep rate of 50 mV s^{-1}.

Amperometric measurements were performed at a constant applied potential of +0.30 V versus Ag/AgCl in 0.15 M NaOH. A few microlitres of a standard solution of monosaccharide or disaccharide were injected into a cell filled with 5 ml of 0.15 M NaOH solution, and the steady state current due to direct oxidation of the sugar was measured.

The enzyme activities of invertase and β-galactosidase were determined by mixing enzyme and substrate in 3 ml of phosphate buffer solution, pH 6.0, for an appropriate time. The enzymatic reaction was then stopped by addition of 1.75 ml of 1.0 M NaOH. The total volume (4.75 ml) was then added to the 5 ml of 0.5 M NaOH solution in the cell. The steady state current, proportional to the enzyme activity and substrate concentration, was recorded.

Experiments on phenylmercury inhibition were conducted in two steps, in the absence and in the presence of inhibitor. In the first, a solution was prepared containing 1 μg ml^{-1} invertase and 10 mM sucrose. After 20 min reaction time, a sample was injected into the cell after adding NaOH solution, the procedure being similar to enzymatic activity measurement. The current due to oxidation of glucose and fructose is directly related to the concentration of sucrose and invertase present in solution. The resulting steady state current, after blank subtraction, is designated I_1.

In the second step, a solution containing the same concentration of invertase as before was spiked with phenylmercury and incubated for 15 min. Sucrose was then added and after 20 min the reaction was stopped with 1.0 M NaOH. The resulting volume was injected into the batch cell and the steady state current, after blank subtraction, was designated I_2. The degree of inhibition was calculated from the peak height with and without phenylmercury using the equation:

$$I\,(\%) = 100\left(\frac{I_1 - I_2}{I_1}\right)$$

2.5. Spectrophotometric procedure for determination of glucose and fructose

Validation of the electrochemical method was carried out by a spectrophotometric method.

A solution of DNS was prepared by mixing 1.0 g of 3,5-dinitrosalicylic acid, 30 g of sodium potassium tartrate, 20 ml of 2.0 M NaOH and 50 ml H_2O. The mixture was heated during a few minutes in order to dissolve the 3,5-dinitrosalicylic acid, and the volume was then adjusted with distilled water to 100 ml [33].

The calibration curve was obtained by mixing 1.0 ml of a suitable concentration of monosaccharides (equimolar solution of glucose plus fructose), 1.0 ml of phosphate buffer solution and 2.0 ml of DNS solution in glass-capped test tubes (1.5 cm × 15.5 cm). These test tubes were placed in a water bath at 98 ± 0.1 °C for 5 min and cooled under running water, then 10 ml of distilled water was added. The aldehyde functional group or ketone functional group of the sugar are oxidized to carboxyl groups while the 3,5-dinitrosalicylic acid is reduced to 3-amino,5-nitrosalicylic acid, which has an orange coloration and is measured at 540 nm [34].

3. Results and discussion

3.1. Influence of modification by copper on gold and glassy carbon electrode voltammetric behavior

The changes in the voltammetric profile of gold and glassy carbon electrodes after electrochemical deposition of copper were studied by cyclic voltammetry and are illustrated in Fig. 1.

Representative cyclic voltammograms at a Au/Cu electrode, with 0.2 mg cm^{-2} copper loading, and at a bulk gold electrode in 0.20 M NaOH are shown in Fig. 1a. The voltammetric profile of the gold electrode agrees with that reported by Casella et al. [18], with anodic and cathodic waves, related to gold oxide formation and reduction, respectively. In the CV for the copper modified electrode a small cathodic peak at +0.61 V, during the reverse scan, is probably associated with the Cu(III)/Cu(II) transition [12,17,21]. During the first scan in the positive direction, a broad anodic wave with a peak potential of +0.40 V versus Ag/AgCl was observed, which decreased rapidly in height upon repetitive potential scanning.

Cyclic voltammograms at a GC/Cu electrode with 0.2 mg cm^{-2} loading, and a bare glassy electrode obtained in 0.2 M NaOH are reported in Fig. 1b. At GC/Cu, as at Au/Cu, there is a small cathodic peak at 0.62 V during the negative scan, attributable to the Cu(III)/Cu(II) transition. A broad anodic wave with a peak potential of +0.40 V versus Ag/AgCl also occurs and decreases with repetitive scanning. A very reproducible voltammogram can be obtained under steady-state conditions after about eight cycles between −0.30 and +0.80 V. The shape of the cyclic

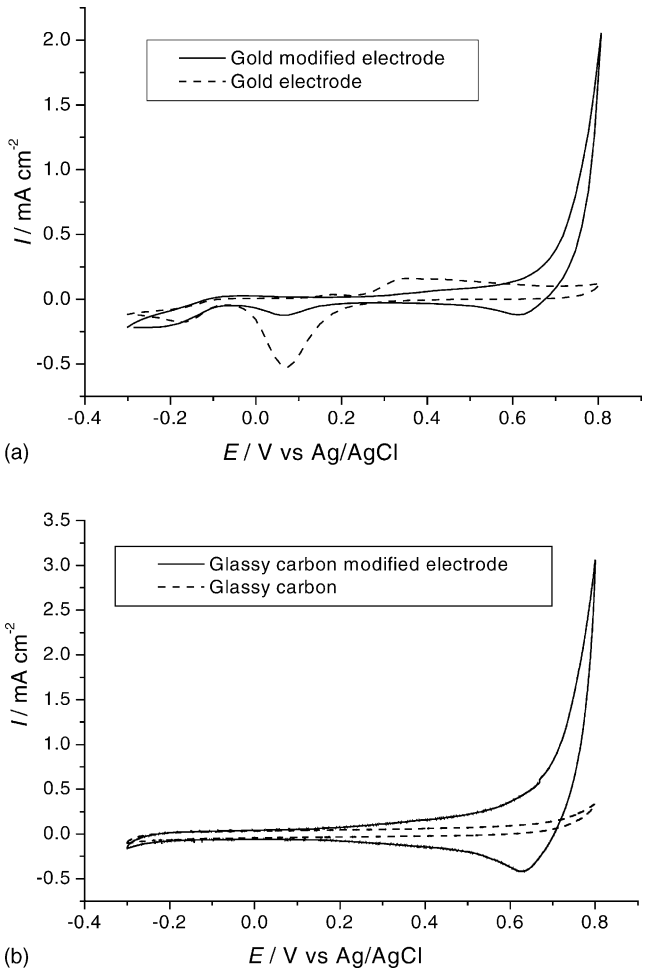

Fig. 1. Steady-state cyclic voltammograms (10th cycle) in 0.2 M NaOH, scan rate 100 mV s^{-1}, at: (a) copper-modified gold electrode (—) and bare gold electrode (- - -); (b) copper-modified glassy carbon electrode (—) and bare GC electrode (- - -).

voltammogram at the modified electrode is very similar to that with gold substrate. Differences can be attributed to the copper microparticle size and number since nucleation and growth are influenced by the surface structure and active sites of substrate electrodes.

3.2. Linear sweep voltammetry of saccharides

The electrooxidation of mono-and disaccharides was examined by linear sweep voltammetry at the different electrode substrates in order to ascertain whether the applied potential and NaOH concentration permit a selective response to monosaccharides.

3.2.1. Copper-modified gold electrode

Electrooxidation of mono-and disaccharides at copper-modified gold electrodes in sodium hydroxide alkaline media at different concentrations is shown in Fig. 2. The response to 20 mM glucose, fructose and sucrose changes with the concentration of NaOH solution, which was varied between 0.05 and 1.0 M. The voltammograms show that the oxidation potentials of glucose, fructose and sucrose are different: an anodic peak appears, at potentials higher than 0.25 V, centered at different values according to the NaOH concentration. The peak potentials were shifted to more positive values as the NaOH concentration increases, but at 1.0 M NaOH shift negatively again. This is probably due to the formation of electroactive species from sugar decomposition, since at this higher pH sugars are more easily oxidized, also explaining the higher currents. The maximum peak separation between mono- and disaccharides was equal to 200 mV and was obtained in 0.15 M NaOH electrolyte.

3.2.2. Copper-modified glassy carbon electrode, by electrodeposition

At the glassy carbon electrode modified electrochemically by a copper film, the oxidation potentials of glucose, fructose and sucrose are relatively close to each other, as occurs at the copper-modified gold electrode. The maximum separation between the mono and disaccharide current peaks was in 0.15 M NaOH with a value of 150 mV.

3.2.3. Copper electrode

A similar behavior was also observed with the bulk copper electrode: the peak potentials were shifted to more negative values with high (1.0 M) and low (0.05, 0.1 M) NaOH concentration. The maximum separation between mono- and disaccharide peaks was equal to 200 mV and was obtained in 0.15 M NaOH solutions.

3.2.4. Other electrodes

No response to oxidation of glucose, fructose or sucrose was observed by linear sweep voltammetry at unmodified gold or glassy carbon electrodes, or at glassy carbon modified by copper at open circuit. In the last case, this is probably due to there being insufficient copper deposited to cause an electrocatalytic effect on the timescale of the experiment.

3.2.5. Choice of applied potential and concentration of NaOH

Although the exact mechanism for the oxidation of carbohydrates at copper electrodes is still not known, Cu(III) species have been suggested to act as an electron transfer mediator [21]. As seen in Fig. 2, the oxidation of sugars occurs in the potential range 0.6–0.8 V where the oxidation wave for Cu(II)/Cu(III) was reported [21,35].

As the applied potential is increased, the current due to sucrose oxidation increases faster and the monosaccharide to disaccharide ratio falls. Since the current due to monosaccharide oxidation is of prime interest (i.e. a large ratio—see also results in Table 1 to be discussed in the next section), +0.30V was chosen for the amperometric study of mono- and disaccharides in 0.15 M NaOH, because this combination led to the greatest separation between mono- and disaccharide oxidation waves. These conditions were selected for the rest of the work.

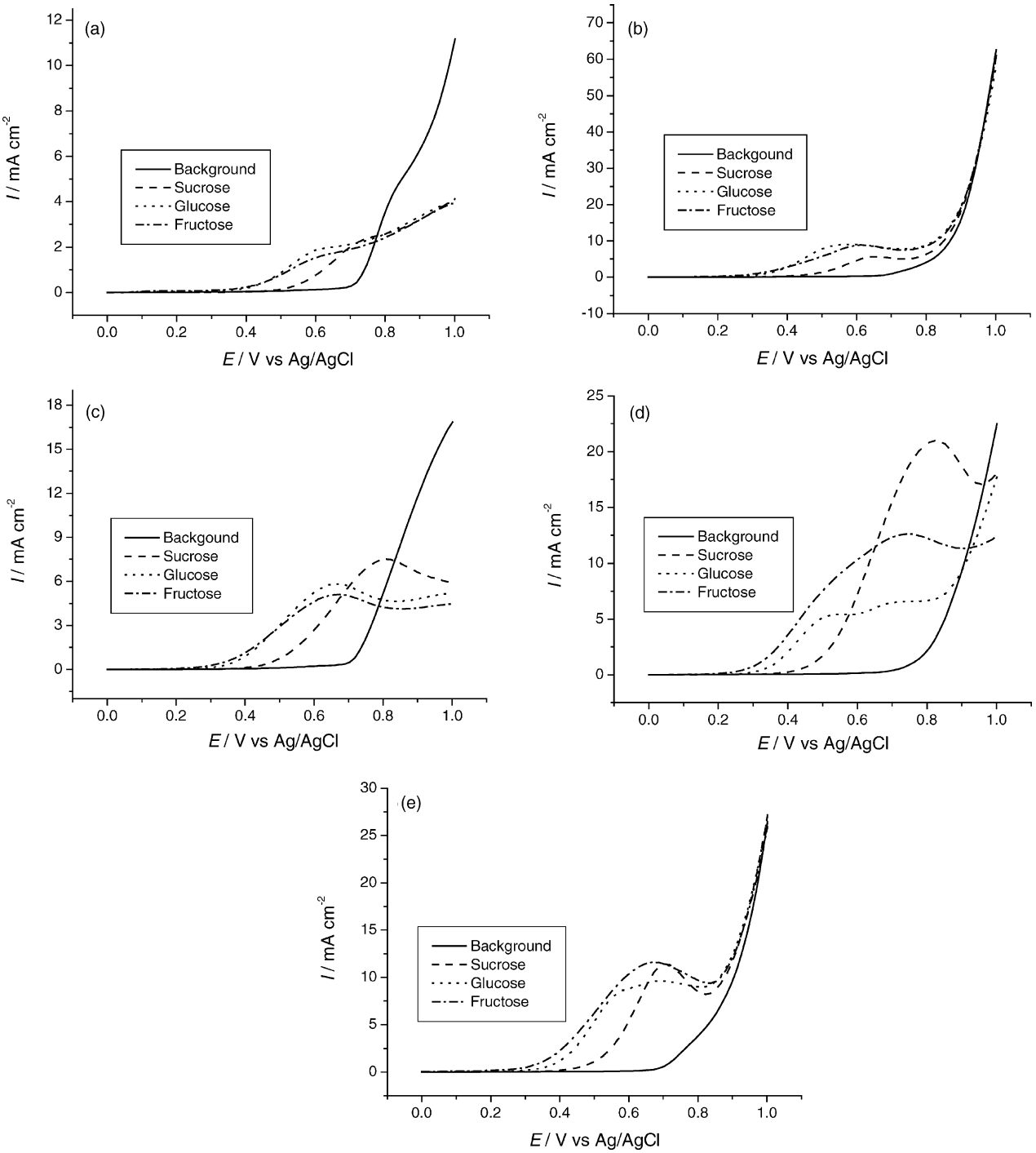

Fig. 2. Linear sweep voltammograms at copper-modified gold electrode of 20 mM glucose, fructose and sucrose in: (a) 0.05 M, (b) 0.1 M, (c) 0.15 M, (d) 0.3 M, (e) 1.0 M NaOH electrolyte. Scan rate: 50 mV s^{-1}.

3.3. Amperometric study

The amperometric response of glucose, sucrose and fructose in alkaline media, at +0.30 V versus Ag/AgCl in 0.15 M NaOH using the different electrodes, is given in Table 1. Although the highest response was recorded at glassy carbon modified by electrodeposition of copper film, the best ratio of monosaccharide to disaccharide steady-state currents, $(I_f + I_g)/I_s$, was obtained at the copper-modified gold electrode.

The oxidation of glucose and fructose was studied at different electrodes, and the results are presented in Table 2. Linearity is obtained in the range of 4–1000 μM for glucose and 1–1000 μM for fructose at the Au/Cu electrode, a larger linear range than at the other electrodes. However, the highest sensitivity and the lowest detection limit (determined as

Table 1
Electrooxidation currents for 1 mM glucose, I_g, fructose, I_f, and sucrose, I_s, on different electrodes in 0.15 M NaOH and at +0.30 V vs. Ag/AgCl

	Background current (μA)	I_g (μA cm^{-2})	I_f (μA cm^{-2})	I_s (μA cm^{-2})	$(I_g + I_f)/I_s$
GC/Cu (open circuit)	0.005	0.11	0.28	0.01	29
GC/Cu (electrodeposition)	0.4	74	271	17	20
Au/Cu	0.1	19	70	1.6	57
Copper	0.6	60	109	5	34

three times the signal to noise ratio) were obtained by GC/Cu modified by copper electrodeposition for both monosaccharides. The reproducibility (RSD) of the experiments, GC/Cu modified at open circuit and by electrodeposition, Au/Cu and copper electrode, is around 10% in all cases.

Although the GC/Cu electrode has the lowest detection limit and highest sensitivity, the stability of the copper film is limited to only 2–3 h. However, the Au/Cu electrode is stable for 3 days and has the largest linear range and the best ratio $(I_f + I_g)/I_s$, so it was chosen for further studies.

3.4. Oxidation of mono- and disaccharides using copper-modified gold electrode

The response of a number of mono- and disaccharides, in addition to glucose and fructose, was studied at the copper-modified gold electrode. The results obtained are grouped in Table 3. It was observed that the oxidation current of monosaccharides is always higher than that of disaccharides.

Therefore, this method can be applied to measure concentrations of monosaccharides, the concentration of disaccharides indirectly after their complete enzymatic conversion to monosaccharides and, finally, the activity of the enzyme involved in the hydrolysis of disaccharides.

3.5. Determination of enzymatic activity

The enzymatic activity of invertase, which hydrolyses sucrose to glucose plus fructose (reaction (1)), and of β-galactosidase, which hydrolyses lactose to glucose plus galactose (reaction (2)):

$$\text{sucrose} + H_2O \xrightarrow{\text{invertase}} \text{glucose} + \text{fructose} \quad (1)$$

$$\text{lactose} + H_2O \xrightarrow{\beta\text{-galactosidase}} \text{glucose} + \text{galactose} \quad (2)$$

was calculated using calibration curves of glucose plus fructose and of glucose plus galactose, respectively.

It was observed that the monosaccharide oxidation current decreased with a high concentration of disaccharides in the batch cell.

The response to glucose plus fructose at concentrations in the range 0–1 mM was recorded, without and with 10 mM sucrose in solution. The calibration curves are described by the equations $I\,(\mu A) = 0.04 + 2.8c$ and $I\,(\mu A) = 0.011 + 0.7c$ in the absence and presence of sucrose, respectively, where c is the concentration of (glucose + fructose) in mM.

A similar study for glucose plus galactose gave $I\,(\mu A) = 0.007 + 0.5c$ in the absence of lactose and $I\,(\mu A) = -0.002 + 0.4c$ in its presence, where, in this case, c represents the concentration of (glucose + galactose) in mM.

It was concluded that a high concentration of disaccharide substrate had a significant influence on the oxidation of glucose plus fructose and oxidation of galactose plus glucose. This should therefore be taken into account.

The enzyme was then mixed with 3 ml of phosphate buffer solution (pH 6) containing 10 mM substrate. The reaction

Table 2
Comparison of glucose and fructose oxidation response obtained with different electrodes in 0.15 M NaOH and at +0.30 V vs. Ag/AgCl

	Linear range	Slope (μA cm^{-2} mM^{-1})	Limit of detection (μM)
Glucose			
GC/Cu (open circuit)	1.2–20 mM	0.11	800
GC/Cu (electrodeposition)	2–500 μM	74	0.3
Au/Cu (electrodeposition)	4–1000 μM	19	2.0
Copper	20–1000 μM	60	15
Fructose			
GC/Cu (open circuit)	1–20 mM	0.28	500
GC/Cu (electrodeposition)	0.5–400 μM	271	0.1
Au/Cu (electrodeposition)	1–1000 μM	70	0.5
Copper	8–1000 μM	109	8

Table 3
Oxidation current, I, of mono and disaccharides in 0.15 M NaOH using copper-modified gold electrode at +0.30 V vs. Ag/AgCl

Sugar	I (μA cm^{-2})
Monosaccharides	
Fructose	70
Glucose	19
Galactose	32
Xylose	25
Disaccharides	
Sucrose	1.6
Maltose	1.9
Lactose	1.6
Trehalose	0.3
Cellobiose	2.5

Sugar concentrations: 1 mM.

was stopped by adding 1.75 ml of 1.0 M NaOH after incubation during different reaction times (3, 6, 9 and 12 min). The mixture was injected into the batch cell containing 5 ml of 0.15 M NaOH solution.

The enzymatic activity of invertase was calculated from the calibration curves of glucose and fructose in the presence of 10 mM sucrose, since enzymatic hydrolysis led to glucose plus fructose. There is a good linear relationship between oxidation current, I, and hydrolysis time, t, with $I\,(\mu A) = 8.0 \times 10^{-3} + 0.008t$ for 0.1 units of invertase, with t in min. Under these conditions the slope also varied linearly with the amount of enzyme used (0.1, 0.2 and 0.4 units). The slopes for 0.2 and 0.4 units were respectively 0.016 and 0.032 $\mu A\,min^{-1}$. The smallest amount of invertase which could be detected was 0.005 units. The concentration of glucose plus fructose produced in the mixture after 12 min reaction in the presence of 0.005 units of enzyme corresponded to 6 μM, which is the lowest concentration detectable in the presence of 10 mM sucrose by the copper-modified gold electrode.

The enzyme activity of β-galactosidase was calculated in a similar way using the calibration curve of glucose plus galactose in the presence of 10 mM lactose. The corresponding equation for 0.1 unit of enzyme is $I\,(\mu A) = -0.006 + 0.006t$. The slopes for 0.2 and 0.4 units were respectively 0.013 and 0.026 $\mu A\,min^{-1}$. The lowest detectable β-galactosidase activity was 0.009 units.

3.6. Comparison with the spectrophotometric method

The response to fructose plus glucose, by the spectrophotometric method based on DNS (see Section 2 for procedure), was measured. The concentrations of glucose plus fructose were varied between 1 and 10 mM. A very good linear relation between the absorbance and the concentration of glucose + fructose added was found, with absorbance $= -0.012 + 0.153c$ and $R = 0.999$, where c is in mM.

The corresponding response to glucose plus fructose, using the amperometric method developed at copper-modified gold electrodes over the concentration range 0.01 to 0.1 mM—a dilution of 100 times—led to $I\,(\mu A) = 0.013 + 2.6c$ and $R = 0.995$.

The proposed amperometric method was compared directly with the spectrophotometric method in seven solutions of different concentration, diluting by a factor of 100 for electrochemical analysis. Fig. 4 shows the comparison between results from both methods: the glucose and fructose found in these solutions was between 1.6 and 7.6 mM with a good correlation of $R = 0.999$. The slope and the intercept of the regression line were equal to 0.98 ± 0.01 and 0.05 ± 0.02 mM respectively, so that the slope of the regression line is not significantly different from 1 nor the intercept significantly different from 0. The correlation coefficient indicates good agreement between the two methods.

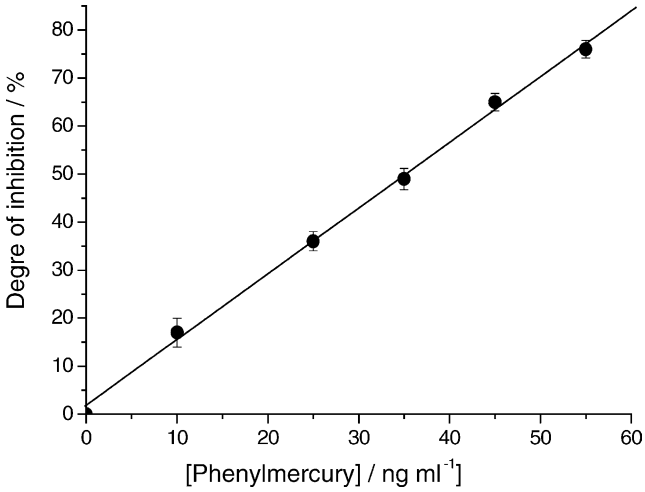

Fig. 3. Calibration curve for inhibition of invertase by phenylmercury after 15 min incubation at copper-modified gold electrode; error bars are for three determinations.

3.7. Inhibitor determination

All inhibition measurements were performed with a concentration of the saturated substrate of 10 mM, a concentration of invertase of 1.0 $\mu g\,ml^{-1}$ and a reaction time of 20 min. These conditions were chosen so that a sufficiently large oxidation peak could be obtained before and after adding inhibitor, keeping in mind that a high percentage of inhibition is often observed with low enzyme concentration [32].

The oxidation current corresponding to glucose and fructose decreased on addition of phenylmercury as expected, owing to reduction in the activity of invertase enzyme. The variation of percentage inhibition was successfully correlated with the concentration of phenylmercury, Fig. 3. Linearity in the range 10–55 $ng\,ml^{-1}$, which corresponds to 17–76% inhibition, was obtained. An inhibition value of 50% corresponds to 35 ± 2 $ng\,ml^{-1}$.

3.8. Analysis of real samples

The copper-modified gold electrode was tested by determination of phenylmercury in two different pharmaceutical samples, Sulfa–Bleu and Polyfra, containing declared amounts of phenylmercury, respectively 33 and 20 $\mu g\,ml^{-1}$, corresponding to 0.116 and 0.070 mM. The samples were diluted in order to give a concentration in the linear range of the proposed method (10–55 $ng\,ml^{-1}$). The calculated

Table 4
Recovery of phenylmercury, PhHg

Initial [PhHg] ($\mu g\,ml^{-1}$)	Added [PhHg] ($\mu g\,ml^{-1}$)	Found [PhHg] ($\mu g\,ml^{-1}$)	Recovery (%)
18	10	25	90
35	10	45	100

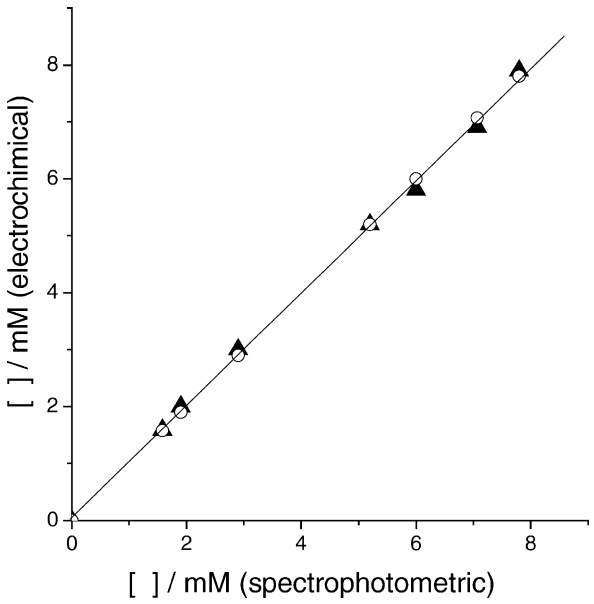

Fig. 4. Comparison between (○) spectrophotometric and (▲) proposed electrochemical method. The line represents perfect correlation.

concentrations are 35 ± 4 and $18 \pm 2\,\mu g\,ml^{-1}$ for Sulfa bleu and Polyfra, respectively, representing very good agreement. Under these conditions, recoveries obtained were (90–100%), as shown in Table 4 (Fig. 4).

4. Conclusions

The development of an electrode modified with a copper film selective for determination of monosaccharides under optimized conditions of pH and applied potential has been achieved with success using a copper-modified gold electrode. The sensitivity of the method makes it a good alternative to the traditional spectrophotometric method based on 3,5-dinitrosalicylic acid for determination of the activity of the enzyme involved in the hydrolysis of disaccharides.

The determination of phenylmercury in two pharmaceutical samples was in good agreement with the declared mercury concentration.

A similar approach can be expected to be fruitful for the determination of disaccharides after their complete enzymatic hydrolysis.

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