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PII: S0304-3894(17)30520-4
DOI: http://dx.doi.org/doi:10.1016/j.jhazmat.2017.07.021
Reference: HAZMAT 18713

To appear in: *Journal of Hazardous Materials*

Received date: 2-2-2017
Revised date: 4-7-2017
Accepted date: 8-7-2017

Please cite this article as: Basant Elsebai, Mariana Emilia Ghica, Mohammed Nooredeen Abbas, Christopher M.A.Brett, Catalase Based Hydrogen Peroxide Biosensor for Mercury Determination by Inhibition Measurements, Journal of Hazardous Materialshttp://dx.doi.org/10.1016/j.jhazmat.2017.07.021

Catalase Based Hydrogen Peroxide Biosensor for Mercury Determination by Inhibition Measurements

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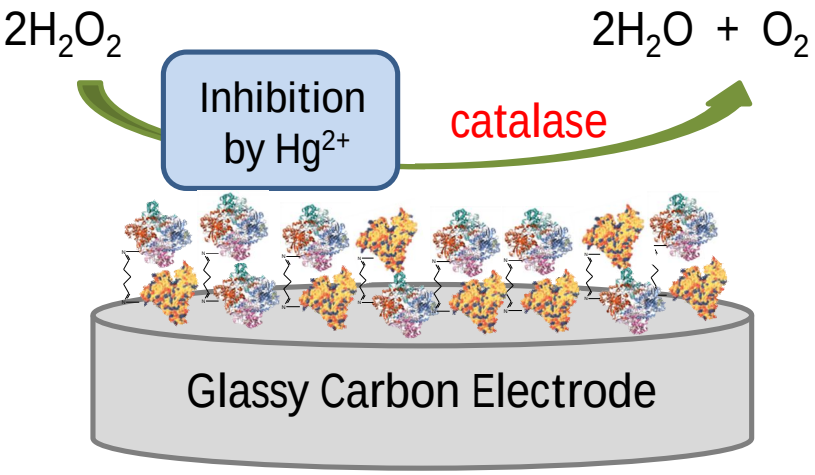
Graphical abstract



HIGHLIGHTS

- Novel amperometric enzyme inhibition biosensor for determination of mercury ions
- Biosensor based on catalase immobilized on glassy carbon electrode
- Evaluation of inhibition characteristics and analytical parameters
- Very low detection limit and high selectivity with respect to other toxic species
- Application to analysis of water samples

Abstract

A new amperometric hydrogen peroxide enzyme inhibition biosensor for the indirect determination of toxic mercury ions, Hg^{2+}, based on catalase immobilized on a glassy carbon electrode surface by cross-linking with glutaraldehyde and bovine serum albumin, is reported. The parameters influencing biosensor performance were optimized, including enzyme loading, the amount of hydrogen peroxide, the applied potential and electrolyte pH. It was shown that the inhibition of catalase by Hg^{2+} species is irreversible, with a linear inhibition response between $5x10^{-11}$ and $5x10^{-10}$ M. The limit of detection calculated as 10 % inhibition was $1.8x10^{-11}$ M and is the lowest reported until now. Electrochemical impedance spectroscopy was successfully used as a diagnostic of inhibition. Interferences from other heavy metal ions and organic pesticides were evaluated and the inhibition showed very good selectivity towards Hg^{2+}. The method was successfully applied to the determination of mercury ions in different types of water sample.

Keywords: amperometric biosensor; catalase enzyme; mercury; enzyme inhibition, electrochemical impedance spectroscopy

1. Introduction

Mercury compounds are widely present in air, water and soil as elemental or metallic mercury, inorganic mercury compounds and organic mercury compounds. Mercury is a very toxic pollutant because of its high volatility and the possibility for it to combine easily with many organic compounds [1]. It is toxic due to its deleterious effects on the central nervous system, disturbing haemin synthesis as well as causing neuropsychiatric disorders [2,3]. Upper limits for total mercury concentrations in waters are stipulated in environmental regulations. For example, the US Environmental Protection Agency states that the maximum surface water contaminant level must not be above 2 $\mu g\ L^{-1}$ (10 $nmol\ L^{-1}$) [4]. The development of sensitive and selective methods for mercury determination is thus a very important need.

Mercury can be detected by various analytical methods such as inductively coupled plasma mass spectrometry (ICP-MS) e.g. [5], atomic absorption spectrometry e.g. [6,7], X-.ray fluorescence e.g. [8] and chromatography coupled with atomic emission e.g. [9]. These techniques are highly sensitive and reproducible; however, they have drawbacks such as taking a long time to carry out, using large amounts of chemical reagents and expensive equipment, needing qualified personnel and they cannot be used for field analysis.

Electrochemical approaches are particularly advantageous because of their high sensitivity, low cost and simple operation and can be made into portable sensing systems. Among them, the classical anodic stripping voltammetry [10], is often used, and potentiometric methods have been also developed for mercury determination [11,12]. A different strategy is based on the influence of metal ions on electrochemical signals from DNA or DNAzymes, reviewed in [13]. Within strategies for recognition of metal ions through monitoring of their interaction with DNA oligonucleotides, e.g. [14-17], an important approach for mercury ions involves thymine-thymine (T-T) base pairs in DNA duplexes with specific recognition ability to bind

Hg^{2+} in aqueous solutions e.g. [18-20]. This can lead to extremely low detection limits but the modified electrode architecture is complex.

Another electrochemical approach for heavy metal ion detection is that of enzyme inhibition based electrochemical biosensors, which have been widely reported in recent years and have attractive advantages such as high efficiency, fast response, high selectivity and low detection limit [21-24]. Such inhibition based electrochemical biosensors have been developed for the determination of heavy metal ions such as Hg^{2+}, Pb^{2+}, Cd^{2+} [25], Cr^{3+} [26]. The most commonly used enzymes are tyrosinase [26], horseradish peroxidase [27], urease [28, 29] and acetylcholine esterase [30]. To our knowledge, catalase has not been previously used in enzyme inhibition biosensors for the determination of heavy metal ions. Catalase functions by catalysing the decomposition of hydrogen peroxide into water and oxygen.

In this work, an amperometric catalase based biosensor for the inhibitive determination of toxic mercury ions has been prepared and used for the first time. The experimental conditions are optimized for maximizing the biosensor response, including the effect of concentration of enzyme, enzyme substrate concentration (H_2O_2), incubation time, pH of the supporting electrolyte and applied potential. Electrochemical impedance spectroscopy is used for the first time as a diagnostic tool for mercury inhibition. Comparison is made with previous results in the literature, as well as selectivity towards mercury ions, and determination of mercury ions in different types of water sample is carried out.

2. Experimental

2.1. Reagents

Reagents were all of analytical grade and were used without further purification. Catalase (Cat), bovine serum albumin (BSA) and glutaraldehyde (GA) were purchased from Sigma-Aldrich, Germany. Solutions of hydrogen peroxide were freshly prepared from H_2O_2, 33 %

w/v, from Panreac Química, Spain, and the concentration determined by potassium permanganate (Merck, Germany) titration. Phosphate buffer solutions with various pH values were prepared by mixing standard stock solutions of 0.2 M Na_2HPO_4 (Merck, Germany) and 0.2 M NaH_2PO_4 (Riedel De Haën, Germany) and adjusting the pH with HCl or NaOH, both from Riedel De Haën, Germany. For the inhibition studies, the appropriate amount of $Hg(NO_3)_2$ (Fisher Scientific, U.S.A.) was dissolved in water.

2.2. Instrumentation

Amperometric and voltammetric experiments were performed with an Ivium CompactStat potentiostat (IviumTechnologies B.V., Eindhoven, Netherlands) and impedance measurements were carried out with potentiostat/galvanostat ZRA-Gamry (Gamry Instruments, U.S.A.) reference 600. A conventional three-electrode system was used for all experiments consisting of a 1 mm diameter glassy carbon electrode (GCE) as working electrode, a platinum wire as counter electrode and an Ag/AgCl (3 M KCl) reference electrode.

2.3. Preparation of the catalase biosensor

Catalase (Cat) was immobilised onto the electrode surface by cross-linking with glutaraldehyde (GA) and bovine serum albumin (BSA) in order for the enzyme to be maintained close to its natural environment [31]. In the optimized procedure, a volume of 1 µL of a mixture containing 10 mg mL^{-1} Cat, 40 mg mL^{-1} BSA was pipetted onto the surface of a GCE, followed immediately by 1 µL of 2.5% (v/v) GA, and allowed to dry for 1 h at room temperature. The enzyme modified electrode was designated Cat/GCE. When not in use, the biosensors were stored in phosphate buffer solution at 4 ºC.

2.4. Biosensor response measurements

The enzyme modified electrodes were immersed into a stirred buffer solution and a chosen concentration of hydrogen peroxide (enzyme substrate) was added in order to record a steady-state current (I_0) before adding the inhibitor. The biosensor was incubated with different concentrations of Hg^{2+} to inhibit the enzyme activity, leading to a lower current (I_1), the decrease in current depending on the concentration of inhibitor in solution. The percentage of inhibition (I (%)) was calculated according to the equation [32]:

$$I\,(\%) = \frac{I_0 - I_1}{I_0}\ x\,100$$

3. Results and discussion

3.1. H_2O_2 biosensor

The catalase enzyme biosensor was characterised electrochemically by cyclic voltammetry in the absence and in the presence of hydrogen peroxide. Fig. 1A shows cyclic voltammograms of the enzyme electrode Cat/GCE measured without and with the addition of 2.4 mM H_2O_2 in 0.1 M phosphate buffer, pH 7.0. In supporting electrolyte, no peaks appeared and when peroxide was added, a large increase in the reduction current starting at around -0.4 V was observed.

The influence of the applied potential was investigated by fixed-potential amperometry in the range −0.5 V to 0.0 V vs. Ag/AgCl, see Fig. 1B. The highest response to H_2O_2 was achieved at -0.5 V and then continuously decreased as the applied potential value became less negative: 67 % of this response was obtained at -0.4 V, 30 % at -0.3 V, and only 3 % at 0.0 V. In light of these results, and in order not to use a very negative potential, but to still ensure a good response to peroxide, an applied potential of −0.4 V was selected for amperometric measurements.

The response to hydrogen peroxide was linear from 0.5 to 4.8 mM with a detection limit (S/N=3) of 0.28 mM and sensitivity of 1.23 μA cm^{-2} mM^{-1}, see calibration plot in Fig. 1C.

3.2. Optimization of experimental conditions for inhibition

Amperometric measurements at fixed potential were first performed by successive injections of mercury cations into a stirred phosphate buffer solution, pH 7.0 in the presence of hydrogen peroxide, but no reduction in biosensor response due to the mercury cations was observed. However, incubation of the enzyme biosensor with mercury ions led to inhibition. The response to H_2O_2 was measured before and after incubation with Hg^{2+} and a decrease in the response to enzyme substrate was observed, corresponding to its inhibition. All further measurements were performed in this way.

Experimental parameters that can influence the performance of the inhibition biosensor using amperometry, such as enzyme and substrate concentration, pH of the supporting electrolyte and incubation time with mercury ions were investigated in order to optimize the inhibition response.

3.2.1. Influence of enzyme concentration

The amount of immobilised enzyme leading to the best response towards hydrogen peroxide and Hg^{2+} was evaluated. Different concentrations of 5, 10, 20 and 30 mg mL^{-1} enzyme solution were used, and the immobilization was performed as explained in the experimental section; the response towards peroxide increases with increase in enzyme loading. The inhibition was assessed using the slopes of the calibration curve for hydrogen peroxide before and after incubation with 5 nM Hg (II). The percentage inhibition decreases with increase of enzyme concentration, being 26.3 % for 5 mg mL^{-1}, 3.9 % for 10 mg mL^{-1}, 0.7 % for 20 mg mL^{-1} and no inhibition was observed for 30 mg mL^{-1}. The highest percentage inhibition was obtained for 5 mg mL^{-1}, as would be expected; however, this also corresponds to the lowest

response to hydrogen peroxide. As a compromise, an enzyme concentration of 10 mg mL^{-1} was chosen for future experiments.

3.2.2. Influence of H_2O_2 concentration

The degree of inhibition can be influenced by the substrate concentration. If the inhibitor competes with the enzyme substrate, then an increase of substrate concentration will lead to a decrease of enzyme inhibition by the inhibitor [24]. On the other hand, when the substrate concentration is low, it is easy for it to be catalytically oxidised, and no clear decrease of the response current is observed with the addition of inhibitor. Thus, for an inhibition biosensor the amount of enzyme substrate needs to be carefully adjusted.

The effect of substrate (H_2O_2) concentration on the inhibition of Hg^{2+} was examined. Concentrations of 0.1, 0.5 and 1.0 mM H_2O_2 were used; the response to Hg^{2+} under these conditions is shown in Fig. 2. The maximum inhibition decreased with increase in peroxide concentration. The detection limit, calculated as the concentration of mercury ions leading to 10 % degree of inhibition, I_{10}, [22,33,34] also increased. Hence, an intermediate value of 0.5 mM H_2O_2 was chosen as the best value for the inhibition biosensor.

3.2.3. Influence of pH

The sensitivity of an enzyme biosensor can depend significantly on the pH of the solution. For this reason, the influence of the pH of the supporting electrolyte on the degree of inhibition at the Cat/GCE incubated with 5.0 nM Hg^{2+} at pH values between 6.0 and 8.0 was investigated. The sensitivity of the hydrogen peroxide biosensor decreased by 4.4 % when incubated with mercury ions in pH 6.0 solution. A value of 35.7% inhibition was obtained at pH 7.0, and at pH 8.0 no inhibition was observed, see Table 1. Hence, pH 7.0 was selected as the best pH for further experiments, in agreement with the optimum pH for catalase activity. This pH value has also been used in enzyme inhibition studies for mercury ion determination using other

enzymes, such as glucose oxidase [35,36] and urease [26,37].

3.2.4. Influence of incubation time

The influence of incubation time on the degree of inhibition was investigated using incubation periods of 2, 5, 10 and 15 min with 0.5 mM H_2O_2, Fig. 3. The detection limit decreased and maximum inhibition increased with increasing incubation time. The increase in sensitivity compared with 2 min of incubation was 40 % for 5 min, 84 % for 10 min and 116 % for 15 min. For 10 and 15 min incubation, the highest inhibition and lowest detection limits were achieved. Taking into account that the increase in sensitivity from 10 to 15 min is much less than from 5 to 10 min and that the detection limits were very similar, 1.5 $x10^{-9}$ and 1.1 $x10^{-9}$ M for 10 and 15 min, respectively, a value of 10 min was selected as the incubation time for further amperometric measurements.

3.3. Analytical determination of mercury ions

Under the optimized conditions described above, mercury ions were determined at Cat/GCE at concentrations between $5x10^{-11}$ and $2.5x10^{-9}$ M, (Figure 4) with maximum inhibition 77 %; the response was linear up to $5x10^{-10}$ M, and the detection limit, I_{10}, was 1.8 $x10^{-11}$ M. In some studies, the detection limit has been considered the lowest concentration tested [24,35,38,39], others calculated it based on a signal-to-noise ratio of 3 (S/N=3) [21,33,35,40] and only a few considered the detection limit concentration as that producing 9 % [35] or 10% [38] inhibition. For comparison, the detection limit was also calculated using S/N=3 and the value found was 3.5 $x10^{-11}$ M. Independently of the method of calculation, the present biosensor exhibited the lowest detection limit achieved up until now. The concentration corresponding to 50 % inhibition was reached at 3.5 $x10^{-10}$ M Hg^{2+}. A more complete comparison with most recent literature for Hg^{2+} determination based on enzyme inhibition is summarised in Table 2.

An enzyme inhibition biosensor with a multiwalled carbon nanotubes (MWCNT) modified GCE on which catalase was immobilized was also prepared and tested but gave only a small increase of ~10 % in the sensitivity and similar detection limit. This is not sufficient to justify the use of a more complex architecture biosensor.

3.4. Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) has rarely been used to characterise inhibition biosensors, the only example known being [43]. EIS was done at Cat/GCE at -0.4 V vs. Ag/AgCl, the same potential as in amperometry. The experiments were carried out in buffer solution, with addition of hydrogen peroxide, without and with 10 min incubation with different concentrations of mercury ion between $5x10^{-11}$ and $2.5x10^{-9}$ M. The change in the charge transfer resistance, $R_{ct,}$ can be monitored as a diagnostic of mercury inhibition.

The spectra are shown in Fig. 5A and were all fitted with the same electrical equivalent circuit, Fig. 5B, comprising the cell resistance, R_Ω, in series with a parallel combination of a constant phase element, CPE, and a charge transfer resistance, R_{ct}. The CPE is modelled as a non-ideal capacitor according to the relation $\mathrm{CPE} = -1/(C\ i\omega)^{\alpha}$, where C is the capacitance (describing the charge separation at the double layer interface), ω is the angular frequency and the α exponent expresses the degree of non-uniformity, heterogeneity and roughness of the surface, having a value of 1.0 for a completely smooth and homogeneous surface and 0.5 for a porous electrode.

The values of the parameters obtained from fitting are shown in Table 3; R_Ω was constant at 5 Ω cm^2. Variations occur in the R_{ct} values, but the CPE and α exponent values are essentially constant, as would be expected, the exponent value of 0.88 representing a small degree of non-uniformity, as commonly observed on these types of modified electrode. A large decrease of the R_{ct} value was observed when H_2O_2 was added, corresponding to the occurrence of

charge transfer, Fig. 5A. With Hg^{2+} incubation, R_{ct} begin to increase gradually and a linear dependence on inhibitor concentration was obtained up to $5x10^{-10}$ M, Fig. 5C, in agreement with amperometry. Thus, the use of R_{ct} values from EIS experiments could be an interesting measurement alternative in enzyme inhibition sensors.

3.5. Type of inhibition and reactivation

The response to hydrogen peroxide was evaluated in measurements made after a series of 8 successive incubations with different mercury concentrations, dropping by around 20-25 %, in the absence of any special treatment.

In previous work, up to 70 % recovery after inhibition [38] was achieved for some enzyme biosensors by immersing in phosphate buffer solution [24,38,39], and other biosensors could be regenerated to up to 70 to 90 % of the initial response by using a metal chelating agent, such as EDTA [23,35,36].

To make repeated use of the biosensor possible, its reactivation either in phosphate buffer solution or with 5 mM EDTA was investigated. Thus, after each incubation with mercury ions, the biosensor was left in buffer or EDTA during 10 min. However, no improvement was observed, the same as happened in a trienzymatic system with invertase, mutarotase and glucose oxidase [40,44].

The inhibition of catalase by mercury ions was considered as irreversible, with sensitivity dropping to 75% after a calibration curve (~8 incubations with mercury ions). Hence, a new electrode was prepared for each set of measurements and the reproducibility of the response between different electrodes maintained a relative standard deviation RSD = 4.5 % (n = 5). A similar irreversible reaction was also found for lactate dehydrogenase [41], invertase, mutarotase and glucose oxidase [40,44]. The inhibition is based on irreversible reactions with some amino acid residues such as sulphur groups in the enzyme, which lead to breaks in its

steric structure [45]. It also suggests that the standard addition method should be employed for measurements on unknown samples.

3.6. Selectivity

Selectivity with respect to different interferents, including the cations Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Na^{+} and K^{+}, the anions PO_4^{3-}, SO_4^{2-} and Cl^{-}, as well as the organic pesticides atrazine, cyanazine, pestanal and terbutryn pestanal was studied, in a ratio of 1:2 of analyte to interferents. The biosensor was incubated for 10 min with each of these possible interferents and the response to hydrogen peroxide was measured before and after incubation. The percentage inhibition was calculated using the same procedure as for mercury ions and the results are illustrated in Fig. 6. None of the species tested led to any significant change in inhibition. Measurable inhibition of catalase activity was only exhibited for the following species: 1.4 % Pb^{2+}, 1.3 % Cd^{2+}, 0.4 % Cu^{2+}, 1.0 % atrazine, 1.2 % cyanazine and 1.5 % terbutryn; all values are much lower than the 21 % for Hg^{2+}. These results ensure excellent selectivity of the new inhibition biosensor for the determination of mercury ions. The literature shows that these same cations did not interfere with mercury determination at glucose oxidase based biosensors, where their detection limits, considered as 4 % inhibition, were 500 times higher than that for Hg^{2+} [23]. On the other hand, at a urease biosensor [37], for the same cation concentrations, copper exhibited 27.8% inhibition, a big interference for mercury ions at 57.4 % inhibition, while Cd^{2+} and Pb^{2+} were less active at 2.3% and 9.2% inhibition. There was no report found in the literature concerning pesticide interference on mercury ion determination by enzyme inhibition.

3.7. Application

To demonstrate the feasibility of the biosensor for environmental use, application to the determination of Hg^{2+} in tap water, mineral water and river water by the standard addition

method was examined. No mercury was found in tap and mineral water; however, a small amount of mercury was measured in river water, 0.2 nM. The samples were then spiked with known amounts of Hg^{2+} and the recoveries calculated. Data obtained are shown in Table 4. The recoveries were in the range of 94 to 105 %, which indicates the efficacy of the biosensor for practical analysis.

4. Conclusions

An enzyme biosensor for Hg^{2+} detection based on the inhibitory effect on the activity of catalase, has been developed for the first time, in which catalase was immobilized by cross-linking with glutaraldehyde on a glassy carbon electrode. The Cat/GCE biosensor described here represents an inexpensive, fast and simple method for the analysis of mercury ions. It allows the selective and sensitive determination of Hg^{2+} by fixed-potential amperometry in the presence of other heavy metal ions and organic pesticides, offering a good alternative method for mercury ion trace analysis. The biosensor exhibited the lowest detection limit reported until now at similar biosensors and is promising for application in environmental analysis. Electrochemical impedance spectroscopy can be successfully used as a diagnostic for enzyme inhibition and for quantitative measurements of mercury ion concentration.

Acknowledgements

The authors gratefully acknowledge financial support from the European Commission 7th Framework Programme Marie Curie Actions People IRSES N°294993 SMARTCANCERSENS and from Fundação para a Ciência e a Tecnologia (FCT), Portugal of projects PTDC/QEQ-QAN/2201/2014, in the framework of Project 3599-PPCDT, and of UID/EMS/00285/2013 (both co-financed by the European Community Fund FEDER). MEG thanks FCT for a postdoctoral fellowship SFRH/BPD/103103/2014.

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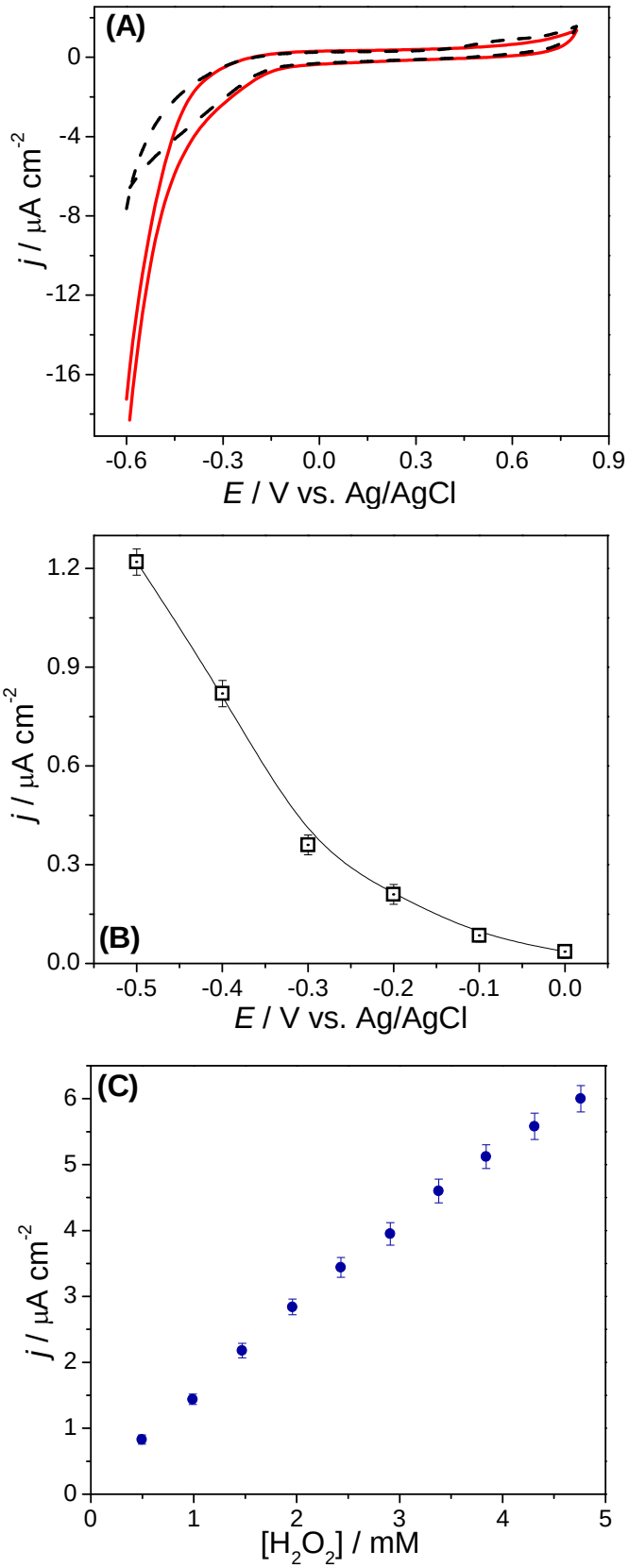

Fig.1. (A) Cyclic voltammograms at Cat/GCE (--) without and (-) with 2.4 mM H_2O_2,scan rate 50 mV s^{-1},(B) Influence of the applied potential vs Ag/AgCl on the response to 0.5 mM H_2O_2, (C) Calibration curve for H_2O_2 at -0.4 V in 0.1 M phosphate buffer, pH 7.0.

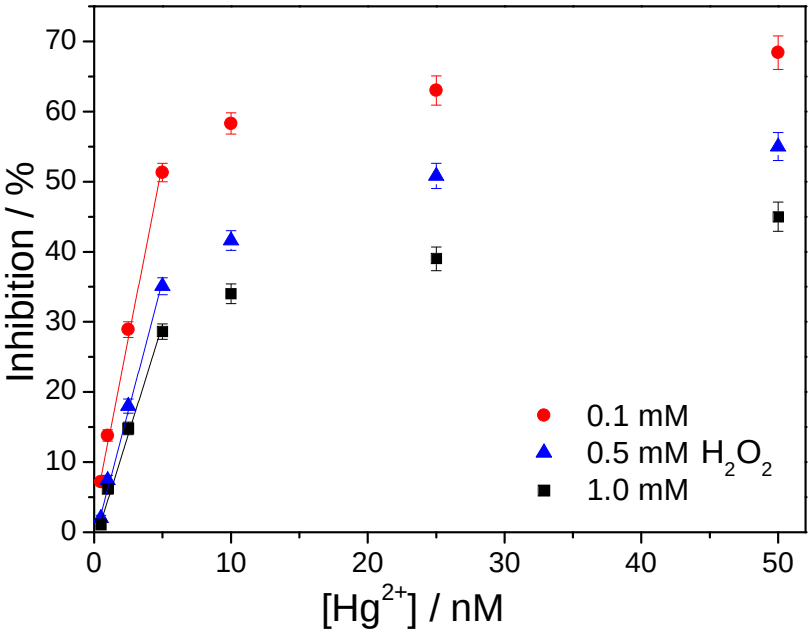

Fig.2. Inhibition by Hg^{2+} at different H_2O_2 (enzyme substrate) concentrations: (●) 0.1, (▲) 0.5 and (■) 1.0 mM in 0.1 M phosphate buffer, pH 7.0 at -0.4 V vs Ag/AgCl.

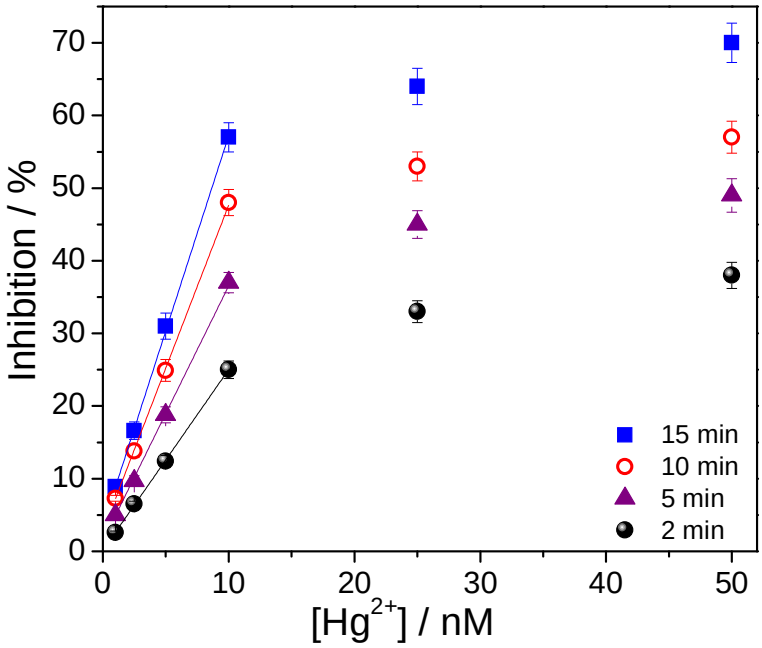

Fig.3. Inhibition by Hg^{2+} after different incubation times (●) 2, (▲) 5, (○) 10 and (■) 15 min, in 0.1 M phosphate buffer pH 7.0 at -0.4V vs Ag/AgCl; [H_2O_2] = 0.5 mM.

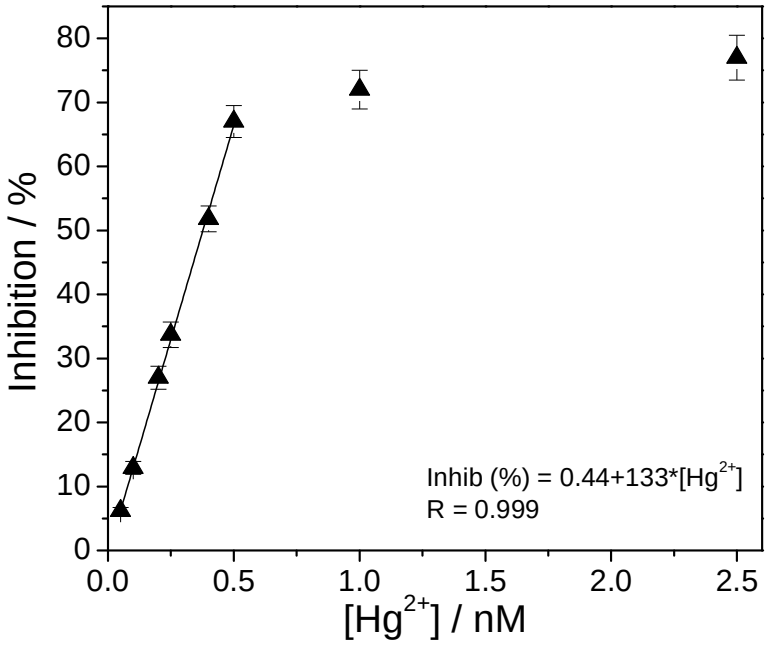

Fig. 4. Calibration curve for catalase inhibition by Hg^{2+} at Cat/GCE in 0.1 M phosphate buffer pH 7.0 at -0.4 V vs Ag/AgCl; 10 min incubation, $[H_2O_2]$ = 0.5 mM.

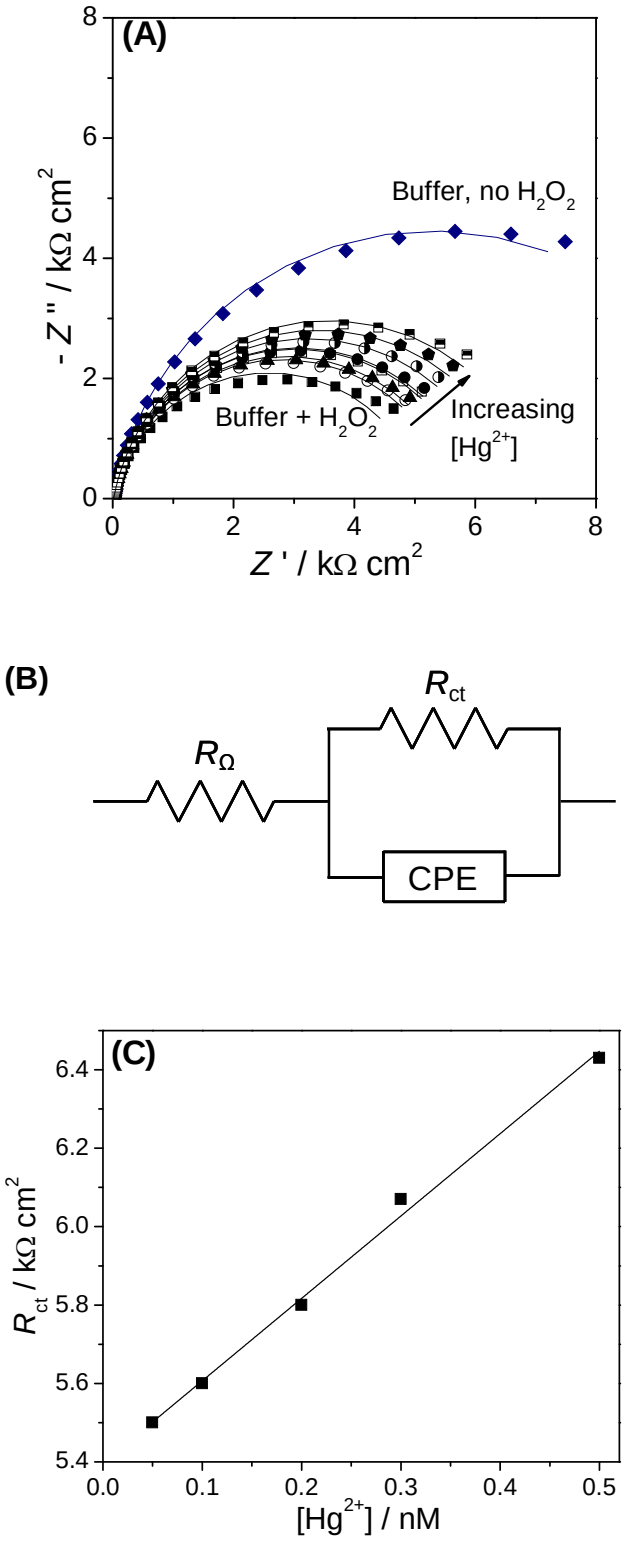

Fig. 5. (A) Impedance spectra of Cat/GCE in phosphate buffer pH 7.0 at -0.4 V vs Ag/AgCl, and in the presence of 1.0 mM H_2O_2 without Hg^{2+} and with increasing Hg^{2+} concentrations of 0.05, 0.1, 0.2, 0.3, 0.5, 1.0, 2.5 nM after incubation for 10 min; (B) Circuit used to fit the spectra in (A); (C) Dependence of R_{ct} on Hg^{2+} concentration. Lines in (A) show fitting to the equivalent circuit in (B).

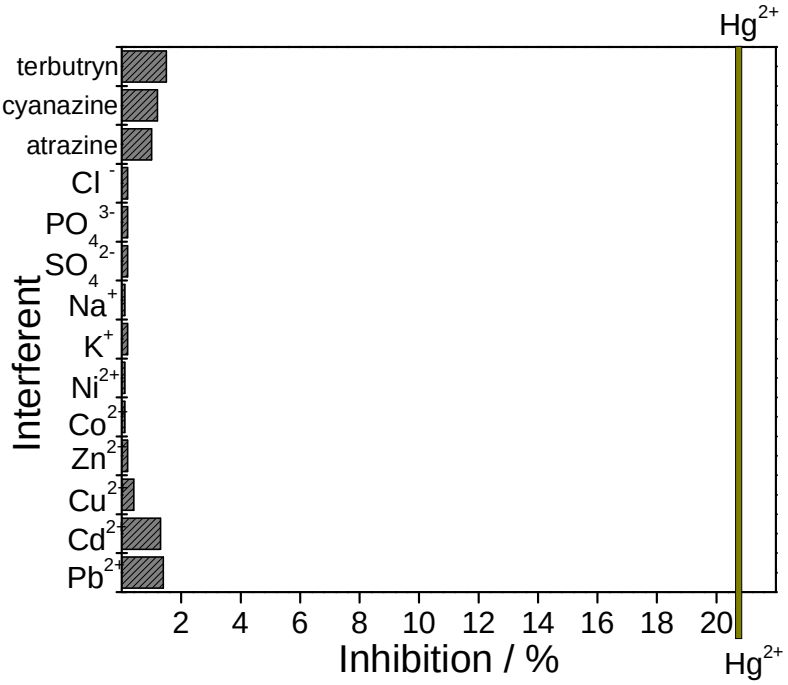

Fig. 6. Inhibition at Cat/GCE caused by different interferents compared with Hg^{2+} (ratio 2:1) in 0.1 M phosphate buffer pH 7.0 at −0.4 V vs Ag/AgCl.

Tables

Table 1. The influence of the pH of the 0.1 M phosphate buffer supporting electrolyte solution on the sensitivity of H_2O_2 determination at Cat/GCE

pH	Sensitivity / μA cm^{-2} mM^{-1}		Inhibition (%)
	$[Hg^{2+}]$ / nM		
	0.0	5.0	
6.0	1.15	1.10	4.4
7.0	1.23	0.79	35.7
8.0	1.05	1.05	0.0

Table 2. Comparison of Hg^{2+} determination at different inhibition-based biosensor configurations. Detection mode is constant potential amperometry.

Biosensor	Applied potential and pH	Linear range / M	Incubation	Detection limit / M	I_{C50} / M	reactivation	Inhibition type	Ref.
GOx/MnO_2/CPE	+0.46 V vs. Ag/AgCl pH 7.0	$6.1x10^{-6}$-$1x10^{-4}$	--	$1.5x10^{-6}$	n.d.	0.1 M EDTA (2 min; 70-90%)	n.d.	[23]
GOx/PANI-Fc/Pt	+0.7 V vs. SCE pH 2.55	$1.5x10^{-9}$-$2.4x10^{-6}$ $2.4x10^{-6}$-$7.8x10^{-5}$	--	$1.5x10^{-9}$	n.d.	phosphate buffer pH 7.0 (8 min)	competitive	[24]
Urease/AuNP/SPE	+1.5 V vs. Ag/AgCl pH 7.0	$6x10^{-9}$-$6x10^{-8}$	--	$5.6x10^{-8}$	n.d.	-	non-competitive	[26]
GOx-PPy/Pt	+0.7 V vs. Ag/AgCl pH 7.0	$4.8x10^{-7}$-$3.3x10^{-6}$	--	$4.8x10^{-7}$	$1.5x10^{-6}$	2 mM EDTA (10s)	non-competitive	[35]
GOx/PPDA/Pt	+0.7 V vs. SCE pH 7.0	$5.0x10^{-6}$-$180x10^{-6}$	--	$2.5x10^{-6}$	$22x10^{-6}$	0.1 M EDTA (1h; 90%)	reversible	[36]
Urease/PVF/Pt	+0.7 V vs. SCE pH 7.0	$9.2x10^{-6}$-$4.2x10^{-4}$	10 min	$7.4x10^{-6}$	~~3~~$31x10^{-6}$	-	n.d.	[37]
Inv/Mut/GOx	+0.6 V vs. Ag/AgCl pH 6.0	$3x10^{-8}$-$1x10^{-4}$	20 min	$3x10^{-8}$	$1x10^{-6}$	10mM cysteine (15 min; 20-40 %)	irreversible	[40]
LDH/PGA-Py/Au	+0.2 V vs. Ag/AgCl pH 7.5	-	-	$5x10^{-10}$	$25x10^{-10}$	-	irreversible	[41]
Urease-GlDH/SPE	+0.3 V vs. Ag/AgCl pH 8.0	3 $x10^{-9}$-$300x10^{-9}$	15 min	$0.2x10^{-7}$	$5.2x10^{-7}$	-	non-competitive	[42]
Cat/GCE	-0.4 V vs. Ag/AgCl pH 7.0	$5x10^{-11}$-$5x10^{-10}$	10 min	$1.8x10^{-11}$	$3.5x10^{-10}$	-	irreversible	This work

PPy – polypyrrole; PPDA – poly-o-phenylenediamine; PGA-Py – poly glutaraldehyde-pyrrole; PANI – polyaniline; PVF – poly(vinylferrocenium); Fc – ferrocene; ZnO-NR – zinc oxide nanorods; AuNP – gold nanoparticles; SPE – screen printed electrode; CPE – carbon paste electrode; GCE – glassy carbon electrode; GOx – glucose oxidase; LDH – lactate dehydrogenase; Inv – invertase; Mut – mutarotase; GlDH – glutamate dehydrogenase; Cat – catalase; n.d.-not determined

Table 3. Data obtained from equivalent circuit fitting of the impedance spectra for the Cat/GCE in 0.1 M phosphate buffer, pH 7.0 at -0.4 V vs. Ag/AgCl.

Solution	R_{ct} / kΩ cm^2	CPE / μF cm^{-2} $s^{\alpha-1}$	α
buffer	10.58	110	0.89
+ 1.0 mM H_2O_2	5.13	114	0.87
+ 0.05 nM Hg^{2+}	5.52	110	0.88
+ 0.1 nM Hg^{2+}	5.62	109	0.88
+ 0.2 nM Hg^{2+}	5.83	106	0.88
+ 0.3 nM Hg^2	6.07	107	0.88
+ 0.5 nM Hg^2	6.43	106	0.88
+ 1.0 nM Hg^2	6.77	106	0.88
+ 2.5 nM Hg^2	7.12	104	0.88

Table 4. Analysis of natural samples and recovery measurements at Cat/GCE at -0.4 V in 0.1 M phosphate buffer, pH 7.0

Sample	Measured / nM	Added / nM	Expected / nM	Found / nM	Recovery (%)
Tap water	0.0	0.2	0.2	0.21±0.01	105.0
		0.5	0.5	0.48±0.02	96.0
Mineral water	0.0	0.2	0.2	0.19±0.01	95.0
		0.5	0.5	0.47±0.03	94.0
River water	0.2	0.2	0.4	0.39±0.02	97.5
		0.5	0.9	0.87±0.05	96.6