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Highly Sensitive Choline Oxidase Enzyme Inhibition Biosensor for Lead Ions Based on Multiwalled Carbon Nanotube Modified Glassy Carbon Electrodes

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Abstract: The determination of lead ions by inhibition of choline oxidase enzyme has been evaluated for the first time using an amperometric choline biosensor. Choline oxidase (ChOx) was immobilized on a glassy carbon electrode (GCE) modified with multiwalled carbon nanotubes (MWCNT) through cross-linking with glutaraldehyde. In the presence of ChOx, choline was enzymatically oxidized into betaine at -0.3 V versus Ag/AgCl reference electrode, lead ion inhibition of enzyme activity causing a decrease in the choline oxidation current. The experimental conditions were optimised regarding applied potential,

buffer pH, enzyme and substrate concentration and incubation time. Under the best conditions for measurement of the lowest concentrations of lead ions, the ChOx/MWCNT/GCE gave a linear response from 0.1 to 1.0 nM Pb^{2+} and a detection limit of 0.04 nM. The inhibition of ChOx by lead ions was also studied by electrochemical impedance spectroscopy, but had a narrower linear response range and low sensitivity. The inhibition biosensor exhibited high selectivity towards lead ions and was successfully applied to their determination in tap water samples.

Keywords: amperometric choline biosensor • choline oxidase • electrochemical impedance spectroscopy • inhibition by lead ions • multiwalled carbon nanotubes

1 Introduction

Lead is used in various applications, such as car batteries, ammunition, piping, paints, anti-radiation screens, tin-based welding alloys [1]; however, many of them are currently being curtailed due to increasing concern about lead toxicity. Lead is not degraded under environmental conditions and can therefore be easily accumulated in bones and tissues [2]. Long exposure to high levels of lead can cause hemotoxic and cardiovascular effects, reproductive dysfunction, gastrointestinal tract alterations and nephropathies [1,2].

Classical methods for lead determination rely on techniques such as flame atomic absorption spectrometry [3] and inductively coupled plasma optical emission spectrometry [4]. Nevertheless, these methods require expensive instrumentation and are difficult to use out of the laboratory, i. e. direct online measurements in the field without pre-treatment are not possible. Electrochemical methods are a good alternative to the classical techniques, in terms of simplicity, relative low cost of analysis and portability, in addition to the possibility of online monitoring. Lead ion-selective electrodes have been reported [5,6], but most of them possess narrow working concentration ranges and suffer serious interferences from various other cations.

As an alternative, or as a complement, to conventional methods, inhibition-based electrochemical enzyme biosensors have emerged for toxicity analysis and environmental monitoring. These biosensors offer several advantages

such as minimum sample pre-treatment, low time of analysis, high sensitivity and selectivity, as well as low detection limits [7–9]. The detection principle of electrochemical enzyme inhibition biosensors is based on the selective inhibition of the immobilised enzyme's activity by the target analyte, resulting in a decrease of the electrochemical signal that is proportional to the amount of target analyte present in the test solution. Several toxic metal ions, such as lead, mercury and cadmium exhibit inhibition effects toward different enzymes; hence, several amperometric [10–13], potentiometric [14,15] and conductometric [16–18] biosensors have been developed for their measurement. Electrochemical determination of lead ions by enzyme inhibition has been reported for several enzymes: urease [16], alkaline phosphatase [17], nitrite reductase [18], peroxidase [11,19] and glucose oxidase [20,21], some [11,19,20] being based on amperometric detection.

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A few mono- and bienzymatic electrochemical biosensors based on choline oxidase (ChOx) have been used for the determination of nicotine [22,23] or mustard agents [24] by inhibition; however, to our knowledge, there is no report on heavy metal ion determination based on inhibition of ChOx. The preparation of the reported ChOx inhibition biosensors is complex, either the enzyme is loaded in microgels and held by a dialysis membrane [22] or a nylon net is placed over the transducer surface [23], which may contribute to loss of enzyme activity.

In the present work, a simple, easy-to-prepare amperometric biosensor for Pb^{2+} assay is described. Choline oxidase was immobilised on the MWCNT/GCE through cross-linking with glutaraldehyde. In this configuration, choline oxidises at a relatively low potential of -0.3 V vs. Ag/AgCl and Pb^{2+} inhibits the enzyme activity by decreasing its oxidation current. The experimental conditions for the analytical determination of Pb^{2+} by fixed potential amperometry were optimised. Electrochemical impedance spectroscopy was also used as diagnostic for enzymatic inhibition. Selectivity towards lead ions and application of the biosensor to tap water analysis are discussed.

2 Experimental

2.1 Reagents and Instrumentation

Choline oxidase (ChOx, from *Alcaligenes sp.*, 14 U/mg), choline chloride, chitosan (Chit) of low molecular weight with a degree of deacetylation of 80%, bovine serum albumin (BSA), glutaraldehyde (GA, 25% v/v in water), were supplied by Sigma-Aldrich, Germany. Multi-walled carbon nanotubes (MWCNT) with ~95% purity, $30 \pm 10\text{ nm}$ diameter and $1\text{--}5\text{ }\mu\text{m}$ length were from NanoLab, U.S.A. $\text{Pb}(\text{NO}_3)_2$ (99%) was purchased from Merck, Germany. All solutions were prepared using Millipore Milli-Q nanopure water (resistivity $> 18\text{ M}\Omega\text{ cm}$). The supporting electrolyte was sodium phosphate buffer saline (NaPBS) at different pH values, prepared from $0.1\text{ M NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ to which 0.05 M NaCl was added. Aliquots of solutions of 2.0 M HCl and 5.0 M NaOH were used for the adjustment of pH. Amperometric experiments were performed with an Ivium CompactStat electrochemical analyser. Electrochemical impedance spectroscopy (EIS) measurements were done with a Gamry potentiostat/galvanostat/ZRA. A sinusoidal voltage perturbation of 10 mV amplitude was applied, scanning from 65 kHz to 0.1 Hz with 10 points per frequency decade.

The three-electrode system consisted of a glassy carbon electrode (GCE) with a surface area of 0.00785 cm^2 as working electrode, a Ag/AgCl as reference electrode and a Pt wire as counter electrode. All experiments were carried out at room temperature ($25 \pm 1\text{ }^\circ\text{C}$).

2.2 Preparation of the Enzyme-Modified Electrode

MWCNT were functionalized with carboxylate groups by using nitric acid, washed until neutral solution obtained and dried in an oven at $80\text{ }^\circ\text{C}$ for 24 h, as described in [25]. A solution of 0.2% MWCNT was prepared by dissolving 2 mg of MWCNT in 1 mL of 1% chitosan solution previously prepared in 1% acetic acid. MWCNT were immobilised by dropping $1\text{ }\mu\text{L}$ of the 0.2% solution on the GCE surface, the resulted electrode was designed as MWCNT/GCE. ChOx was immobilized by cross-linking with glutaraldehyde and bovine serum albumin. For this, $1\text{ }\mu\text{L}$ of the solution containing 30 mg mL^{-1} ChOx and 40 mg mL^{-1} BSA was dropped onto the MWCNT/GCE and immediately $1\text{ }\mu\text{L}$ of GA (2.5%) was added, then left to react for at least 1 h at room temperature. The ChOx/MWCNT/GCE electrode exhibited no response if used immediately, but responded well after keeping overnight in 0.1 M NaPBS ($\text{pH}=8.5$) at $4\text{ }^\circ\text{C}$ before use.

2.3 Procedure for Enzyme Inhibition Studies

The ChOx-modified MWCNT/GCE was dipped into a stirred 0.1 M NaPBS solution ($\text{pH } 8.5$) to which an appropriate amount of enzyme substrate was added and the steady-state current was recorded, I_0 . Following this, the electrode was incubated with different concentrations of Pb^{2+} solution (from 1 to 50 nM) for different incubation times (2, 5, 10 and 15 min) then placed again into 0.1 M NaPBS solution ($\text{pH}=8.5$); the same amount of substrate was added and the current again registered, I_1 . The functioning of the inhibitor by blocking the active site of the enzyme is shown schematically in Figure 1. The current decrease is proportional to the final concentration of inhibitor in solution. The percentage inhibition ($I\%$) due to Pb^{2+} was calculated using the relationship [7],

$$I(\%) = \frac{I_0 - I_1}{I_0} \times 100 \quad (1)$$

In the case of electrochemical impedance spectroscopy (EIS), the inhibition was calculated considering the values of the charge transfer resistance, R_{ct} . Since in this case, there is an increase in charge transfer resistance value after inhibition, the equation was modified to:

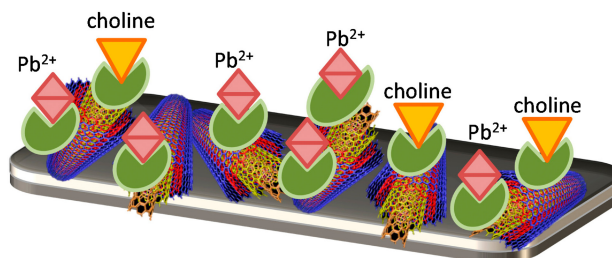


Fig. 1. Schematic functioning of the biosensor showing Pb^{2+} inhibition by blocking the active site of the enzyme.

$$I(\%) = \frac{R_{ct1} - R_{ct0}}{R_{ct0}} \times 100 \quad (2)$$

3 Results and Discussion

3.1 Optimization of the Inhibition Biosensor

Experimental Conditions

In order to prepare a simple inhibition biosensor, the choline oxidase enzyme was first immobilised on top of a glassy carbon electrode without any modification, ChOx/GCE. The performance of this electrode for choline determination and, implicitly, for lead ion measurement by incubation was poor. For this reason, the glassy carbon electrode was modified with carbon nanotubes and then enzyme immobilised on top, ChOx/MWCNT/GCE. Under the same experimental conditions, the response to choline greatly increased and hence this biosensor configuration was used for further inhibition measurements.

Different parameters were studied for optimised Pb^{2+} determination by inhibition, including applied potential, pH of the supporting electrolyte, enzyme and substrate concentration and incubation time.

3.1.1 Effect of the Applied Potential

The effect of operating potential on the performance of the electrode based on ChOx/MWCNT/GCE was optimized in 0.1 M NaPBS, pH 8.5 in the range from -0.3 to $+0.4$ V vs. Ag/AgCl. The response to choline decreased with less negative potential up to 0.0 V and then increased again for more positive potential values, Figure 2 A. Choline oxidase catalyses the oxidation of choline into betaine, with production of hydrogen peroxide. The response at $+0.3$ and $+0.4$ V was due to hydrogen peroxide oxidation. Between -0.2 and $+0.2$ V reduction occurs, and at -0.3 V an anodic change in current was observed.

To understand these observations, the response to choline in the absence of only residual oxygen, as well as in oxygen-saturated solution was studied, at -0.3 V. When N_2 was bubbled through the solution to remove dissolved oxygen, the response observed was a small cathodic change in the current. When O_2 was bubbled through the solution to saturate it with oxygen, there was a large increase in the anodic response by a factor of 5. Deoxygenation can never remove all oxygen, especially at CNT-modified electrodes where it can be trapped within the nanotube network under the enzyme layer; hence, there is small amount of hydrogen peroxide produced from this oxygen, which can be further reduced to water. Choline oxidase needs oxygen to function properly and so in the absence of oxygen, the reaction of FAD regeneration is also decreased. In oxygen-saturated solution FAD regeneration is increased. The overall process at the electrode is the result of a competition between enzyme cofactor FAD regeneration (oxidation current) and hydrogen peroxide reduction, as proposed for other oxidase enzymes [25,26].

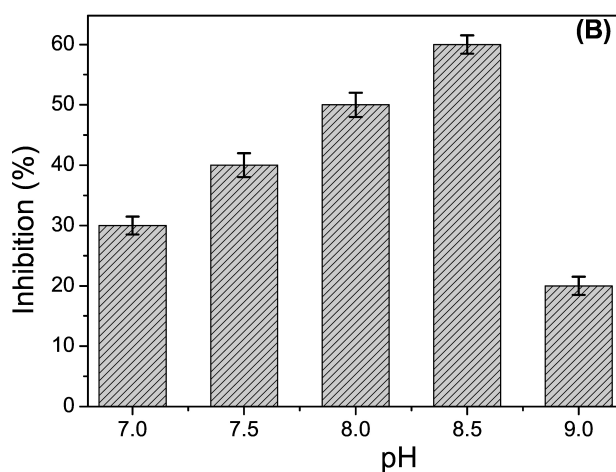
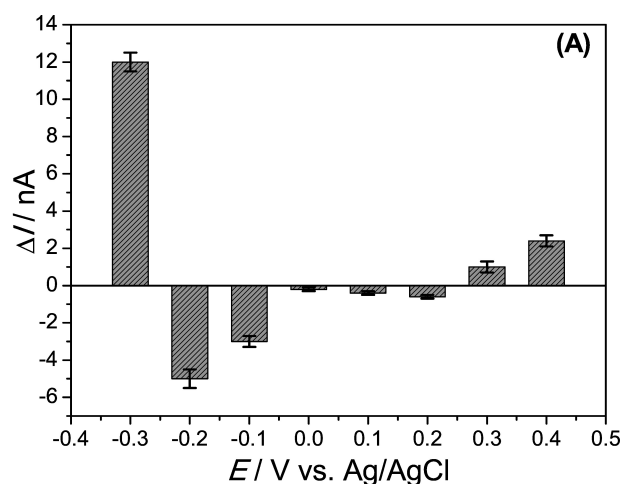


Fig. 2. (A) Response to $20 \mu\text{M}$ choline at ChOx/MWCNT/GCE at different applied potentials in 0.1 M NaPBS, pH 8.5; (B) Influence of pH on the inhibition at ChOx/MWCNT/GCE in 0.1 M NaPBS after incubation for 5 min with 2.5 nM Pb^{2+} in the presence of $20 \mu\text{M}$ choline.

In the range of potentials tested, the highest signal was achieved at -0.3 V, dropping by 58% at -0.2 V; since -0.3 V was independently investigated and shown to be free of interferences [27], this potential was chosen for further study.

3.1.2 Influence of pH

The influence of the pH of the supporting electrolyte over the range 7.0 – 9.0 on the amperometric response of the biosensor to a fixed concentration of $20 \mu\text{M}$ choline, after incubated for 5 min with 2.5 nM Pb^{2+} was studied, Figure 2B. The results indicate an increase of inhibition with increase in pH, up to pH 8.5, and then a decrease. This pH value is also in agreement with the best value for

choline oxidase in the absence of inhibitor, found in independent studies [27].

3.1.3 Influence of ChOx Concentration

An increase in enzyme concentration can enhance the electrode response; however, a thick enzyme layer can also act as diffusional barrier [28]. In order to verify the influence of the enzyme concentration on lead inhibition, its loading was varied. Different concentrations of ChOx enzyme (10, 20, 30 and 40 mg/mL) were studied, and the inhibition for 1.0 nM Pb^{2+} measured, Figure 3 A. When the concentration of ChOx increases, the inhibition decreased: 44% for 10 mg mL⁻¹, 36% for 20 mg mL⁻¹, 13% for 30 mg mL⁻¹ and no inhibition occurred for 40 mg mL⁻¹. Taking into account this behaviour and that lower loading of enzyme is desirable for reducing biosensor cost, 10 mg mL⁻¹ choline oxidase was used for preparing the biosensor.

3.1.4 Influence of Choline Concentration

For an inhibition biosensor, the concentration of substrate has to be carefully adjusted in order to have a reliable response. Low substrate concentrations can lead to an unclear inhibition effect, whereas high concentrations of substrate can lead to significant competition in the case of competitive inhibition. The influence of substrate concentration on the determination of lead ions was evaluated. The response to three concentrations of choline: 20, 50 and 100 μM was measured after the biosensor was incubated for 5 min with different Pb^{2+} concentrations between 0.1 and 2.5 nM. The inhibition caused by lead ions tends to decrease on increasing the choline concentration, Figure 3B. With 50 and 100 μM choline, it was not possible to reach 50% inhibition; hence, it is preferable to perform the measurements with lower concentration and 20 μM choline was chosen.

3.1.5 Effect of Incubation Time

Different incubation times of 2, 5, 10 and 15 min were tested and the inhibition for different lead ion concentrations from 1.0 to 50 nM evaluated. In Figure 3C, inhibition in the presence of 1.0 and 2.5 nM Pb^{2+} is illustrated and the behaviour was similar. The inhibition increased with increasing incubation time from 2 to 5 min incubation, above which only a small increase was observed, hence not justifying the increase of the measurement time. In the light of these results, 5 min was chosen as incubation time in further experiments.

3.2 Analytical Inhibition Studies with Pb^{2+}

3.2.1 Fixed Potential Amperometry

Under optimised conditions, namely 0.1 M NaPBS, pH 8.5, applied potential -0.3 V, 10 mg mL⁻¹ choline oxidase,

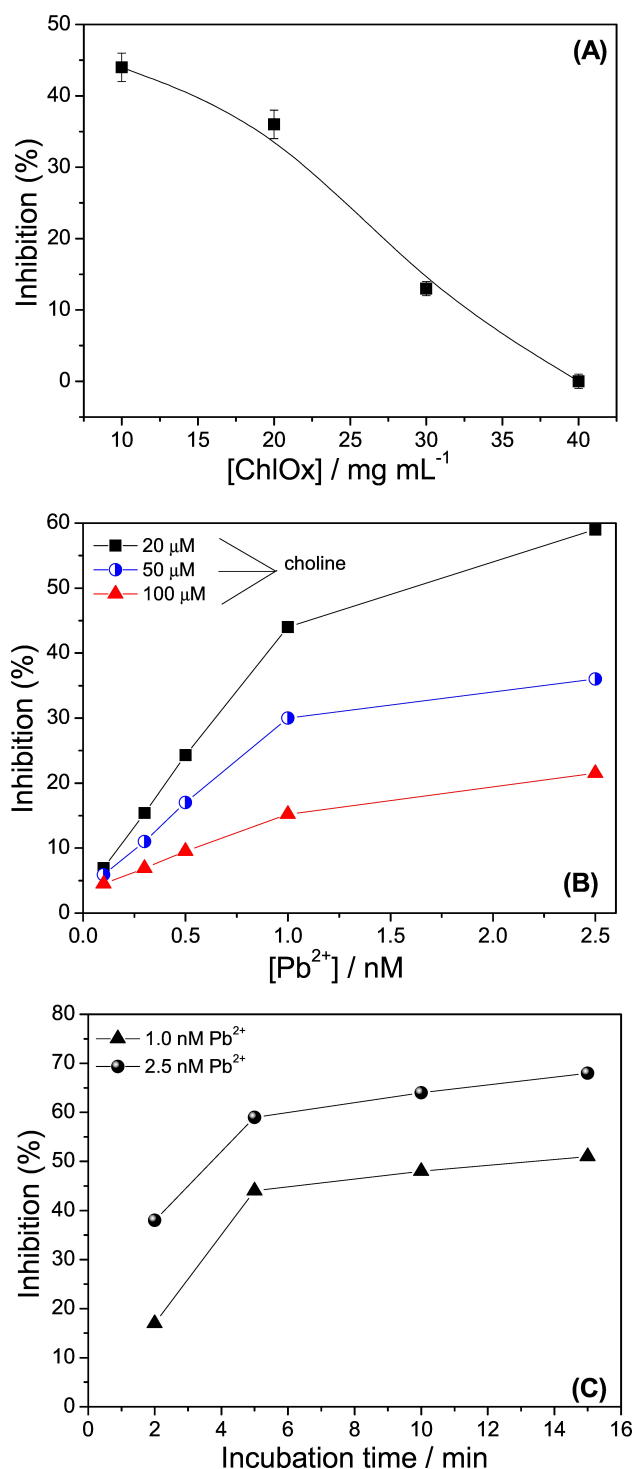


Fig. 3. The influence of (A) enzyme concentration; (B) substrate concentration and (C) incubation time on inhibition at ChOx/MWCNT/GCE in 0.1 M NaPBS, pH 8.5, after incubation with (A) 1.0; (B) 2.5 and (C) 1.0 and 2.5 nM Pb^{2+} . Incubation time in (A) and (B) 5 min. Concentration of choline in (A) and (C) 20 μM .

20 μM choline and 5 min incubation time, the determination of lead ions was possible from 0.1 to 50 nM, Figure 4, exhibiting a maximum inhibition of 97%. The

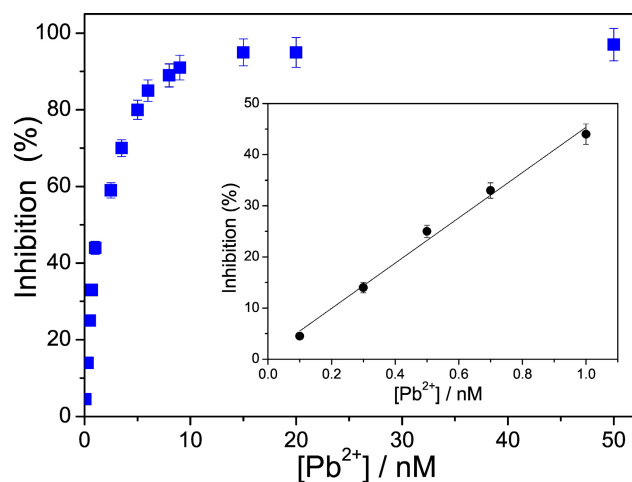


Fig. 4. Inhibition at ChOx/MWCNT/GCE in 0.1 M NaPBS, pH 8.5, after 5 min incubation with different concentrations of Pb^{2+} in the presence of 20 μM choline. Inset, linear response for lead ion inhibition.

response was linear up to 1.0 nM with a sensitivity of 44% nM^{-1} and the detection limit was 0.04 nM ($S/N=3$). In inhibition studies, different suggestions exist for the calculation of the limit of detection (LOD). The LOD has been reported as 5% [29], 10% [30] or even 20% [31] enzyme inhibition, since the enzyme substrate concentration has an influence on the detection limit. In order to avoid confusion, Amine et al. [7] recommend that 10% inhibition, I_{10} , should be considered as detection limit. Hence, I_{10} was determined for this biosensor and the value was 0.2 nM. Comparison with the literature for lead ion determination by enzyme inhibition is presented in Table 1; there is no other biosensor based on enzyme inhibition with a lower detection limit than that achieved

by the present biosensor. The concentration of inhibitor producing 50% inhibition, I_{50} , can be easily determined from the plot of degree of inhibition versus inhibitor concentration, and from Figure 4 this was estimated to be 1.55 nM. In [34], it was demonstrated that exists a unique relationship between the degree of inhibition, y , and the concentration of inhibitor, $[I]$, and I_{50} :

$$y = \frac{[I]}{[I] + [I_{50}]} \quad (3)$$

From equation 3, considering the maximum inhibition of 97% which was achieved for an inhibitor concentration of 50 nM, the I_{50} value was calculated to be exactly 1.55 nM, the same value as that obtained from Figure 4.

The mechanism of inhibition was assessed by using two complementary plots, the Dixon [35] and the Cornish-Bowden [36], in the presence of three different choline concentrations. From the Dixon plot, Figure 5 A, in which the inverse of the enzyme activity is represented versus the inhibitor concentration, it can be deduced that inhibition by lead ions is competitive or mixed, since there is an interception of the lines on the left side of the y axis. However, by using the Cornish-Bowden representation of the ratio of the substrate concentration and enzyme activity versus the inhibitor concentration, parallel lines resulted, Figure 5B showing that the inhibition is competitive. Competitive inhibition was also observed in [33] for lactate dehydrogenase. Different mechanisms were found for lead inhibition when using various enzymes: mixed [11] or non-competitive [19] inhibition for horseradish peroxidase, mixed [20] or irreversible [28] inhibition for glucose oxidase. Further evidence that the mechanism of inhibition by lead cations is competitive is the fact that inhibition decreases on increasing the amount of substrate, from 59% in the presence of 20 μM choline to 36%

Table 1. Determination of lead by enzyme inhibition with different biosensors

Electrode configuration	Experimental conditions	Linear range / nM	LOD / nM	I_{10} / nM	Type of inhibition	K_i / nM	Ref
HRP/MT-MWCNT/GCE	pH 7.0 -0.3 V (Ag/AgCl)	277-1660	7.5	-	mixed	7800	[11]
HRP/PANI/PtE	pH 7.0 -0.2 V (Ag/AgCl)	14.3-122	0.09	-	non-competitive	-	[19]
GOx/PNR/CFE	pH 7.0 -0.35 (SCE)	250-1388	9.0	-	mixed	13500	[20]
GOx-Inv/PtUME	pH 5.5 +0.35 V (Ag/AgCl)	50-250	30	112	irreversible	-	[28]
LDH/Clark Electrode	pH 7.0 -0.7 V (Ag/AgCl)	-	200	-	competitive; irreversible	20000	[32]
Urease/NSPN/Au/Al ₂ O ₃ E	pH 7.0 -0.27 (Ag/AgCl)	300-3000	300	-	irreversible	-	[33]
ChOx/MWCNT/GCE	pH 8.5 -0.3 V (Ag/AgCl)	0.1-1.0	0.04	0.2	competitive	0.9	This work

GOx-glucose oxidase; HRP-horseradish peroxidase; Inv-invertase; LDH-lactate dehydrogenase; ChOx-choline oxidase; PPD-poly-phenylenediamine; AuNP-gold nanoparticles; MT-MWCNT-maize tassel-multi walled carbon nanotubes; PANI-polyaniline; PNR- poly (neutral red); NSPN-nanostructured polyaniline-Nafion; GCE-glassy carbon electrode; PtE-platinum electrode; PtUME-platinum ultra-microelectrode; CFE- carbon film electrode; Al₂O₃E-alumina electrode

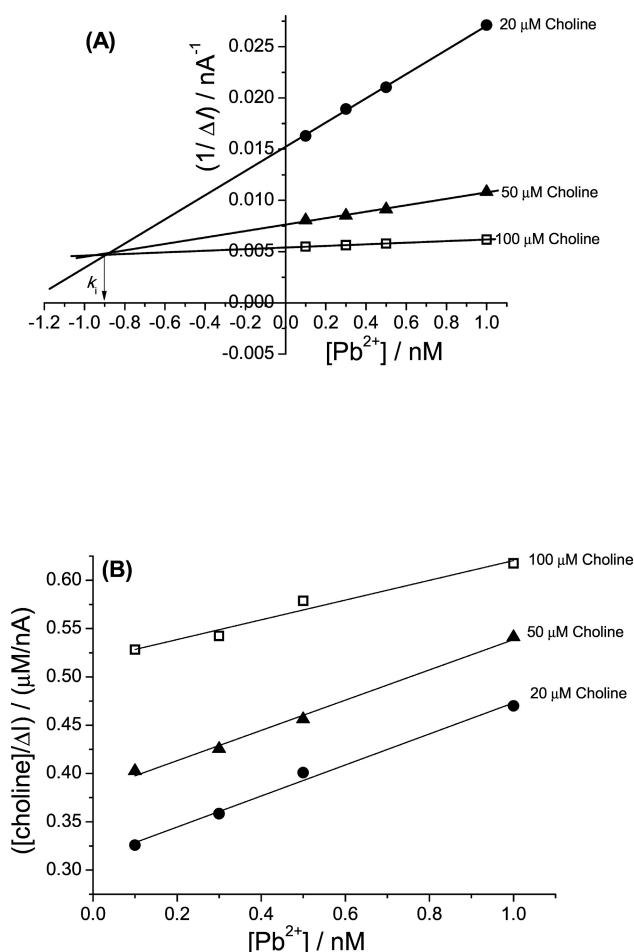


Fig. 5. (A) Dixon and (B) Cornish-Bowden plots for lead ion inhibition for three different choline concentrations: 20, 50 and 100 μM .

in the presence of 50 μM choline and 21% for 100 μM choline. The inhibition constant, K_i , which measures the affinity of the enzyme to the inhibitor, was determined from the Dixon plot and was 0.9 nM.

3.2.2 Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy was used to characterise the modified electrode and also to assess the possibility of using EIS as a sensing technique for quantitative determination of the degree of inhibition.

Impedance spectra were recorded at ChOx/MWCNT/GCE in 0.1 M NaPBS, pH 8.5 and after addition of choline, plus different concentrations of lead ion in the range 0.2 to 8.0 nM, with 5 min incubation, Figure 6 A. The spectra were fitted to the electrical equivalent circuit presented in Figure 6B. This comprises the cell resistance, R_Ω , in series with a parallel combination of a charge transfer resistance, R_{ct} , and a double layer capacitor, C_{DL} (for the high frequency region semicircle). This parallel combination is followed in series by an open Warburg element (for intermediate frequencies) and finally by a

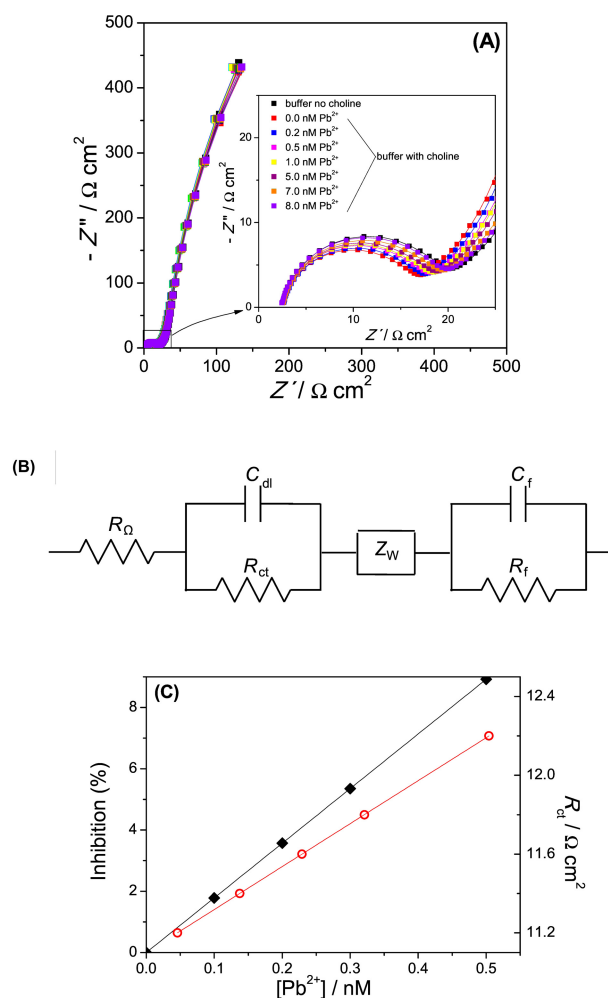


Fig. 6. (A) Impedance spectra at ChOx/MWCNT/GCE in 0.1 M NaPBS, pH 8.5 and in the presence of 20 μM choline without and with incubation for 5 min for increasing Pb^{2+} concentrations; (B) Circuit used to model the spectra in (A). (C) Dependence of R_{ct} and degree of inhibition on Pb^{2+} concentration. Lines in (A) show equivalent circuit fitting.

second parallel combination of a film resistance, R_f , and the film capacitance, C_f (low frequency region) representing the electrode's modifier layers. The open Warburg element is expressed by $Z_w = R_w \text{cth}[(\tau i \omega)^\alpha](\tau i \omega)^{-\alpha}$, where $\alpha \leq 0.5$, τ is the diffusional time constant and R_w the diffusional resistance.

Values of the parameters obtained by fitting the spectra are shown in Table 2. The value of R_Ω is constant at 2.1 $\Omega \text{ cm}^2$. Except for R_{ct} , values of all parameters remain constant within experimental error. Values of R_f and C_f , corresponding to the modifier film, are much larger than those of R_{ct} and C_{DL} , as predicted. There is a decrease in R_{ct} when choline is added to the buffer solution, owing to the occurrence of the enzymatic reaction. After incubation with lead ions, the value of R_{ct} increased with lead concentration, as would be expected if there is enzyme inhibition. A plot of R_{ct} vs. lead ion concentration gave a linear response up to 0.5 nM Pb^{2+}

Table 2. Values of parameters from fitting of impedance spectra to the equivalent circuit in Fig.6B, for lead ion determination using ChOx/MWCNT/GCE. The value of R_{Ω} is $2.1 \Omega \text{ cm}^2$ in all cases.

	$R_{ct} / \Omega \text{ cm}^2$	$C_{dl} / \mu\text{F cm}^{-2}$	$Z_w / \Omega \text{ cm}^2 \text{ s}^{\alpha-1}$	τ / s	α	$R_f / \text{k}\Omega \text{ cm}^2$	$C_f / \text{mF cm}^{-2}$
Buffer	14.2	16.6	39.8	0.52	0.38	1.86	5.48
+ 20 μM Choline	11.2	17.6	39.0	0.63	0.37	1.68	5.36
+ 0.1 nM Pb^{2+}	11.4	17.7	39.7	0.65	0.38	2.00	5.85
+ 0.2 nM Pb^{2+}	11.6	17.7	39.2	0.72	0.36	2.02	5.19
+ 0.3 nM Pb^{2+}	11.8	17.4	38.6	0.60	0.37	1.91	5.40
+ 0.5 nM Pb^{2+}	12.2	17.4	37.5	0.50	0.38	1.79	5.59
+ 1.0 nM Pb^{2+}	12.4	17.5	39.2	0.68	0.36	2.00	5.28
+ 2.5 nM Pb^{2+}	12.6	17.1	38.6	0.55	0.38	1.84	5.49
+ 3.5 nM Pb^{2+}	12.8	17.0	38.7	0.53	0.38	1.68	5.56
+ 5.0 nM Pb^{2+}	13.0	17.2	39.1	0.61	0.36	1.85	5.33
+ 6.0 nM Pb^{2+}	13.3	16.9	37.5	0.43	0.39	1.54	5.88
+ 7.0 nM Pb^{2+}	13.4	16.8	39.0	0.48	0.38	1.65	5.69
+ 8.0 nM Pb^{2+}	14.1	16.5	39.1	0.44	0.39	1.53	5.86

with a sensitivity of $18 \% \text{ nM}^{-1}$, Figure 6C; above this concentration there is a further, but non-linear, increase. From this plot and equation (2) it was possible to deduce the degree of inhibition by lead ions, also shown in Fig. 6C. It is therefore possible to measure the concentration of lead ions by EIS using inhibition of choline oxidase, but the linear range is narrower than by fixed potential amperometry (up to 0.5 nM rather than 1.0 nM). It represents a complementary method and can also be extremely useful as a diagnostic, during prolonged use of the sensor, of any changes to the modified electrode architecture, to which impedance spectra are highly sensitive.

3.3 Interferences

Several cations, including Cr^{3+} , Cd^{2+} , Hg^{2+} , Cu^{2+} and Mn^{2+} , were tested to examine whether they interfere to the determination of Pb^{2+} . The biosensor was incubated with 50 nM of each of these cations separately and the inhibition response was compared with that for Pb^{2+} at 5.0 nM, for which the inhibition was 80%. Only a small amount of inhibition was achieved for the cations tested here: 2.0% for Cr^{3+} , 1.3% for Cd^{2+} , 1.0% for Cu^{2+} and 0.5% Mn^{2+} ; the highest inhibition among the interferents tested was by Hg^{2+} of 7.0% which still represents less than 10% interference. The results obtained indicate a good selectivity for lead ion determination.

3.4 Tap Water Analysis

The practical use of the ChOx-modified biosensor was assessed by the determination of Pb^{2+} in tap water samples spiked with different concentrations of Pb^{2+} and incubated with the biosensor during 5 min. The standard addition method was used and the recoveries obtained were within the range 97–105%, which augurs well for use of this inhibition biosensor in lead ion detection.

4 Conclusions

An easy to fabricate, low-cost choline biosensor for the determination of lead ions by inhibition of choline oxidase has been developed for the first time. A lower limit of detection than those reported in the literature based on enzyme inhibition has been achieved. The inhibition of lead by electrochemical impedance spectroscopy was also possible, but with lower performance than by amperometry. The biosensor was used for the selective determination of Pb^{2+} in the nanomolar range and was successfully applied to the recovery of Pb^{2+} from tap water.

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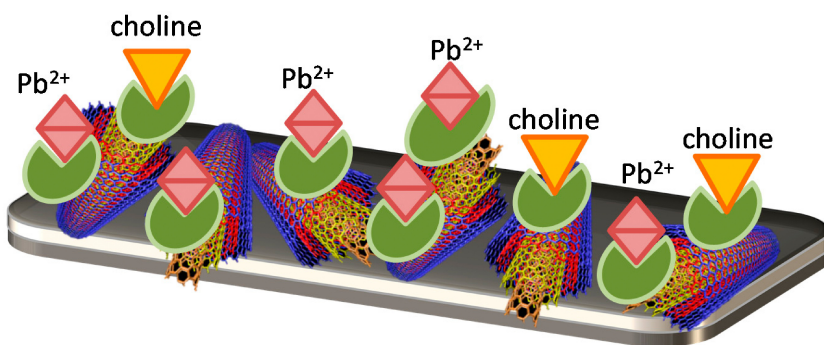
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Highly Sensitive Choline Oxidase Enzyme Inhibition Biosensor for Lead Ions Based on Multiwalled Carbon Nanotube Modified Glassy Carbon Electrodes
