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Electrochemical sensor for amino acids and glucose based on glassy carbon electrodes modified with multi-walled carbon nanotubes and copper microparticles dispersed in polyethylenimine

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ABSTRACT

This work reports the analytical performance of glassy carbon electrodes (GCE) modified with multi-walled carbon nanotubes (CNT) and copper microparticles dispersed in polyethylenimine (PEI) (GCE/CNT-PEI-Cu) for the quantification of amino acids, albumin and glucose. The best analytical performance was obtained with CNT-PEI-Cu prepared by sonicating for 15.0 min a mixture of 1.0 mg mL^{-1} PEI, 1.0 mg mL^{-1} CNT and 3.0 mg mL^{-1} copper microparticles. In the case of amino acids and albumin, the analytical signals were obtained from the increase of the copper oxidation signal produced as a consequence of the complex formation between Cu(II) and the amino acids. The sensor allowed the highly sensitive (submicromolar levels) and reproducible (3.9%) amperometric quantification of histidine, serine and cysteine at very low potentials (0.000 V) and pH 7.40. Albumin was quantified by Square Wave Voltammetry after 10.0 min interaction at −0.100 V with detection limits of 1.2 mg mL^{-1}.

GCE/CNT-PEI-Cu was also used for the quantification of glucose by amperometry at 0.700 V in a 0.100 M sodium hydroxide solution through the known catalytic activity of copper towards the oxidation of glucose, with highly competitive detection limits (182 nM). GCE/CNT-PEI-Cu was successfully used for the quantification of amino acids and albumin in pharmaceutical products and carbohydrates in beverages.

1. Introduction

Electrochemical (bio)sensors have received considerable attention in the last years due to their known advantages such as low cost, high sensitivity, versatility, simplicity, and portability [1]. Since 1996 [2], we have witnessed an explosive growth of carbon nanotube (CNT)-based electrochemical (bio)sensors mainly due to the unique properties of this nanomaterial [3,4], especially those connected with electrochemistry like the strong electrocatalytic activity, the large decrease of surface fouling and the noticeable increase of the electroactive area [3,5,6].

The incorporation of CNTs in biosensors requires minimizing their trend to aggregation. To overcome this inconvenience CNTs have been dispersed in solvents [7], ionic liquids [8] and polymers [9–18]. Particularly, polyethylenimine (PEI) has demonstrated to be highly efficient to disperse CNTs [9]. Glassy carbon electrodes (GCE) modified with MWCNT-PEI dispersions have been successfully used for the quantification of dopamine in the presence of ascorbic acid and serotonine [19], phenols [20], and herbicides like amitrol [20]. PEI-CNT modified GCE has been also used for the adsorption of oligo and polinucleotides [21].

This work is focused on the development of an electrochemical sensor for the quantification of amino acids and glucose based on the modification of GCE with a dispersion of multi-walled carbon nanotubes (MWCNT), copper microparticles and PEI (GCE/CNT-PEI-Cu).

The quantification of amino acids using electrochemical sensors has been based on the direct electrooxidation of tyrosine, tryptophan and cysteine [22,23], or the catalytic oxidation in 0.10 M NaOH at Cu [24], Ni [25] or carbon electrodes modified with these metals [26,27]. Luque et al. [28] have reported the sensitive quantification of amino acids and albumin using carbon nanotube paste electrodes (CNTPE) modified with copper microparticles through the facilitated dissolution of copper in the presence of the amino acids. The direct quantification of electroactive amino acids in strong alkaline medium using copper electrodes in connection with HPLC has been also reported [29].

The non-enzymatic quantification of carbohydrates has been mainly based on the use of spectroscopic techniques with a previous step of derivatization [30]. The direct electrooxidation at platinum and gold electrodes has been also used; nevertheless, the procedure requires a continuously pulsed program to reduce the surface fouling [31]. In the last years, CNTs modified with metallic particles like CuO, Pt or copper

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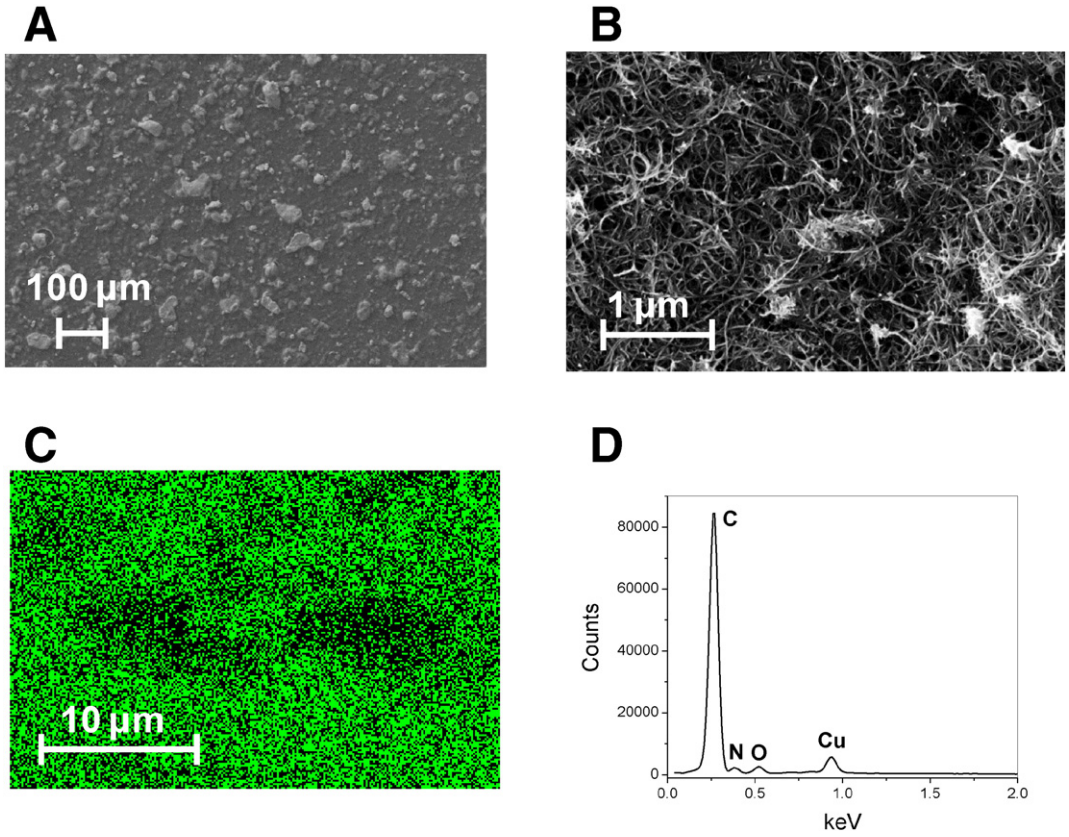

Fig. 1. FE-SEM pictures for CNT-PEI-Cu obtained with different magnifications: (A) 70× and (B) 18k×. (C) EDX mapping for CNT-PEI-Cu, magnification: 10k×; (D) EDX spectrum of CNT-PEI-Cu.

nanoclusters have been also proposed for the direct electrochemical quantification of glucose [32–35].

In the following sections, we discuss the more relevant aspects about the characterization of GCE/CNT-PEI-Cu using Scanning Electron Microscopy (SEM), X-Ray Dispersion Energy (EDX), amperometry and cyclic voltammetry, and the analytical performance of the resulting electrodes for the quantification of amino acids, albumin and glucose.

2. Experimental

2.1. Reagents

Polyethylenimine (PEI, average MW 750,000, catalog number P-3143), bovine serum albumin (Alb, A-4503), L-cysteine, L-histidine, and L-serine were purchased from Sigma. Copper microparticles (99% purity, −325 mesh, 10% max, +325 mesh, 99%) were acquired from Alfa-Aesar. Multiwalled carbon nanotube powder (MWCNT) (diameter (30 ± 15) nm, length 1–5 µm and >95% of purity) was obtained from NanoLab (U.S.A.). Glucose and sodium hydroxide were from Merck. Human serum albumin 20% intravenous injection was received from "Laboratorio de hemoderivados", UNC (Córdoba, Argentina) while L-cysteine capsules (Tricomax 2) were obtained from Cassara Laboratory. The beverages were purchased in a local supermarket.

Amino acids stock solutions were daily prepared using a 0.050 M phosphate buffer solution pH 7.40 as supporting electrolyte. All solutions were prepared with ultrapure water ($\rho = 18$ M Ω cm) from a Millipore-MilliQ system.

2.2. Apparatus

The measurements were performed with EPSILON (BAS) and TEQ_04 potentiostats. The electrodes were inserted into the cell (BAS, Model MF-1084) through holes in its Teflon cover. A platinum wire and Ag/AgCl, 3 M NaCl (BAS, Model RE-5B) were used as counter and reference electrodes, respectively. All potentials are referred to the latter. A magnetic stirrer provided the convective transport during the amperometric measurements. Scanning Electron Microscopy (FE-SEM) images were obtained with a Field Emission Gun Scanning Electron Microscopy (FE-SEM Zeiss, ΣIGMA model). EDX mapping was obtained

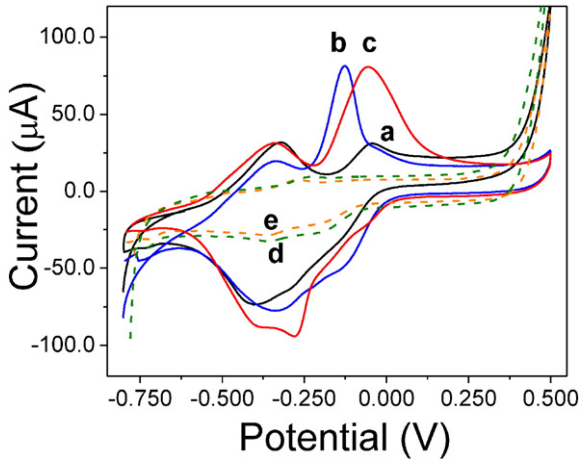

Fig. 2. Cyclic voltammograms obtained at GCE/CNT-PEI-Cu in a 0.050 M phosphate buffer solution pH 7.40 (a, —) and in a 0.050 M phosphate buffer solution pH 7.40 containing 5.0×10^{-4} M histidine (b, —) and 5.0×10^{-4} M L-serine (c, —). The corresponding voltammograms obtained at GCE/CNT-PEI in a 0.050 M phosphate buffer solution pH 7.40 containing 5.0×10^{-4} M histidine (d, —) and 5.0×10^{-4} M L-serine (e, ----) are also included. Scan rate: 0.100 V/s.

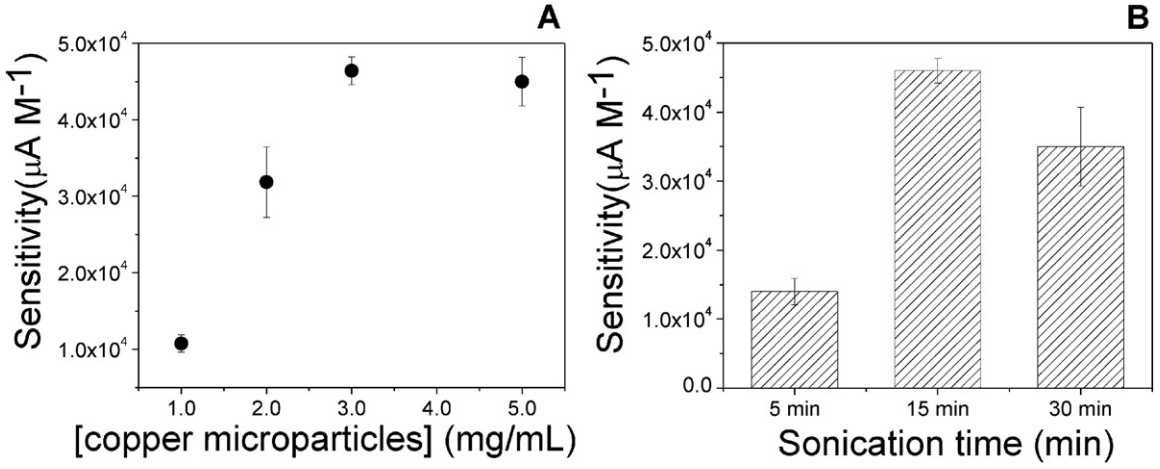

Fig. 3. (A) Effect of the amount of copper microparticles in the dispersion of CNT (1.0 mg mL^{-1}) and PEI (1.0 mg mL^{-1}) deposited at GCE on the sensitivity towards L-histidine. (B) Effect of the sonication time of CNT(1.0 mg mL^{-1})-Cu(3.0 mg mL^{-1})-PEI (1.0 mg mL^{-1}) on the performance of GCE modified with the resulting dispersions. Working potential 0.000 V. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

with a SDD EDX detector from Oxford Instruments X-Max model. The sonication was performed with a Testlab 160 W ultrasound bath.

2.3. Preparation of GCE modified with CNTs and copper microparticles dispersed in PEI

2.3.1. Preparation of the dispersion

CNT-PEI-Cu: the dispersion was obtained by mixing 1.0 mg of CNT with 3.0 mg copper microparticles and 1.0 mL of 1.0 mg mL^{-1} PEI solution (prepared in 50:50 *v/v* ethanol/water) followed by sonication for 15 min with ultrasonic bath. CNT-PEI dispersion was prepared in a similar way without copper.

2.3.2. Modification of glassy carbon electrodes with CNT-PEI-Cu

The GCE was first polished with alumina slurries of 1.0, 0.30, and 0.05 μm for 2.0 min each. The electrodes were then cycled between −0.300 V and 0.800 V at 0.050 V/s in a 0.050 M phosphate buffer solution pH 7.40 (10 cycles). After that, they were modified by drop coating with 30 μL of CNT-PEI-Cu dispersion followed by the evaporation of the solvent at room temperature. Control experiments were also performed using GCE modified with CNT-PEI dispersions following the same protocol.

2.4. Procedure

Amino acids quantification: performed by amperometry in a stirred 0.050 M phosphate buffer solution pH 7.40 by applying a potential of 0.000 V and allowing the transient currents to decay to a steady-state value prior to the addition of the analyte and subsequent current monitoring.

Albumin quantification: performed by Square Wave Voltammetry (SWV) in a 0.050 M phosphate buffer solution pH 7.40 after holding the GCE/CNT–Cu-PEI at −0.100 V for 10.0 min in a stirred solution of albumin. The voltammetric parameters were: frequency: 2 Hz, pulse amplitude: 25 mV, potential step: 4 mV.

Glucose quantification: performed by amperometry in a stirred 0.100 M sodium hydroxide solution by applying a potential of 0.700 V and allowing the transient currents to decay to a steady-state value prior to the addition of the analyte and subsequent current monitoring.

All measurements were performed at room temperature.

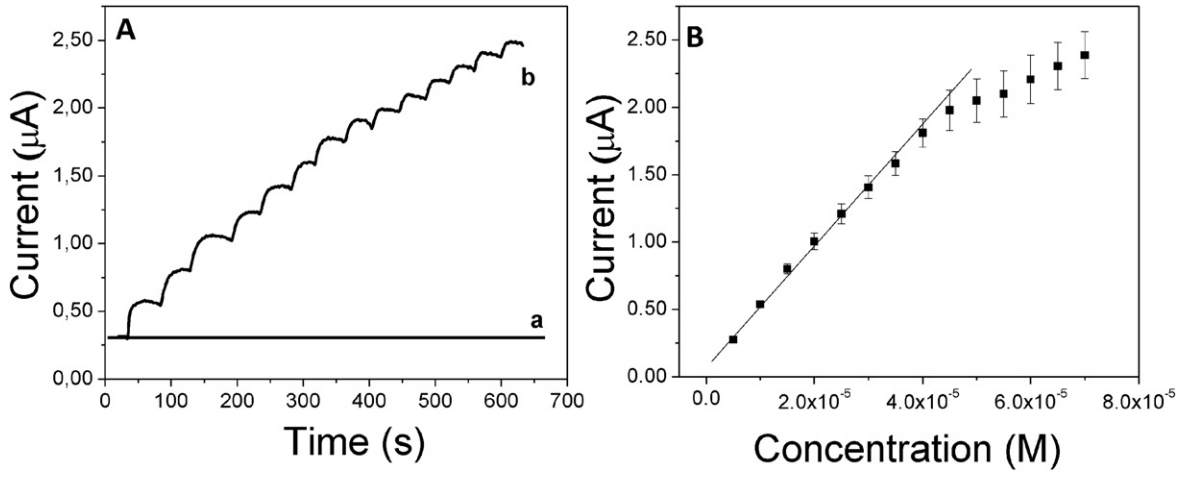

Fig. 4. A) Amperometric recordings for successive additions of 5.0×10^{-6} M L-histidine at GCE/CNT-PEI (a) and at GCE/CNT–Cu-PEI (b). Working potential 0.000 V. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40. B) Calibration curve for histidine obtained from the amperometric recording shown in Fig. 4A.

Table 1
Analytical parameters obtained from the amperometric determination of amino acids. Working potential: 0.000 V. Other conditions as in Fig. 4.

Amino acid	Sensitivity ($\mu A\ M^{-1}$)	Detection limit (μM)	Linear range (μM)	pK_f
L-cystine	$(6.5 \pm 0.4) \times 10^4$	0.10	0.30 to 45.0	19.2
L-histidine	$(4.6 \pm 0.2) \times 10^4$	0.14	0.42 to 50.0	10.6
L-serine	$(1.8 \pm 0.2) \times 10^4$	0.37	1.12 to 45.0	7.4

3. Results and discussion

3.1. Surface characterization of GCE/CNT-PEI-Cu

Fig. 1A and B displays FE-SEM images of GCE covered by CNT-PEI-Cu obtained at 70× (A) and 18k× (B). Both images show that, although the dispersion of CNT–Cu-PEI completely covers the glassy carbon disks, there are some regions with different density. EDX mapping of GCE/CNT-PEI-Cu (Fig. 1C) confirms the distribution of Cu microparticles in the CNT-PEI net, suggesting an efficient and intimate contact between the different materials. Fig. 1D depicts the EDX spectrum where it is possible to detect the presence of C (0.28 KeV), Cu (0.90 KeV) and N (0.39 KeV) that come from CNT, Cu microparticles and PEI, respectively. These results confirm the close contact of the different components of the dispersion (CNTS, PEI and Cu), which is a critical aspect for the development of an electrochemical sensor.

3.2. Amino acids and albumin quantification

Fig. 2 displays cyclic voltammograms obtained at GCE/CNT–Cu-PEI under different conditions: in a 0.050 M phosphate buffer solution pH 7.40 in the absence of amino acids (a-black line) and in the presence of 5.0×10^{-4} M histidine (b-blue line) and L-serine (c-red line). In the absence of the amino acids there are two anodic peaks, the peak I due to the oxidation of Cu to Cu_2O (peak I, peak potential (E_{pI}) = −0.320 V) and the peak II due to the oxidation of Cu_2O to CuO (peak II, E_{pII} = −0.042 V). The negative scan shows a broad peak associated with the reduction of CuO to Cu. In the presence of histidine and L-serine there is an enhancement in the currents associated with the peak II, indicating that the complex formation between the amino acids and Cu(II) facilitates the dissolution of Cu, in agreement with previous reports [24,26–28]. In the presence of serine, the peak II appears at potentials more positive than that for histidine. This shifting in the potential and the higher constant for the complex formation (K) with Cu(II) [36], suggest some correlation between the facilitated dissolution of copper in the presence of the amino acid and the stability of the complex in solution. The bi-dentate amino acid ligand is first chelated with Cu^n, followed by the reversible reduction of $Cu^{II}O$ to Cu^{I}_2O. As soon as Cu^{I}_2O is regenerated back to $Cu^{II}O$, the same cycle can be repeated again [26–28]. These results indicate that Cu is the responsible for the detection of amino acids, in agreement with the results reported by Zen et al. [27] who demonstrated that a reversible 1:1 Cu^{II}-amino acid complex formation takes place at the Cu-electrode interface and that the interaction is not as strong as that of Cu^{II} ion in aqueous solution. It is important to mention that no electrochemical response was observed for the amino acids at GCE/CNT-PEI. For comparison, the i-E profiles for 5.0×10^{-4} M histidine (d-blue dashed line) and L-serine (e-red dashed line) at GCE/CNT-PEI are also shown for comparison. No response is observed in this case, clearly demonstrating the interaction between the amino acids and Cu(II).

The working potential for amperometric experiments was selected from a hydrodynamic voltammogram for 2.0×10^{-5} M L-histidine obtained at GCE/CNT-PEI-Cu using 0.050 M phosphate buffer solution pH 7.40 as a supporting electrolyte (not shown). The selected value was 0.000 V, considering that the current started rising at −0.150 V due to the facilitated oxidation of Cu to CuO and that at potentials higher than 0.100 V, the current decreased probably due to the formation of other copper compounds that passivate the electrode surface.

The influence of the amount of copper microparticles and the sonication time on the efficiency of CNT–Cu-PEI dispersion and on the response of GCE modified with the resulting dispersion were evaluated from amperometric experiments performed at 0.000 V for successive additions of 5.0×10^{-6} M L-histidine. Fig. 3A shows the effect of the amount of copper microparticles in the CNT-PEI-Cu dispersion on the sensitivity towards L-histidine once immobilized at GCE. The sensitivity increases with the amount of copper up to 3.0 mg mL^{-1} remaining constant thereafter. These results suggest that at such level, the amount of polymer is not enough to disperse the Cu microparticles and CNTs. Fig. 3B illustrates the influence of the sonication time of the dispersion (1.0 mg mL^{-1} CNT, 3.0 mg mL^{-1} Cu, and 1.0 mg mL^{-1} PEI) on the performance of GCE modified with the resulting dispersions. The sensitivity

Table 2
Comparison of the analytical parameters for the electrochemical quantification of amino acids obtained with different electrochemical sensors.

Electrode	Detection	Sensitivity	Linear range	LOD	Ref.
L-histidine					
CNTPE–Cu	Amp 0.00 V	$(29 \pm 6)\ 10^2$ ($\mu A\ mM^{-1}$)	1 to 10 μM	2 μM	[28]
SWNT-modified	Amp +0.55 V	–	–	0.6 μM	[26]
DNA duplex/end-truncated ETHH Au NCs/GCE	SWV	–	0.1 pM to 0.1 μM	0.01 pM	[38]
DNA duplex/AuNP/HDT/Au electrode	SWV	–	0.1 pM to 50 nM	0.1 pM	[39]
GNPs–GNSs nanocomposite	SWV	0.050 (μA/ng mL^{-1})	10 pM to 10 μM	0.1 pM	[40]
CIP-modified PGE	DPASV	–	10–343 (ng mL^{-1})	12.8 μM	[41]
gold electrode modified with Fe(III)-porphyrin	OSWV	–	1.0×10^{-9} to 1.0×10^{-4} M	0.49 nM	[43]
MIPs/MWNTs/Si–ITO electrode	DPV		2.0 μM to 1.0 mM	5.8 nM	[44]
CNT-PEI-Cu	Amp 0.00 V	$(4.6 \pm 0.2)\ 10^4$ $\mu A\ mM^{-1}$	0.42 to 50 μM	0.14 μM	This work
L-cystine					
CNTPE–Cu	Amp 0.00 V	$(28.8 \pm 0.1)\ 10^2$ $\mu A\ mM^{-1}$	1 to 10 μM	3 μM	[28]
CNT-PEI-Cu	Amp 0.00 V	$(6.5 \pm 0.4)\ 10^4$ $\mu A\ mM^{-1}$	0.30 to 45 μM	0.10 μM	This work
L-serine					
CNTPE–Cu	Amp 0.00 V	353 ± 2 $\mu A\ mM^{-1}$	–	100 μM	[28]
NiONPs/GCE	Amp +0.42 V	12.4 nA μM^{-1}	1 to 400 μM	0.85 μM	[42]
CNT-PEI-Cu	Amp 0.00 V	$(1.8 \pm .2)\ 10^4$ $\mu A\ mM^{-1}$	1.12 to 45 μM	0.37 μM	This work

Abbreviations: ETHH: elongated tetrahexahedral. NCs: Au nanocrystals. HDT: 1,6-hexanedithiol. GNPs–GNSs composite: switching structure of aptamer and gold nanoparticles–grapheme nanosheets. Amp: amperometry. CIP: complex imprinted polymers. PGE: pencil graphite electrode. DPASV: differential pulse anodic stripping voltammetry. OSWV: Osteryoung square-wave voltammetry. DPV: differential pulse voltammetry. MIPs: film of molecularly imprinted polymers.

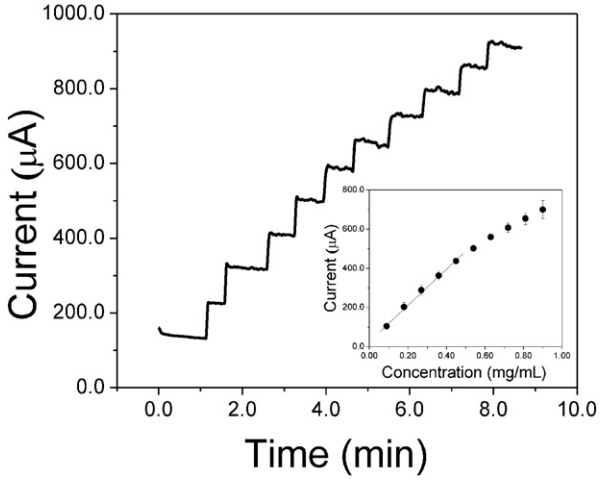

Fig. 5. Amperometric response obtained at GCE/CNT–Cu-PEI at 0.700 V for successive additions of 0.10 mM glucose in a stirred 0.10 M sodium hydroxide solution. The inset shows the corresponding calibration plot.

for histidine increases with the sonication time up to 15 min and then slightly decreases, indicating that there is a critical time necessary to efficiently disperse the microparticles and nanotubes with the polymer and to allow an effective integration of the different components.

Fig. 4A depicts the amperometric response at 0.000 V for successive additions of 5.0×10^{-6} M L-histidine obtained at GCE/CNT-PEI (a) and GCE/CNT–Cu-PEI (b). Since L-histidine is non-electroactive, there is no response at GCE/CNT-PEI after the additions of the amino acid. On the contrary, at GCE/CNT–Cu-PEI, a sensitive and fast response is obtained, demonstrating that the complex formation between histidine and Cu(II) generated at 0.000 V facilitates the copper dissolution and produces an increase in the oxidation current (peak II, Fig. 2). Fig. 4B shows the calibration plot for histidine at GCE/CNT–Cu-PEI obtained from amperometric experiments like those shown in Fig. 3A. The average sensitivity obtained using 20 electrodes and 5 different dispersions was $(4.6 \pm 0.2) \times 10^4$ μ AM^{-1} ($r^2 = 0.997$) and the detection limit (taken as 3.3 × standard deviation of the blank signal/sensitivity) was 0.14 μM. The R.S.D. for the average sensitivity was 3.9%, demonstrating the high reproducibility of the overall protocol from the preparation of the dispersion to the immobilization on the top of the GCE. The sensitivity for 10 successive calibration plots using the same surface presented an R.S.D. of 7.7%, demonstrating an excellent repeatability and short-term stability.

The response of the electrode was also evaluated using serine and cysteine. Table 1 summarizes the analytical parameters for the quantification of these amino acids obtained from amperometric recordings at 0.000 V. In general, a correlation between sensitivity and stability constants for complex formation with Cu(II) in solution is observed for cysteine, histidine and serine, as indicated in Table 1. L-cysteine presents the largest constant for the Cu(II)-complex formation in solution and shows the highest sensitivity at GCE/CNT–Cu-PEI.

Table 2 summarizes the analytical parameters obtained for the quantification of amino acids using other electrochemical sensing schemes. It is important to notice that the response obtained with the electrochemical sensor proposed here is by far more sensitive than that reported in 2006 using a carbon nanotubes paste electrode containing copper microparticles (CNTPE–Cu) [28]. In fact, the sensitivities obtained at GCE/CNT-PEI-Cu are at least one order of magnitude higher and the detection limits one order of magnitude smaller than those obtained with CNTPE–Cu, demonstrating the great advantage of incorporating copper microparticles in the CNT-PEI dispersion. Our sensor presents competitive detection limits for histidine, serine and cysteine compared to those reported in the last years. Some works reported detection limits lower than the one obtained here [37–42]; however, it is important to remark that our sensor presents the advantage of using a very simple transduction scheme and faster and easier electrode preparation, without involving several steps to build the sensor platform or additional biorecognition elements. The sensor was used for the quantification of L-cysteine in a commercial product (Tricomax 2 (Cassara Laboratory). The amount of L-cysteine per capsule obtained with our sensor was (104 ± 2) mg, demonstrating an excellent agreement with the value reported by the laboratory (100 mg). In summary, this new sensor offers the possibility to perform the electrochemical determination of electroactive and non-electroactive amino acids at 0.000 V at pH close to the physiological one, representing a good alternative to most of the traditional methods.

We have also investigated the quantification of albumin at pH 7.40 from the complex formation between Cu(II) and the amino acid residues present in the protein using GCE/CNT-PEI-Cu. The detection was performed in a 0.050 M phosphate buffer solution by SWV-stripping analysis after 10.0 min interaction with albumin at −0.100 V. The calibration plot shows a linear relationship ($r^2 = 0.991$) between 2.5 and 10.0 mg·mL^{-1} albumin, with a detection limit of 1.2 mg mL^{-1} (obtained from the standard deviation of y-residuals (Sy/x)).

Table 3
Comparison of the analytical parameters for the electrochemical quantification of glucose obtained with different electrochemical sensors.

Electrode	Detection potential	Sensitivity	Linear range	LOD (μM)	Ref.
CuO/MWCNTs	+0.40 V	2596 μA mM^{-1} cm^{-2}	0.4 μM to 1.2 mM	0.2	[33]
nafion/CuNPs/AuNPseed/CNTs/chit	+0.65 V	–	1.0×10^{-4} to 5 mM	0.03	[57]
CuO nanowires	+0.33 V	0.49 μA mM^{-1} cm^{-2}	0.4 μM to 2.0 mM	0.049	[56]
Cu–CNTs–GCE(composite)	+0.65 V	17.76 μA mM^{-1}	7.0×10^{-4} to 3.5 mM	0.21	[35]
CNT–CuNP hybrid	+0.60 V	63.751 nA mM^{-1}	0.001–2.0 mM	1.18	[50]
DWCNTs/Cu_2S	+0.5 V	35 μA cm^{-2} mM^{-1}	–	1	[49]
MWCNTs/Cu_2S	+0.5 V	5 μA cm^{-2} mM^{-1}	–	5	[49]
S-AuCu/CNTs/C	+0.34 V	22 μA mM^{-1}	0.08–9.26 mM	4	[48]
CuO–MWCNTs	+0.55 V	2190 μA mM^{-1} cm^{-2}	0.2–3.0 mM	0.8	[46]
CuO nanorods–graphite	+0.60	371.4 μA mM^{-1}	Up to 8.0 mM	4.0	[45]
CuO nanospheres	+0.60	404.5 μA mM^{-1} cm^{-2}	Up to 2.6 mM	1.0	[44]
Cu nanoparticles	+0.65 V	–	1 μM to 5 mM	0.5	[47]
Cu/graphene	+0.50 V	0.1234 μA mM^{-1}	Up to 4.5 mM	0.5	[51]
MWCNT/PEI/Cu	+0.35 V	50.47 μA mM^{-1}	10 μM to 0.3 mM	0.5	[52]
CuO-nanofibers	+0.40 V	431.3 μA mM^{-1} cm^{-2}	6×10^{-3} to 2.5 mM	0.8	[53]
Cu_xO/Cu	+0.50 V	1.62 mA mM^{-1} cm^{-2}	Up to 4.0 mM	49	[54]
Cu-MWCNT	+0.55 V	1096 μA mM^{-1} cm^{-2}	Up to 7.5 mM	1.0	[55]
CNT-PEI-Cu	+0.70 V	(109 ± 8) μA mM^{-1}	Up to 2.5 mM	0.18	This work

The proposed sensor was also used for the quantification of albumin in a pharmaceutical product (Human serum albumin 20% intravenous injection, "Laboratorio de Hemoderivados", UNC). The amount of albumin obtained with our sensor was (10.7 ± 0.8) g/50 mL, demonstrating an excellent agreement with the value informed by the laboratory (10.0 ± 0.6) g/50 mL.

3.3. Glucose quantification

GCE/CNT–Cu-PEI was also used for the quantification of glucose based on the known catalytic activity of copper oxide on the electrooxidation of glucose in highly alkaline media [32]. Although the exact mechanism for the oxidation of carbohydrates in alkaline media at Cu modified electrodes is still not perfectly known, Cu(III) has been proposed as an electron transfer mediator [43]. Fig. 5 shows the amperometric response of GCE/CNT–Cu-PEI at 0.700 V in 0.100 M NaOH for successive additions of 0.10 mM glucose while the corresponding calibration plot is shown in the inset. There is a fast response, with a sensitivity of (109 ± 8) μA mM^{-1} (r^2 = 0.998), a detection limit of 182 nM and a quantification limit of 552 nM (calculated as 3.3 and 10 times the ratio between the standard deviation of the blank signal and the sensitivity, for the detection and quantification limits, respectively).

Table 3 compiles the analytical parameters for the most relevant non-enzymatic electrochemical glucose sensing obtained in the last years. Our sensor demonstrated to be highly competitive, with detection limits lower [44–55] or comparable [33,35,56] to those obtained in most of the cases, without needing additional metallic nanoparticles or polymers [57,58].

The sensor was used for the direct quantification of carbohydrates in two beverages, "Coca Cola" and orange juice (Baggio). The concentration obtained for "Coca Cola" was (23 ± 2) g/200 mL, demonstrating an excellent correlation with the value reported by the company, 22 g/200 mL. The sensor was also challenged with the orange juice (Baggio) and the concentration obtained with GCE/CNT-PEI-Cu ((19.9 ± 0.5) g/200 mL) presents an excellent agreement with the value reported by the company (20 g/200 mL, respectively).

4. Conclusions

The efficient dispersion of Cu microparticles in the CNT-PEI net and the robust deposition at glassy carbon surfaces allowed the development of a simple, sensitive and practical electrochemical sensor for the quantification of amino acids, albumin and glucose. The sensor was successfully used for the highly sensitive and stable amperometric detection of electroactive and non-electroactive amino acids at very low potentials and pH close to the physiological value through the facilitated copper dissolution due to the amino acid-Cu(II) complex formation. The catalytic activity of Cu(II) in alkaline medium allowed the highly sensitive non-enzymatic detection of glucose. The sensor was challenged with beverages and medicines with excellent performance without any pretreatment.

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