Surface functionalization of natural hydroxyapatite by polymerization of β-cyclodextrin: Application as electrode material for the electrochemical detection of Pb(II)

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Abstract

A composite material prepared by polymerization of β-cyclodextrin (β-CD) on the surface of natural hydroxyapatite using citric acid as cross linker, was employed as electrode material for the detection of Pb(II). Hydroxyapatite was obtained from bovine bones, following a three-step procedure including pre-calcination, chemical treatment with (NH₄)₂HPO₄ and calcination. The structure of the pristine hydroxyapatite (NHAP₀.₅) and its functionalized counterpart (NHAP₀.₅·CA·β-CD) materials was examined using XRD and FTIR. Upon deposition as thin film on a glassy carbon electrode (GCE), the ion exchange ability of NHAP₀.₅·CA·β-CD was exploited to elaborate a sensitive sensor for the detection of lead. The electroanalytical procedure was based on the chemical accumulation of lead ions under open-circuit conditions, followed by the detection of the preconcentrated species using differential pulse anodic stripping voltammetry. The reproducibility of the proposed method based on different measurements in a solution containing 2 µM lead gave a coefficient of variation of 1.27%. Significant parameters that can affect the lead stripping response were optimized: a linear calibration curve for lead (II) in the concentration range of 2 × 10⁻⁸ mol L⁻¹ - 20 × 10⁻⁸ mol L⁻¹ (R² = 0.998) was obtained. The detection limit (DL = 3S/M) and the sensitivity of the proposed sensor were 5.06 × 10⁻¹⁰ mol L⁻¹ and 100.80 µA.µM⁻¹, respectively. The interfering effect of several ions expected to affect the stripping determination of lead was evaluated, and the proposed sensor was also successfully applied in the determination of Pb²⁺ ions in spring water and well water.

Introduction

Due to their non-biodegradability and especially their toxicity, inorganic pollutants such as heavy metals pose a major threat to the environment because of their involvement in many natural and industrial processes (Adebisi et al. 2017). Among heavy metals, lead is particularly toxic, with negative effects on human health. Yet, it is known that the ingestion of lead can induce damages on the nervous system, the main pathologies being severe headaches, encephalopathy, sleepiness, psychosis, memory deterioration and reduced consciousness (Flora et al. 2012). The safe disposal of lead is always a concern in industrialized countries. The World Health Organization (WHO), the European Union (EU), and the US Environmental Protection Agency (USEPA) set the permissible level of lead in drinking water at 0.01, 0.01 and 0.015 mg L⁻¹ respectively (Awual 2019). To quantify and monitor lead ions, the development of analytical devices has attracted increasing interest in recent decades. For this purpose, a range of different analytical methods have been utilized, such as inductively coupled plasma-mass spectrometry (Longerich et al. 1987), X-ray fluorescence spectrometry (Lau and Ho 1993), and atomic absorption spectrometry (Chen and Teo 2009). These techniques operate quiet well but usually they require expensive equipment, highly trained staff for their implementation and long time for analyses. To overcome these limitations, the development of electrochemical techniques based on modified electrodes has been promoted as these techniques can be easily implemented, and are characterized by high selectivity and sensitivity, good repeatability and cheap instrumentation (Ngassa et al. 2014; Tonle et al. 2015). Moreover, modified electrodes offer to the researcher the advantage of using a wide range of
HAPs have on their surface numerous hydroxyl groups (P-OH and Ca-OH) that can allow their modification by grafting of either organic or inorganic compounds (Saoiabi et al. 2010). Therefore, the modification of HAP surface with various organic substances has been the subject of many studies (Liu et al. 1998; Leprêtre et al. 2009; Othmani et al. 2013; Tang et al. 2013; Yala et al. 2013; Tchoffo et al. 2021). These modifications are of great interest as they induce to the material new properties. Among the organic modifiers generally used, natural polymers are increasingly solicited. Nowadays, many reports focus exclusively on β-cyclodextrin (β-CD) which is a natural macrocyclic oligosaccharide composed of seven glucose molecules. It is toroidal in shape, with a hydrophilic outer side which provides water solubility and a hydrophobic inner cavity (Niu et al. 2018; Tcheumi et al. 2019). These interesting characteristics provide the materials functionalized by β-CD a wide field of application, especially in electroanalysis (Alam et al. 2018; Tcheumi et al. 2019; Tchoffo et al. 2021; Yang et al. 2015; Zhu et al. 2016). Thus, Alam et al. (2018) used multi-walled carbon nanotube and β-cyclodextrin for electrochemical sensing of acetaminophen. Liu et al. (2017) exploited a SH-β-cyclodextrin functionalized graphene-palladium nanoparticles for sensitive electrochemical detection of rutin and isoquercitrin. In the recent past years, our unit is involved in the valorization of β-CD for the protection of environment and against pollution. In these lines, the exploitation of smectite/β-CD composite material for the detection of paraquat has been achieved (Tcheumi et al. 2019). Also, we have reported the use of a hydroxyapatite/β-CD hybrid material for the electrochemical detection of diquat (Tchoffo et al. 2021). In the framework of
that program, the present study describes the electroanalysis and detection of Pb\textsuperscript{2+} ions in aqueous medium. To our knowledge, no study on the use of a composite material based on natural hydroxyapatite and β-CD as electrode modifier for the electrochemical quantification of Pb\textsuperscript{2+} has been reported in the literature. Modifying the electrode surface using such a material should create a favorable environment for the absorption of Pb\textsuperscript{2+} ions, due to the complexing nature of β-CD, the electrostatic interaction between the Pb\textsuperscript{2+} ions and the free carboxylate functions of citric acid available at the surface of the material, and the cation exchange properties of natural hydroxyapatite.

**Experimental**

**Chemicals and reagents**

All chemicals and reagents used in both the material synthesis and the electrochemical experiments were of analytical grade, and used as received. The 0.01 M stock solution of Pb(II) (Prolabo) was prepared by dissolving Pb(NO\textsubscript{3})\textsubscript{2} in deionized water. Ru(NH\textsubscript{3})\textsubscript{6}Cl\textsubscript{3} (98%) was purchased from Aldrich. (NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4} (Anachemia) was used for the chemical treatment of precursor. β-cyclodextrin (98%, Aldrich), Citric acid (99%, Sigma-Aldrich) and Na\textsubscript{2}HPO\textsubscript{4} (Anachemia) were used for the surface modification of hydroxyapatite. CH\textsubscript{3}COOH (99.8%, Scharlau) and CH\textsubscript{3}COONa (99%, Synth) were mixed with different ratios for the preparation of the 0.1 M acetate buffer at different pH values. The pH of the accumulation medium was adjusted using dilute solutions of KOH (BDH) or HNO\textsubscript{3} (60%, Scharlau). All solutions used were prepared with deionized water (18 M\textsuperscript{2} cm\textsuperscript{-1}) obtained from a Millipore milli-Q water purification system.

**Preparation and characterization of the β-cyclodextrin/hydroxyapatite composite**

**Starting bovine bones material: pre-treatment, calcination and impregnation**

The preparation and characterization of β-CD natural hydroxyapatite composite material is fully described elsewhere (Tchoffo et al. 2021). Briefly, the starting materials were bovine bones collected at local market. They were carefully cleaned and then cut into small pieces of about 2 to 5 cm, then boiled for 30 minutes in deionized water and then washed several times in order to remove all traces of remaining meat and fat. Upon drying at room temperature for about 30 days, the bone marrow was scraped, and the resulting cleaned bone was pre-calcined in an electric oven with the heating rate set at 12.5°C.min\textsuperscript{-1}; followed by a residence time of 3 hours at 450°C. The bones were cooled slowly to room temperature and the calcined material was then crushed, sieved and the particles smaller than 50 µm were retained for later use. This pre-calcined material (referred as precursor) was chemically treated as follows: 2 g of precursor were added to 40 mL of a 0.5 M solution of activating agent ((NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4}) chosen to avoid the destruction of hydroxyapatite structure. The impregnation step was achieved in a
glass vial under constant stirring at 150 rpm at (20 ± 2) °C for 48 h. The sample was then washed several times with deionized water, filtered and then dried in an oven for 24 h at 80°C. The final material was washed with deionized water until the neutral pH of solution was reached. The fine particles of the impregnated material were then collected. For this purpose, the particles obtained in the previous step were dispersed in 200 mL of deionized water and the suspension was placed in a sedimentation tube, the fraction below 3 µm was separated by sedimentation according to the Stokes law and then recovered by centrifugation. Afterwards, more deionized water was added in the tube and the procedure repeated until a clear liquid was obtained at the top of the tube. The resulting material (hereafter denoted as NHAP$_{P0.5}$) and not impregnated material (hereafter denoted as NHAP) were dried in an oven for 24 h at 80°C and stored in plastic containers for later use.

**Coupling of β-cyclodextrin to hydroxyapatite**

The synthesis of the β-CD natural hydroxyapatite composite was adapted from a published procedure (Leprêtre et al. 2009). An amount of 0.500 g of NHAP$_{P0.5}$ was carefully dispersed in 5 mL deionized water in an ultrasonic bath for 5 min. Afterwards, 0.250 g of β-CD, 0.250 g citric acid (cross linker agent) and 0.083 g of Na$_2$HPO$_4$ (catalytic agent) were added to this mixture. The mass ratio of β-CD and natural hydroxyapatite was set at 1:2 (w/w). The resulting mixture was sonicated for 5 min and the solution obtained was transferred to an electric oven at 100°C for 120 min. The obtained residue was then placed in an electric oven at 180°C for 45 minutes, followed by cooling at ambient temperature. The solid residue obtained was carefully crushed, washed with deionized water and dried in an oven. The final composite material thus synthesized was referred as NHAP$_{P0.5}$-CA-β-CD. All steps involved in the preparation of NHAP$_{P0.5}$-CA-β-CD are given in Scheme 1. For comparison purposes, the same protocol was followed to prepare the β-CD polymer (without hydroxyapatite, denoted CA-β-CD), excepted that natural hydroxyapatite was no longer used.

**Physic-chemical characterization of materials**

The structural characterisation of the starting hydroxyapatite, the precursor and the lysine/NHAP materials were determined by Powder X-Ray diffraction (PXRD) and Fourier Transformed Infrared (FTIR) spectroscopy. The PXRD patterns were recorded at room temperature using powder diffractometer (Stoe Stadi-P) operating with a Cu-Kα radiation ($\lambda = 1.54056$ Å), while FTIR spectra were registered also at room temperature using KBr pellets, in the spectral range from 4500 to 400 cm$^{-1}$ on an Alpha IR spectrometer from Bruker.

**Electrochemical experiments**

Electrochemical measurements were performed by a computerized Potentiostat instrument (Metrohm μ-Autolab, Netherlands) using the GPES software. All voltammograms were recorded at room temperature with a three-electrode comprising a platinum grid as counter electrode, an Ag/AgCl/KCl (3.0 M) reference electrode and a working electrode that was either a bare glassy carbon electrode (GCE), or the GCE modified by a thin film of CA-β-CD, NHAP$_{P0.5}$ or NHAP$_{P0.5}$-CA-β-CD material. The GCE was first polished
on billiard cloth covered by alumina paste (0.05 µm particle size), then ultrasonically cleaned for 5 minutes in deionized water. Dispersions of the materials (NHAP\textsubscript{P0.5}, NHAP\textsubscript{P0.5}-CA-β-CD or CA-β-CD) were prepared by diluting 4 mg of each material in 2 mL of deionized water. 8 µL of the dispersion were then casted on the active surface (3 mm in diameter) of the GCE. The thin film electrode was dried at room temperature for about two hours to ensure complete drying and used without any pre-treatment in the electrochemical cell. These electrodes will be hereafter referred as GCE/NHAP\textsubscript{P0.5}, GCE/CA-β-CD and GCE/NHAP\textsubscript{P0.5}-CA-β-CD for the GCE modified by NHAP\textsubscript{P0.5}, CA-β-CD, and NHAP\textsubscript{P0.5}-CA-β-CD; respectively.

The electrochemical analysis of Pb\textsuperscript{2+} ions was carried out using differential pulse anodic stripping voltammetry (DPASV). The procedure was based on two successive steps: (i) the open-circuit accumulation of the analyte was achieved by dipping the working electrode in a beaker kept at constant stirring and containing the Pb\textsuperscript{2+} solution at a given concentration; and (ii) the voltammetric detection of the accumulated analyte between −0.90 and −0.20 V in 0.1 M acetate buffer solution. DPASV measurements were performed by applying the following optimized parameters: 0.00 s equilibration time, 5 mV potential step increments and 100 mV potential amplitude. After each voltammetric detection and before the next accumulation, the surface of the working electrode was renewed by simply shaking it in the detection medium for approximately 3 minutes.

**Results And Discussion**

**Physicochemical characterization of materials**

Figure 1A presents the FTIR spectra between 3800 and 380 cm\textsuperscript{-1} of NHAP, NHAP\textsubscript{P0.5} and NHAP\textsubscript{P0.5}-CA-β-CD materials. On all spectra, fundamental peaks characteristic of hydroxyapatites are observed (Koutsopoulos 2002). The absorption bands which appear around 3450 and 1638 cm\textsuperscript{-1} correspond to the bending vibrations of water molecules adsorbed on the materials (Koutsopoulos 2002; Patel et al. 2015; Kanchana and Sekar 2014). The visible vibration bands of OH groups were identified at 3570 and 630 cm\textsuperscript{-1} (Kanchana and Sekar 2014). The absorption peaks observed at 1458, 1419 and 873 cm\textsuperscript{-1} are attributed to the binding vibrations of carbonate (CO\textsubscript{3})\textsuperscript{2−} in the structure of the material (Koutsopoulos 2002; Kanchana and Sekar 2014). The vibrations bands of phosphate PO\textsubscript{4}\textsuperscript{3−} were also detected at 1090, 1039, 961, 601 and 569 cm\textsuperscript{-1}, respectively (Yala et al. 2013; Koutsopoulos 2002). On the basis of these results, it can be deduced that all the collagens and other organic compounds present in the raw bones have been eliminated during calcination. After treatment with dibasic ammonium phosphate, the spectrum of the resulting material (NHAP\textsubscript{P0.5}) displays bands similar to those of NHAP. This confirms that this treatment had not affected the structure of the material. Furthermore, comparing the spectra of pristine HAP and NHAP\textsubscript{P0.5}-CA-β-CD samples clearly showed additional peaks at 2933 and 1729 cm\textsuperscript{-1} that are respectively attributed to the vibrations bands of C-H groups of β-CD and C = O bands of ester bonds formed during the polymerization (Leprêtre et al. 2009; Tang et al. 2013; Tcheumi et al. 2019; Heydari and Sheibani 2015). The observed absorption bands of ester groups indicated that hydroxyl
groups of β-CD have reacted with the carboxyl groups of citric acid, leading to the formation of a three-dimensional polymer network (Tcheumi et al. 2019).

The crystalline phase analyses of obtained materials were carried out by XRD as shown in Fig. 1B. The pattern of NHAP starting material (Fig. 1B(a)) presents well-defined and thin diffraction peaks, proving that the material has a good crystallinity. Some typical and specific crystallographic plane (002), (102), (112), (211), (300), (130), (202) and (310) appearing at 25.80°, 28.92°, 31.71°, 32.21°, 32.90°, 34.13°, 40°, 46.70° and 49.44° 2θ angles were matched with standard ICDD Diffraction File No. 00-009-0432 of hydroxyapatite (Kanchana and Sekar 2014). This confirms the results obtained in FTIR, from which it appeared that the material obtained from bovine bones after calcination was pure hydroxyapatite.

Upon the impregnation of NHAP with ammonium phosphate dibasic, the material becomes somewhat amorphous (Fig. 1B(b)) although the main diffraction peaks remain visible. By comparing the XRD pattern of the hybrid material (NHAP<sub>P0.5</sub>-CA-β-CD) with that of unmodified hydroxyapatite (NHAP<sub>P0.5</sub>), it can be seen that the characteristic diffractions of two materials are almost the same, which shows that the grafting of β-CD had not affected the crystalline phases of the hydroxyapatite particles as previously observed by Leprêtre et al. (2009) in similar materials. By exploiting the Scherrer’s equation (Elkabouss et al. 2004) expressed by \( D = \frac{K \cdot \lambda}{(2 \cdot \beta \cdot \cos \theta)} \) where K is the shape factor (0.9), \( \beta \) the full width at half-maximum of diffraction peaks measured in radians, \( \lambda \) the wavelength of the used anticathode (\( \lambda(Cu) = 1.54056\)Å) and \( \theta \) is Bragg’s diffraction angle, the average crystallite size of NHAP, NHAP<sub>P0.5</sub> and NHAP<sub>P0.5</sub>-CA-β-CD were estimated as 279.65, 36.61 and 99.22 nm respectively. The treatment of NHAP with dibasic ammonium phosphate induces a significant reduction of the crystal size. Yet, this treatment caused a structural reorganization of the material making it better dispersed. When the treated material was functionalized with β-CD, particle size increased, probably due to the grafting of β-CD on the surface of the material.

**Preliminary studies on the electrochemical detection of Pb<sup>2+</sup> ions**

Useful preliminary investigations were performed to explore the ability of investigated materials as convenient GCE modifier for the electrochemical sensing of Pb<sup>2+</sup> ions, in view of selecting the most sensitive material. Thus, GCE/NHAP<sub>P0.5</sub>, GCE/CA-β-CD and GCE/NHAP<sub>P0.5</sub>-CA-β-CD devices were used in turn to analyse a 2 × 10<sup>-6</sup> M Pb<sup>2+</sup> solution, and the DPASV curves obtained are shown in Fig. 2. As one can observe, a peak current of about 14.04 µA in intensity was generated at the GCE electrode modified by raw hydroxyapatite (NHAP<sub>P0.5</sub>). This signal is due to the ability of hydroxyapatite to easily adsorb positive ions by a cation exchange mechanism (El Mhammedi et al. 2009b; Mobasherpour et al. 2011).

When the bare GCE was modified by β-cyclodextrin polymer, the anodic peak current obtained was enhanced (23.98 µA), giving rise to intensity more than 1.7-fold larger than that obtained in the same conditions on GCE/NHAP<sub>P0.5</sub>. This result is due in one hand to the capacity of β-CD to form reversible inclusion complexes with Pb<sup>2+</sup> metal ions (Zhao et al. 2019), and in the other hand to the ability of the
free carboxylate functions of citric acid to trap Pb$^{2+}$ ions (He et al. 2017; Celebioglu et al. 2019). Finally, when the film on the GCE was formed by the composite material (NHAP$_{0.5}$-CA-β-CD), the anodic peak current obtained was substantially improved. The peak current obtained (38.60 µA) was 1.61-fold and 2.75-fold more intense than the signals recorded on GCE/CA-β-CD and GCE/NHAP$_{0.5}$, respectively. This observation can be reasonably attributed to three major factors: (i) the complexation of Pb$^{2+}$ ions by β-CD; (ii) the ability of free carboxylate functions of citric acid that can also contribute by electrostatic attraction to accumulate Pb$^{2+}$ ions; and (iii) the adsorption of Pb$^{2+}$ ions by natural hydroxyapatite present in the film, following an ion exchange process. From these investigations, the composite material was thus chosen for further experiments as it led to the most sensitive detection of Pb$^{2+}$ ions.

The reproducibility and stability of the composite material coated on the GCE were then evaluated. For this purpose, a series of repetitive measurements (series of 8 experiments) were performed by using the same GCE/NHAP$_{0.5}$-CA-β-CD sensor for the detection of 2 µM Pb$^{2+}$ ions. As shown on Fig. SI(1) (Electronic Supporting Material), highly reproducible stripping currents were obtained with a relative standard deviation of 1.27%. Moreover, a series of measurements on five electrodes prepared with an identical procedure gave a relative standard deviation of 3.75%. This indicates a good reproducibility of the proposed sensor for the detection of Pb$^{2+}$ ions. The long-term stability of the proposed sensor was also evaluated by recording daily the signal of a 2 µM Pb$^{2+}$ solution, the electrode being kept in 0.1 M acetate buffer (pH 5) in a fridge. It was found that GCE/NHAP$_{0.5}$-CA-β-CD can retain more than 96% of its original response, without regenerating or reactivating the surface during the process of successive determinations. In order to obtain the highest sensitivity of the modified electrode for the electrochemical determination of Pb$^{2+}$, the operating parameters of the accumulation and detection steps have been optimized in the coming sections.

**Optimization of key parameters involved in the accumulation step**

The pH of the accumulating medium is an important parameter, expected to affect the electrode signal. It was investigated on GCE/NHAP$_{0.5}$-CA-β-CD in the range between 4.0 and 6.5 by DPASV in aqueous solution containing 2µM of Pb$^{2+}$. It is important to mention that in highly acidic media (pH < 3), no real signal was obtained, probably due to the mineralization of the hydroxyapatite backbone. Basic media were not studied as they may induce the precipitation of Pb$^{2+}$. The obtained results are given in Fig. 3A: in moderate acidic media, the stripping peak current of Pb$^{2+}$ increased as the pH was changed from 4.0 to 5.0, then it decreased to 6.5. The poor sensor signal obtained for pH values less than 4 was attributed to a competition between H$^+$ and the Pb$^{2+}$ ions, and to the protonation of the free carboxylic acid functions available on the surface of the composite material. As noticed, the optimal pH for the detection of Pb$^{2+}$ ions is 5, that was chosen for further investigations.

The duration of the accumulation step is the second factor that can influence the sensor response. It was thus necessary to study its effect on the peak current of Pb$^{2+}$. The DPASV curves recorded at
GCE/NHAP_{P0.5}-CA-β-CD dipped in an aqueous solution (pH 5) containing 2 µM Pb^{2+} for accumulation time varied between 1 and 30 min are shown in Fig. 3B. It can be noted the peak current increased linearly with increasing accumulation time up to 8 min, showing a rapid and progressive occupation of the binding sites on the working electrode. Afterwards, the peak current leveled as these sites are almost all occupied. For further measurements, an accumulation time of 600 seconds was employed.

**Optimization of the detection parameters**

**pH of the detection solution**

Since the deposition of Pb^{2+} ions on GCE/NHAP_{P0.5}-CA-β-CD strongly depends on the pH, the effect of the acidity of the detection solution on the stripping peak current was studied in 0.1 mol L^{-1} acetate buffer with pH ranging from 3.5 to 6.5. The results given in Fig. 4 show that the pH of the detection medium greatly affects the peak current of Pb^{2+} as reported by some previous studies (Lv et al. 2013; Mohammadi et al. 2020). The electrode response increased from pH 3.5 to reach a maximum at pH 5, then decreased for pH value between 5 and 6.5. Thus, pH 5 was selected as optimum value and used in coming sections.

**Deposition potential and electrolysis time**

In electroanalysis technique based on adsorptive stripping voltammetry, the deposition potential and its duration are determining factors to achieve high sensitivity. The effect of both parameters was studied and the data obtained are shown on Fig. 5. Varying the deposition potential in the range from −0.1 to -1.0 V revealed that the peak currents increased slightly when the deposition potential was decreased from 0 to -0.5 V, then increased significantly for potential values between −0.6 V and −0.8 V, to stabilize beyond (Fig. 5A). Thus, for a quantitative reduction of Pb^{2+} ions, the deposition should be operated at a potential lying between −0.8 V and −1.0 V.

Furthermore, the effect deposition duration on the electrode signal of 2 µM Pb^{2+} was investigated in the range from 0 to 30 s (after 3 min of preconcentration). The results on Fig. 5B indicated that the electrode signal increased rapidly with the electrolysis time to reach a maximum from 20 s. This phenomenon is ascribed to the progressive and rapid occupation of the analyte on the accumulation sites at the working electrode. Upon saturation of these sites, the peak current became constant. Therefore, for subsequent experiments, the deposition of Pb^{2+} ions was achieved at -0.8 V for 30 s.

**Influence of lead concentration and calibration curve**

By using the optimized parameters selected in previous sections on the optimization of accumulation and detection steps, DPASV experiment were conducted for Pb^{2+} concentration varied between 2.00 × 10^{-8} mol L^{-1} to 20.00 × 10^{-8} mol L^{-1}. As shown in Fig. 6, the stripping peak current increased with increasing Pb^{2+} concentration. As shown by the inset in Fig. 6, a linear relationship was obtained for the peak current (Ip) and concentration of Pb^{2+}, that obeyed to the equation Ip(µA) = 100.80 [Pb^{2+}](µM) + 0.15
$10^{-6}$ ($R^2 = 0.998$). The sensitivity of the electrode was 100.80 $\mu$A.$\mu$M$^{-1}$, while a limit of detection of $5.06 \times 10^{-10}$ mol L$^{-1}$ was calculated for a signal-to-noise ratio equal to 3.

Such analytical performances of GCE/NHAP$_{P0.5}$-CA-β-CD sensor for Pb$^{2+}$ detection were confronted with some other electrochemical sensors, and the data were summarized in Table 1 (El Mhammedi et al. 2009b, Pan et al. 2009, Li et al. 2016, Lv et al. 2013, Mohammadi et al. 2020, Zhan et al. 2016, Oliveira et al. 2020, Xu et al. 2020).

Table 1
Comparison of the performance of some recent modified electrodes, towards the voltammetric detection of lead (II)

| Electrode configuration | (a) LOD (mol.L$^{-1}$) | Linear range (mol.L$^{-1}$) | Reference |
|-------------------------|------------------------|-----------------------------|-----------|
| Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ modified (b) CPE | $7.68 \times 10^{-8}$ | $2.0 \times 10^{-9} - 2.4 \times 10^{-7}$ | El Mhammedi et al. 2009b |
| Nanosized HA nanion modified (c) GCE | $1.00 \times 10^{-9}$ | $5.0 \times 10^{-9} - 8.0 \times 10^{-7}$ | Pan et al. 2009 |
| β-CD (d) reduced GO (e) hybrid nanosheets | $9.42 \times 10^{-11}$ | $1.0 \times 10^{-10} - 9.0 \times 10^{-9}$ | Lv et al. 2013 |
| CPE modified with magnetic eggshell nanocomposite and (f) MWCNTs | $4.52 \times 10^{-7}$ | $1.5 \times 10^{-6} - 6.0 \times 10^{-4}$ | Mohammadi et al. 2020 |
| GCE modified with β-CD and chemically reduced GO | $5.00 \times 10^{-10}$ | $1.0 \times 10^{-9} - 1.0 \times 10^{-7}$ | Zhan et al. 2016 |
| GCE modified with conductive polypyrrole nanoparticles | $55.00 \times 10^{-9}$ | $1.0 \times 10^{-7} - 50.0 \times 10^{-6}$ | Xu et al. 2020 |
| **GCE covered by Zinc oxide nanofibers functionalized by L-cysteine** | $1.20 \times 10^{-9}$ | $3.0 \times 10^{-8} - 4.2 \times 10^{-7}$ | Oliveira et al. 2020 |
| GCE covered by engineered MWCNTs | $1.88 \times 10^{-8}$ | $0.4 \times 10^{-6} - 80.0 \times 10^{-6}$ | Li et al. 2016 |
| GCE modified with β-CD hydroxyapatite composite | $5.06 \times 10^{-10}$ | $2.0 \times 10^{-8} - 2.0 \times 10^{-7}$ | This work |

(a) LOD: Limit of detection (b) CPE: Carbon paste electrode (c) GCE: Glassy carbon electrode
(d) β-CD: β-cyclodextrin (e) GO: Graphene oxide (f) MWCNT: Multi-walled carbon nanotubes
By analysing these data, it appeared that GCE/NHAP$_{0.5}$-CA-β-CD has a higher or comparable sensitivity to the other electrodes. In addition, the detection limit achieved herein is much lower than $4.83 \times 10^{-8}$ mol L$^{-1}$, the value recommended by the World Health Organization (Awual 2019). This suggested that the method developed in this work could be applied to the monitoring and quantification of Pb$^{2+}$ in food and in aqueous environment.

**Interference study and real sample determination**

The selectivity of the sensor was tested under optimal conditions, by analysing the effect of potential interfering cations and anions on the detection of Pb$^{2+}$ ions. Thus, each of the selected ions was intentionally introduced at known concentrations into the accumulation solution containing 2 µM Pb$^{2+}$. After 3 min preconcentration at open-circuit, the electrode response was recorded and compared to that of Pb$^{2+}$ previously registered alone in the same conditions. The results obtained are summarized in Table 2. As observed, the studied chemical species did not interfere when their concentration was less than Pb$^{2+}$ concentration.
Table 2
Variation in the peak current of 2 µM Pb\(^{2+}\), recorded in the absence (100%) and in the presence of others ionic species introduced in relation to Pb\(^{2+}\) ions concentration

| Interference ions | Added amount over Pb\(^{2+}\) concentration | Variation (%) in the peak current of Pb\(^{2+}\) ions |
|-------------------|---------------------------------------------|---------------------------------------------------|
| Cd\(^{2+}\)       | 0.1                                         | 101.3                                             |
|                   | 1                                           | 98.60                                             |
|                   | 10                                          | 96.53                                             |
| Ni\(^{2+}\)       | 0.1                                         | 102.65                                            |
|                   | 1                                           | 96.96                                             |
|                   | 10                                          | 97.30                                             |
| Cu\(^{2+}\)       | 0.1                                         | 99.14                                             |
|                   | 1                                           | 101.11                                            |
|                   | 10                                          | 49.24                                             |
| K\(^{+}\)         | 1                                           | 100.24                                            |
|                   | 10                                          | 101.30                                            |
|                   | 100                                         | 96.70                                             |
| Mg\(^{2+}\)       | 1                                           | 103.60                                            |
|                   | 10                                          | 99.24                                             |
|                   | 100                                         | 98.10                                             |
| SO\(_4^{2-}\)     | 1                                           | 103.60                                            |
|                   | 10                                          | 99.24                                             |
|                   | 100                                         | 98.10                                             |
| Cl\(^{-}\)        | 1                                           | 100.24                                            |
|                   | 10                                          | 101.30                                            |
|                   | 100                                         | 96.70                                             |

However, for much higher concentrations (about 10-fold), a decrease in the expected current was noticed for some ions. Indeed, there was a current drop, for about 3.47% for Cd\(^{2+}\), 2.70% for Ni\(^{2+}\), and 50.76% for Cu\(^{2+}\). This observation can be explained by the relative good affinity displayed by these ions towards the carboxylate groups of free carboxylic acid present on the composite material (He et al. 2017; Celebioglu et al. 2019). It also appeared that for K\(^{+}\), Mg\(^{2+}\), SO\(_4^{2-}\) and Cl\(^{-}\) the electrochemical response of Pb\(^{2+}\) was slightly disturbed, even for concentrations 100-fold greater.
In order to evaluate the potential application of GCE/NHAP$_{P0.5}$-CA-β-CD sensor, the detection of Pb$^{2+}$ ions in spring water and well water (collected in downtown Yaounde, Cameroon) was conducted. A volume of 50 mL of each sample was taken and used without further treatment as the accumulation medium. It should be noted that for both real samples, no Pb$^{2+}$ signal was found in operational conditions, indicating the absence of any trace of Pb$^{2+}$ ion above the detection limit previously obtained. The determination by DPASV of Pb$^{2+}$ was thus performed using the optimized parameters, by the standard addition method. Each sample was spiked with $1.5 \times 10^{-7}$ mol L$^{-1}$ Pb$^{2+}$ and analysed in triplicate, and the results achieved are listed in Table 3. The obtained values are in good agreement with the spiked ones, indicating that the proposed method could serve for the determination of lead (II) in real samples.

| Added (10$^{-7}$ mol L$^{-1}$) | Found$^a$ (10$^{-7}$ mol L$^{-1}$) | Recovery (%) |
|-------------------------------|----------------------------------|--------------|
| Spring water 1.5              | (1.45 ± 0.04)                    | 96.67        |
| Well water 1.5                | (1.47 ± 0.04)                    | 98.00        |

$^a$ Number of samples assayed = 3

**Table 3**

Recovery rate of Pb$^{2+}$ in spring and well water samples at GCE/NHAP$_{P0.5}$-CA-β-CD

**Conclusion**

A novel and sensitive sensor for Pb$^{2+}$ detection, based on a glassy carbon electrode modified by a thin of hydroxyapatite/β-cyclodextrin composite material was proposed. The organohydroxyapatite exploited as electrode modifier was synthesized by grafting β-cyclodextrin on the surface of natural hydroxyapatite, using citric acid as a cross-linked. Upon, physico-chemical characterization by several techniques, the hydroxyapatite-based composite was used to build a highly sensitive, selective and reliable sensor that was successfully applied for the electrochemical determination of Pb$^{2+}$ ions in spring water and well water samples. Considering the results obtained, this study demonstrated the resort to hydroxyapatite as low-cost for the preparation of analytical devices that may find application in pollution control and environment protection.

**Declarations**

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**Ethics declarations**

**Conflict of interest:** The authors declare that they have no conflict of interest.
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Figures
Figure 1

(A) FTIR spectra and (B) PXRD patterns of (a): NHAP, (b): NHAPP0.5 and (c): NHAPP0.5-CA-β-CD materials. (Panel c is not available in this version.)
Figure 2

DPASV recorded after 3 min accumulation from an aqueous $2 \times 10^{-6}$ M Pb$^{2+}$ solution at pH 6 using, (a): GCE/NHAPP05, (b): GCE/CA-β-CD, and (c): GCE/NHAPP05-CA-β-CD. Detection in 0.1 M acetate buffer (pH 5) after 15 s electrolysis at -0.8 V. Other experimental conditions: 0 s equilibration time, 5 mV potential step and 100 mV amplitude.

Figure 3
(A): Evolution of peak current at GCE/NHAPP0.5-CA-β-CD as a function of the pH of accumulating medium, after 3 min accumulation from a 2 µM Pb2+ aqueous solution. (B) DPASV curves recorded at GCE/NHAPP0.5-CA-β-CD at various accumulation times in an aqueous solution (pH 5) containing 2 µM Pb2+. The inset shows the evolution of peak current as a function of accumulation time. Detection in 0.1 M acetate buffer (pH 5) after 30 s electrolysis at -0.8 V.

**Figure 4**

(A): DPASV recorded at GCE/NHAPP0.5-CA-β-CD for different pH of detection medium after 3 min accumulation from an aqueous 2 × 10⁻⁶ M Pb2⁺ solution (pH 6). Detection after 15 s electrolysis at -0.8 V. (B): the corresponding peak current variation.

**Figure 5**
(A): Dependence of Pb(II) peak current on electrolysis potential, after 3 min preconcentration in 2 µM Pb2+ (pH 5), on GCE/NHAPP0.5-CA-β-CD. Detection in 0.1 M acetate buffer (pH 5) after 15 s electrolysis. (B): Variation of Pb(II) peak intensities at GCE/NHAPP0.5-CA-β-CD with electrolysis duration. Other conditions as in (A).

Figure 6

DPASV signal recorded at GCE/NHAPP0.5-CA-β-CD under optimized conditions, after 10 min accumulation in an aqueous medium containing Pb2+ in the range from 2 × 10^-8 to 2 × 10^-7 M. Other experimental conditions are as follows: 0 s equilibration time, 5 mV potential step and 100 mV amplitude. The inset shows the corresponding calibration curve.

Supplementary Files

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