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The Preparation, Morphological Characterization and Possible Electroanalytical Application of a Hydroxyapatite-Modified Glassy Carbon Electrode

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Abstract: By simple modification of a GC electrode with biofunctional material, hydroxyapatite (HAp), an efficient electroanalytical tool, was designed and constructed. Modification of the GC surface includes two steps in synthesis: electrochemical deposition and chemical conversion. The properties, structure, and morphology of a nanosized material formed on a surface and absorbability were studied by electrochemical impedance spectroscopy, Fourier-transform infrared spectroscopy and scanning electron microscopy with energy-dispersive spectroscopy analysis. Numerous methods in this work confirmed that the developed method for controlled HAp deposition results in a HAp open structure and uniform morphology, which is capable of the selective absorption of the target species. The main goal of this study was the possibility of using a HAp-modified electrode for the fast screening of copper, cadmium, and lead content in honey and sugar samples. The electrochemical behavior and potential of the electroanalytical determination of heavy metals using the HAp/GC electrode were studied using cyclic voltammetry and square wave anodic stripping voltammetry. The HAp/GC electrode exhibited great performance in the determination of heavy metals, based on the reduction of target metals, because of the high absorbability of the HAp film and the electroanalytical properties of GC. A linear response between 10 and 1000 μ g/L for Cu and Pb and 1 and 100 μ g/L for Cd, with an estimated detection limit of 2.0, 10.0, and 0.9 µg/L, respectively, was obtained.

Keywords: hydroxyapatite; glassy carbon electrode; electroanalytical application; heavy metals; food analysis

1. Introduction

Environmental contamination is one of the main health problems in industrial countries [1]. Metal pollution has become an increasingly important ecological problem in developed and developing countries. Metal and metalloid pollution in the air, soil, and water comes from mining activities; industrial production; industrial wastes and wastewater; the engines of passenger cars, trucks, and off-road vehicles; areas used for the illegal disposal of hazardous waste; the burning of leaded gasoline, chemicals, and manure-based fertilizers; some pesticides; geological processes; plastic films containing metals, etc. Heavy metals in dust and gases can also enter soil and water by natural sedimentation, rainfall, or infiltration when released into the air [2]. Toxic metals can easily enter the food chain if contaminated water, soils, air, or plants are used for food production.

The term "heavy metals" refers to a metallic element that has a relatively high density and is toxic or poisonous even at low concentrations. Heavy metals include elements such as lead (Pb), cadmium (Cd), nickel (Ni), iron (Fe), zinc (Zn), chromium (Cr), and copper (Cu). Some of them, such as Cd, are exclusively toxic, whereas some are biogenic, such as Cu [3]. There are different sources of heavy metals in the environment such as natural,



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). agricultural, industrial, domestic effluent, and atmospheric sources, as well as other sources. They are stable in the environment, difficult to eliminate, and cause irreversible damage to the survival, feeding, growth, and behavior of organisms. Activities such as mining and smelting operations and agriculture have contaminated extensive areas of the world, mostly by heavy metals such as Cd, Cu, and Zn [4]. Several authors have indicated that honeybees and honey can serve as environmental bioindicators for metal pollution, as biomarkers for the identification of botanical and geographical origins represent a honey fingerprint [5–7].

Honey bees can be exposed to heavy metals when foraging contaminated honey and pollen resources and, in some cases, by airborne exposure. Traditional analytical methods have been proposed for the detection and quantification of heavy metals in honey samples [8] such as flame atomic absorption spectrometry (FAAS) for Ca, Mg, and Zn [9]; atomic absorption spectrometry for Pb, Cd, and Cu [8,10]; or inductively coupled plasma mass spectrometry to uncover metal content in honey samples [11–13]. However, some of the above-mentioned methods are nonspecific and laborious and needed harmful and expensive substances, which, in combination with a long sample preparation time and well-trained operators, drastically increased the time and cost of analysis. Thus, the development of different electroanalytical methodologies has been applied to explore metal contamination. Stripping voltammetric techniques are powerful analytical tools that are becoming widely used in various chemical analyses of food contaminants or essential elements, additive dyes, and other organic compounds of biological significance [14] such as anodic stripping voltammetry at Hg microelectrodes for the determination of Cu, Pb, Cd, and Zn [15]; Cd and Pb at bismuth-film-modified glassy carbon electrodes using square wave anodic stripping voltammetry [16]; or Zn, Cd, Pb, and Cu in raw propolis samples at pencil lead–bismuth film electrodes [17,18]. These are successively applied for the analysis of metals in honey samples.

In our previous research, we reported on the preparation of hydroxyapatite (HAp) coatings on a glass carbon electrode by electrochemical deposition combined with the chemical precipitation of Ca/P phases and subsequent treatment in alkaline (NaOH) solution (physical and chemical process). Thin HAP coatings formed on the surface of GC electrodes have excellent electroanalytical performance [19]. Hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂, HAp) is a new type of bioceramic with great bioactivity and particular multiadsorbing sites analogous to the mineral components of bone and teeth. Due to its "open structure", great varieties of cationic and anionic substitutions in HAp are possible, including its application for electroanalytical purposes. Many different applications are reported in the literature, mostly in bioscience, because of its acid–base properties, ion-exchange ability, biocompatibility, and adsorption capacity [20–23].

The morphology of HAp coatings has been studied using Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and atomic force microscopy (AFM). The prepared film electrode has been used as a sensing material for the detection of heavy metals in honey and sugar. Additionally, for the preparation of real samples, a method for preparing natural organic materials has been developed.

Different approaches for the synthesis of Ca/P phases are presented in the literature, including physical techniques (different performances of spraying, sputtering, evaporation, or deposition) and chemical methods (sol–gel, immersion coating, hot-isostatic pressing, electrophoretic deposition, electrochemical deposition, (micro)emulsion routes, dip coating, or sintering) [24–27]. Different ratios of the crystalline/amorphous phase of HAp coatings can be achieved due to the different methods of synthesis employed in the synthesis of HAp [28,29]. The chemical composition, structure, and property of the amorphous phase are dependent on the structure of its clusters. The formation of amorphous calcium phosphate and its subsequent transition to crystalline hydroxyapatite lead to the formation of a cluster structure [30] and the possible formation of a coordination complex of HAp and heavy metals.

Electrochemical deposition, an environmentally friendly method for coating formation, represents an ecologically clean and safe approach with unique advantages due to its

The analytical performances of the electrochemical methods depending on the sensor's construction and some numerical data of the selected metals at HAp-modified electrodes are presented in Table 1. From the presented results for the detection limit, it can be seen that the electrochemical determination of heavy metals is comparable to the results achieved with flame atomic absorption spectroscopy (FAAS).

Table 1. Electroanalytical and analytical performances reported for the electrochemical determination of selected heavy metals at the HAp-modified electrode.

Electrode Modification/Substrate	Electroanalytical Methods	Analytes	Linear Range (µg/L)	Analyzed Samples	LOD (µg/L)	Ref.
HAp-CILE/CP ¹	CV SWASV	Pb Cd	0.11-0.121	Wastewater sample	0.041 0.056	2009 [33]
nHAp/ionophore/Nafion/GCE ²	CV ASDPV	Pb	1 to 166	Real water sample	0.21	2009 [34]
CNT-HAp/GCE ³	CV DPV	Cd	2.25–33,723	Real tap water sample	0.45	2009 [35]
Bi/HAp–CME ⁴	CV SWASV	Pb Cd	Up to 150	Spiked lake water sample	5 5	2013 [36]
HAp/Pt ⁵	CV SWASV	Pb	4.14–2277	River water samples	4.2	2013 [37]
FAAS ⁶	FAAS	Cu Zn Pb			0.72 0.55 0.512	2015 [38]
HAp/GCE ⁷	CV SWASV	Cu Pb Cd	10–1000 10–1000 1–100	Real sugar and honey samples	2.0 10.0 0.9	2021 This work

¹ HAp-modified carbon ionic liquid electrode (HAp–CILE): ionic liquid N-octylpyridium hexafluorophosphate (OPPF(6)) with a ratio of 50/50 (w/w = mixing the graphite powder with HAp to give an appropriate ratio of HAp/CP) and heating for 2 min at >65 °C. ² Mixture of lead ionophore (IV) (4-tert-butylcalix[4]arene-tet-rakis(N,N-dimethylthioacetamide)) with nanosized hydroxyapatite (nHAp) prepared by one-step sonication for 20 min (from Ca(NO₃)₂ and (NH₄)₂HPO₄; the molar ratio of Ca and P was equal to 1.67) and Nafion dropwise on the GCE. ³ One-step sonication: CNTs dispersed in solution with the aid of ultrasonication for 20 min with Ca(NO₃)₂ and (NH₄)₂HPO₄; the molar ratio of Ca and P was equal to 1.67; 10 mL of 1 mg/mL carbon nanotube–HAp ethanol solution was added on the surface of the GCE and dried under an IR lamp. ⁴ Bismuth (in situ)-modified HAp carbon-modified electrode by adding different content ratios of HAp and graphite to the formation. ⁵ Electrodeposition of HAp on the surface of a bare platinum electrode. ⁶ After preconcentration with hydroxyapatite nanorods originating from an eggshell. ⁷ Electrodeposition of HAp on the surface of a glassy carbon electrode via the electrochemical deposition of and chemical conversation to HAp.

2. Materials and Methods

Chemicals, Solutions, and Apparatus All used reagents were of analytical reagent grade. All of the solutions were prepared with double-distilled water. The Sigradur[®] glassy carbon was supplied from Hochtemperatur-Werkstoffe GmbH, Thierhaupten, Germany.

Preparation of the GC Electrode Before use, the glassy carbon (GC) electrodes (5 mm in diameter), which served as substrate electrodes in all experiments, were mechanically polished with emery paper (2000 grit), followed by polishing with alumina powder down to 0.05 μ m. After polishing, the electrodes were treated ultrasonically in redistilled water, rinsed with ethanol, and finally rinsed with redistilled water.

In order to achieve a reproducible and active electrode surface, the electrodes were activated in $0.5 \text{ mol } \text{dm}^{-3} \text{ HNO}_3$, as noted in our previous study [32].

Preparation of HAp/GC Electrode The Ca/P-phase electrodeposition solution was prepared by mixing 50 cm³ of 0.05 mol dm⁻³ Ca(NO₃)₂ · 4H₂O and 50 cm³ of 0.04 mol dm⁻³ (NH₄)₂HPO₄. The pH of the solution was adjusted to 5.0 by adding 0.5 mol dm⁻³ HNO₃. Electrodeposition was performed at a potential of -850 mV for 60 min at room temperature. The electrolyte was stirred with a magnetic stirrer at a constant speed. In that way, the concentration of Ca^{2+} and HPO_4^{2-} ions was constant during electrodeposition.

Alkaline treatment was performed in a bath with 1.0 mol dm⁻³ NaOH at 80 °C for 60 min after electrodeposition. Between each step, the electrode was cleansed with redistilled water.

As a result of the preparation procedure, a HAp/GC electrode was obtained.

Instruments All experiments were carried out at 25 °C.

The surface morphology was studied using a FEG–SEM Thermo Scientific[™] Quattro ESEM scanning electron microscope (SEM). Obtained images were quantified using ImageJ software (Rasband, U.S. NIH, Bethesda, MD, USA). Preliminary examination of the sample surface was conducted using a metallographic microscope (MMT 800BT, Mikrolab, Warsaw, Poland), and the surface topography of the samples was evaluated using an atomic force microscope (AFM, Dimension Icon, Bruker, Santa Barbara, CA, USA) in tapping mode using a standard silicon probe model TESPA-V2 (Bruker, Santa Barbara, CA, USA). Achieved images were quantified with ImageJ software (Rasband, U.S. NIH, Bethesda, MD, USA). Electrochemical impedance measurements were made using Solartron SI 1287 and SI 1255 HF frequency response analyzer (FRA) instruments. The Solartron instruments were controlled using Zplot and CorrWare computer software, while ZView and CorrView software packages were used as tools for data analysis. For electrochemical voltammetric measurements, a three-electrode cell was used with Ag/AgCl/3M KCl as the reference electrode, a platinum electrode as the counter electrode, and GC or HAp/GC as the working electrode with a potentiostat (AutoLab PGSTAT 302N) connected to a PC and driven by GPES4.9 software (Eco Chemie).

Preparations of Sugar and Honey Samples A representative sample (sample of sugar purchased from a local store and two samples of honey purchased from a local beekeeper from two localities: mountainous and coastal) of up to 2.0 g was digested in concentrated nitric acid, and the mixture was left in the digester for 24 h. The mixture was transferred to porcelain pots, which were annealed at 160 °C for 30 min. After cooling, 8 mL of H₂O₂ and 2 mL of HNO₃ were added. The resulting solution was completely clarified. A 10 mL volume of aliquots of the prepared samples (pH = 4, adjusted with HNO₃) was transferred to a 100 mL flask and filled with 0.2 mol dm⁻³ solution of KNO₃.

3. Results

3.1. Electrodeposition of Ca/P Phases on the GC Electrode and Surface Morphology of the HAp Coating on the GC Electrode

3.1.1. Deposition

For the surface modification of the HAp coating on the GC electrode, a two-part method was performed [19]. The first part comprised the electrochemical and chemical reactions. Electrodeposition was performed at a potential of -850 mV at room temperature for 60 min. Electrochemical reactions involve the reduction of hydrogen (electrochemical reaction) and the spontaneous deposition of Ca/P phases (chemical reaction) when the Ca²⁺ ion reacts with HPO₄²⁻ and PO₄³⁻ and forms CaHPO₄ × 2H₂O (brushite, calcium hydrogen phosphate dihydrate) and Ca₃(PO₄)₂ (calcium phosphate, β -TCP). Brushite and β -TCP are precursors to the formation of stable-phase HAp and Ca₁₀(PO₄)₆(OH)₂. The second part is chemical conversation via reactions that convert deposited Ca/P phases into hydroxyapatite (HAp) through alkaline treatment. Scheme 1 presents the process of deposition under the given experimental conditions.

3.1.2. Atomic Force Microscopy (AFM)

An example optical microscopy image of the surface of each sample and AFM images of the scanning areas of $10 \times 10 \,\mu\text{m}$ are shown below in Figure 1. The optical microscopy images indicate that the surfaces of the tested samples are heterogeneous and not totally covered. From the microscopic images, it is possible to conclude that part of the GC surface is unmodified carbon, an uncovered surface, or microcracks with carbon in a small gap,

probably due to a nonformed nucleus during the electrodeposition step. The formed film was evaluated with ImageJ software. A cross-sectional view is generated from the optical image and presented as the ZY or ZX planes. The obtained AFM images characterize the surface locally, and scans at different locations show noticeable differences in surface roughness. According to the AFM images, the mean image data plane (Rq) calculated on 100 μ m² regions for two different positions on the surface varies from 107.3 down to 35.5 nm for different film thicknesses (900.8 and 280.8 nm; Figure 1C,D).



Scheme 1. Process of deposition under the given experimental conditions.



Figure 1. Optical image of the HAp film at $500 \times$ magnification: (**A**) cross-sectional view generated from the optical image (**B**) and film thickness measurements using AFM (**C**,**D**).

3.1.3. Scanning Electron Microscopy (SEM)

Figure 2 shows the surface morphologies of HAp on the GC surface with different magnifications: $35 \times$ (Figure 2A), $1000 \times$ (Figure 2B), and $8000 \times$ (Figure 2C). For the HAp coating with a magnification of $8000 \times$ (Figure 2C), it can be seen that a porous structure formed on the GC surface with HAp, with a macropore diameter between 0.5 and 3 μ m. The macropores led to the development of a large specific surface area and an "open structure", which is of great importance for good electroanalytical performance.



Figure 2. SEM results with three different magnifications of the electrode surface: (**A**) $35 \times$ (**B**) $1000 \times$ (**C**) $8000 \times$; film was electrodeposited on a polished glassy carbon electrode ($E_{dep} = -0.850$ V; $t_{dep} = 60$ min).

In our previous study [19], we reported that this method of modifying results in a relatively uniform topology and almost completely covered surfaces with an average content of elements derived by energy-dispersive spectroscopy (EDS) analysis corresponds to the formation of a P-deficient topology (Ca-fortified apatite), with a Ca/P ratio of 2.12, which was not found to be a biologically relevant Ca/P form. In the natural bone, the Ca/P ratio is close to 1.67 with an oxygen atomic content of ~62% [24].

3.1.4. Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is a useful method to verify the presence of functional groups in synthesized compounds or materials in a wide wavenumber region. Figure 3 shows the FTIR spectra of the bare (blue line) and HAp-modified (red line) GC electrodes prepared as described in the Materials and Methods section (Section 2).



Figure 3. FTIR spectra of the bare (blue line) and HAp-modified GC electrodes (red line).

The observed peaks on the bare GC electrode are identified as follows. One strong shoulder peak observed between 2830 and 3050 cm⁻¹ can be connected to the absorption of C–H stretching that corresponds to the frequency of asymmetric (2919 cm⁻¹) and symmetric (2848 cm⁻¹) stretching of the C–H bond from the methyl moiety (-CH₂), which originated from organic impurities. The peak at 2330 cm⁻¹ is due to O–H stretching, and the peaks at 1472 and 1400 cm⁻¹ can be associated with the vibration of the traces of organic substances on the surface, or the vibration of a CO₃^{2–} group, formed as a consequence of anodic or cathodic pretreatment in nitric acid. After modification, FTIR of the HAp/GC electrode shows some peaks characteristic of HAp [19,35].

The surface of HAp on the GC electrode showed some characteristic peaks [19,39]:

- (1). The strong characteristic band in the 3400–3900 cm⁻¹ frequency range and at 2310 cm⁻¹ is assigned to O–H stretching.
- (2). The peaks at 2848 and 2919 cm⁻¹ of C–H stretching from the methyl moiety (–CH₂), which originated from organic impurities, suggest a noncovered GC surface.
- (3). The peak at 1623 cm⁻¹ was due to adsorbed atmospheric CO₂, and the peaks at 1540 and 1380 cm⁻¹ can be attributed to the vibration of traces of organic substances on the surface or the vibration of a CO_3^{2-} group.

- (4). The most prominent peaks at 1015 cm⁻¹ are due to the asymmetrical P–O stretching mode of HAp in phosphate.
- (5). The slight shoulders at 961 and 560 cm⁻¹ and the peak at 604 cm⁻¹ are ascribed to the P–O bending mode from phosphate.

The FTIR results confirm the presence of a HAp film on the GC surface.

3.1.5. Electrochemical Impedance Spectroscopy (EIS) Studies of HAp/GC

Interference properties, as well as the surfaces of modified and nonmodified electrodes and the absorption of an optimized HAp film on the GC electrode surface, were investigated using electrochemical impedance spectroscopy (EIS) in 0.2 M KNO₃ (pH = 4). Figure 4 shows the impedance data as Nyquist plots, obtained for bare GC and modified HAp/GC via the electrodeposition process, as described in the Materials and Methods section (Section 2). Impedance data were fitted by a suitable electrical equivalent circuit (EEC) model, employing the complex nonlinear least-squares fit analysis [40] offered by Solartron ZView[®] software. The fitting quality was evaluated by the chi-squared and relative error values, which were of the order of 10^{-3} – 10^{-4} and below 5%, respectively, indicating that the agreement between the proposed EEC model and the experimental data was good. The total impedance, Z of the investigated electrochemical system is the sum of the ohmic resistance and the impedance of the electrochemical interface ($Z_{el} + Z_{HF} + Z_{LF}$) and is described by the transfer function in Equation (1).



Figure 4. Nyquist plot of the unmodified and modified GC electrodes recorded at E_{OCP} in 0.2 M KNO₃ (pH = 4) solution. Inset: The EEC used to fit the EIS data (E_{OCP} for bare the GC electrode = 0.0505 V and for the HAp/GC electrode = 0.0727 V).

The Nyquist plots suggest the presence of the diffusion process. EIS data were modeled by the electrical equivalent circuit (EEC) with three time constants, as shown in the inset in Figure 4. In the EEC, constant phase element CPE_1 is attributed to the double layer capacitance, R_1 to the charge transfer resistance, CPE_2 to the capacitance of the species traveling through the film, and CPE_3 to the diffusion processes [41]. The numerical values of the EEC elements were obtained by the fitting procedure. The presented recorded plots reveal the diffusion-controlled processes that took place at the HAp/GC interface. The electrolyte resistance was 6.1 Ω cm². The numerical values of the EEC elements obtained by the fitting procedure indicate an increase in charge transfer resistance in the case of the

modified electrode, suggesting that the adsorbed layer inhibits charge transfer (from 47 to 113 Ω cm²). The EIS results are in agreement with the FTIR results.

$$Z_{TOTAL}(j\omega) = R_{el} + \left\{ Q_1(j\omega)^{n1} + \left\{ R_1 + \left[Q_2(j\omega)^{n2} + Q_3(j\omega)^{n3} \right]^{-1} \right\}^{-1} \right\}^{-1}$$
(1)

3.1.6. Electrochemical Methods

As the main inorganic constituents of biological hard tissues such as bones or teeth, HAp has a hexagonal structure, which can be self-polymerized during alkaline treatment. The sorption mechanisms of the heavy metals on HAp are diverse and mainly include ion exchange, dissolution/precipitation, and the spontaneous formation of surface complexes due to its unique channel structure [38]. The possible exchange of calcium ions with the target metal can be present as follows (Scheme 2).



Scheme 2. Spontaneous formation of surface complexes due to its unique open-channel structure.

Prior to the investigation of the possibilities for the application of a prepared HAp/GC electrode for an electroanalytical purpose, the electrochemical properties of the prepared HAp/GC electrode were examined. Cyclic voltammograms obtained at the HAp/GC electrode are presented in Figure 5.



Figure 5. Cyclic voltammograms obtained on a bare GC electrode and a HAp/GC electrode in 0.2 M KNO₃ (pH = 4) with a scan rate of 25 mV/s, going from 0.0 V to the cathodic direction.

These measurements were carried out without oxygen removed from the solution. The overpotential of hydrogen reduction on the HAp/GC electrode was very low. The presence of O_2 affects the background. The obtained voltammograms revealed an intensive reduction signal at -0.5 V and an oxidation signal at -0.2 V, corresponding to the full reduction of oxygen. The oxygen reduction reaction ideally takes the four-electron pathway ($O_2 + 4H^+ + 4e^- \leftrightarrow 2H_2O$). The potential of this reaction is similar to that reported for the oxygen reduction reaction at a bare glassy carbon electrode [42]. Afterward, the modification of the electrode reduction/oxidation peak that corresponds to oxygen is significantly reduced, which suggests a low sensitivity of the electrode to the oxygen present in the solution. The overpotential of hydrogen reduction at the HAp/GC electrode was very low. The presence of dissolved O_2 could also reduce the potential window of the bare GC electrode, but the modified electrode could be used in a wider potential window with regard to the bare GC electrode.

4. Discussion

4.1. Analytical Applicability

A methodology for the determination of Cu, Pb, and Cd by square wave anodic stripping voltammetry (SWASV) at proposed electrodes in honey and sugar samples was proposed.

Such modified electrodes were subsequently used for the determination of heavy metal cations individually with optimized parameters. The SWCSV procedure was established by monitoring the influence of the applied potential increment (ΔE_s), frequency (f), pulse height (ΔE_p), accumulation potential (E_{acc}), and accumulation time (t_{acc}) on peak currents (I_p), and the obtained results are shown in Table 2. The SWCS voltammograms were obtained by the standard addition method (Figure 6). The calibration plots of the HAp/GC electrode towards copper, lead, and cadmium were derived from the obtained voltammograms, and the results are shown as insets of Figure 6.

Table 2. Optimization parameters for the individual determination of copper, lead, and cadmium.

	Cu ²⁺	Pb ²⁺	Cd ²⁺	
Supporting electrolyte	0.2 M KNO3	0.2 M KNO3	0.2 M KNO3	
pH	4	4	4	
Accumulation potential	-500 mV	-1000 mV	-1200 mV	
Accumulation time	300 s	240 s	120 s	
Frequency	50 Hz	50 Hz	50 Hz	
Potential increment	5 mV	5 mV	5 mV	
Pulse height	50 mV	50 mV	20 mV	
Stripping potential	~100 mV	~400 mV	~700 mV	
Concentration region with linear responses	10 μg L ⁻¹ –100 μg 100–1000 μg L ⁻¹	10 µg L ⁻¹ –100 µg 100–1000 µg L ⁻¹	1.0 µg L ⁻¹ –10 µg 10–100 µg L ⁻¹	
Correlation coefficient, R ²	0.998 0.994	0.989 0.993	0.986 0.994	
Sensitivity	0.03 μA μg L^{-1} 0.04 μA μg L^{-1}	0.09 μΑ μg L ⁻¹ 0.19 μΑ μg L ⁻¹	0.11 μA μg L ⁻¹ 0.03 μA μg L ⁻¹	

During accumulation, selected potential from the adsorbed metal–apatite-targeting metal ion is reduced and then "stripped" from the surface. The change in slope, with linearity preserved, can be attributed to the increased amount of target metal accumulated at the electrode surface, resulting in saturation of the electrode surface. However, this will not take effect on the analytical performances of the modified electrode, as these ranges are well defined and reproducible. For higher metal concentrations, some authors suggest a shorter accumulation time.



Figure 6. SWCSVs (baseline corrected), recorded at the HAp/GC electrode in 0.2 M KNO₃ (pH = 4), with corresponding calibration plots derived by subtracting the background current from the corresponding voltammograms of copper, lead, and cadmium.

4.2. Analytical Application of HAp/GC Electrode in Real Samples

In order to evaluate the analytical applicability of the prepared modified electrode, the proposed method was applied for the detection of Cu, Pb, and Cd in the real sample (honey and sugar) purchased from a local store, and the results are presented in Table 3, with examples of the SWCSVs of the prepared real sample showed presented in Figure 7. The concentrations of metals were obtained using the standard addition method. Besides being able to overcome the sample's matrix interference, this method can also be used to determine low concentrations of analytes with excellent linearity. The results of the proposed method were compared with the results obtained by the standard analytical method (not shown). The method showed good agreement for copper and cadmium. Large deviations appear in the case of lead, suggestive of potential metal interferences in the determination of lead. For lead determination in sugar, another method must be performed.

Real Sample	Honey 1			Honey 2			Sugar		
	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd
$\mu g k g^{-1}$	1775 ± 47	587.5 ± 22.1	64.3 ± 1.7	373.0 ± 12.0	4410 ± 66	287.4 ± 12.6	693.7 ± 23.5	823.7 ± 13.5	360.0 ± 2.7
Maximum Qty Allowed	2000	300	300	2000	300	300	1000	500	500

Table 3. Obtained concentrations of Cu, Pb, and Cd in sweeteners (honey and sugar).



Figure 7. Examples of the SWCSVs of the prepared real sample (baseline corrected), recorded at the HAp/GC electrode in 0.2 M KNO_3 solution (pH = 4) together with the background response: lead in Honey 1, cadmium in Honey 2, and copper in sugar. The insets show the corresponding calibration plots.

5. Conclusions

In the current work, a chronoamperometric procedure for the preparation of the openchannel structure of HAp was presented, and a possible coordination structure (HAp/GC electrode) with heavy metals was introduced.

High porosity, a film with an open structure, good mechanical characteristics, and reproducibility in terms of the deposited film are achieved with the combined electrochemical and chemical steps for deposition and conversion. The FTIR study confirmed the existence of functional groups, characteristics of the HAp film, and the possibility of the adsorption/accumulation of heavy metals on the electrode surface. The developed method for controlled HAp deposition results in the HAp morphology of a nanosized material with a uniform pore size distribution, which was confirmed with numerous methods for surface analysis, suitable for selective adsorption.

Under the optimized conditions, a linear response between 10 and 1000 μ g/L for Cu and Pb and 1 and 100 μ g/L for Cd with an estimated detection limit of 2.0, 10.0 and 0.9 μ g/L, respectively, were obtained. The development method was applied in complex matrices such as honey and sugar samples. In conclusion, commercial honey and sugar are safe as the amount of daily allowance without any harmful contamination of heavy metals.

Surface modification of the GC electrode with the HAp film significantly increased its sensitivity toward metal determination. However, the future prospects of this preparation and procedure can provide easy-to-handle, rapid, low-cost, highly sensitive, stable, and, with appropriate modification, even highly selective biosensors.

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