High-Content Hydroxyapatite Carbon Composites for the Electrochemical Detection of Heavy Metal Cations in Water

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The great performance of nanostructured hydroxyapatite (Hap) in adsorbing heavy metal cations with the good electrical conductivity of high-surface carbon materials was here combined for the crafting of high-content hydroxyapatite electrodes (Hap 84–96 wt.%) endowed with the synergistic capability of detection and adsorption of heavy metal cations by water solutions. To improve the sustainability of the sensor, a mesoporous carbon (derivable by bio-waste) was selected as scaffold for depositing Hap by simply co-precipitation. The composite with 92 wt.% of Hap, which exhibited ca. 1:1 ratio between the exposed area of Hap and carbon (by Brunauer-Emmett-Teller method), invariably showed an average stripping oxidation peak intensity of ca. 250 µA cm^{-2} and 150 µA cm^{-2} for a 50 µM Pb^{2+} and Cu^{2+} solution, respectively. Control experiments showed that the above sensor outperformed the sensibility of two low-content Hap electrodes (4 and 8 wt.%), representative of the best performing Hap-carbon composites available in literature.

Introduction

Monitoring of heavy metal ions concentration in wastewater and in drinkable water, together with their eventual remediation, is of paramount importance to protect both environment and consumers.[1,2] Indeed, the concentration thresholds for the majority of these pollutants are strictly regulated by dedicated national and/or international laws. Among the analytical techniques developed over the years, electroanalytical methods are particularly appealing for economical and practical reasons (cheap and portable instrumentation, disposable electrodes, etc.).[3] The easy reduction of many metal ions has made the anodic stripping voltammetry (ASV) one of the election techniques for their detection.[4,5] The technique offers i) the capability to pre-concentrate the analyte on the electrode surface, by means of a metal deposition under potentiostatic cathodic polarization, and ii) a high signal-to-noise ratio, by a differential voltammetry run (DPV or SWV) that detects the analyte(s) by recording the dissolution anodic current(s).[6]

Thanks to the almost unique physical and chemical features of mercury (easy surface regeneration, ability to form amalgam, high hydrogen evolution overpotential, etc.), this liquid metal is largely used in the routine environmental analysis, in the form of both drop and thin film electrodes.[5,7] However, due to the toxic nature of mercury, researchers have aimed at finding safer electrode materials while retaining Hg-like performance. Among metallic-like electrodes, solid bismuth and antimony, and liquid galistan (an eutectic mixture of Ga–In-Sn) represent promising alternatives for the detection of heavy metal cations.[8–10] In parallel, the research opened the way to non metallic, carbonaceous materials encompassing a large amount of studies on the so-called carbon paste electrodes due to their easy fabrication and their easy tailoring with proper modifiers.[11,12] To improve the sensor figures of merit, many examples of carbon paste electrodes modified with adsorbing materials appeared in the dedicated literature.[13–15] Inorganic insulating modifiers (SiO$_2$, Al$_2$O$_3$, etc.) are particularly appealing as they are usually characterized by high chemical stability and large surface area. In addition, electric insulating features also increase signal to noise ratio (adsorbent does not contribute to capacitive current) and hence improved detection limits of the electrode. However, one of the limits of common inorganic adsorbents is the relative low selectivity towards metals cations, a drawback that can be overcome only by grafting specific ligands on the surface of the adsorbent.[13]

Hydroxyapatite (Hap, Ca$_{10}$(PO$_4$)$_6$(OH)$_2$) combines the above-mentioned features of inorganic adsorbents with remarkable selectivity and retention ability towards heavy metal cations that are hence efficiently removed from the treated solution.[16–19] The selectivity mainly derives from several mech-

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anisms, which are active in the retention of metal ions (i.e., pure ion exchange, surface complexation, surface precipitation, dissolution precipitation, reductive complexation[20]). The predominance of a certain mechanism depends on the surface features of Hap (Ca/P ratio, facet exposure, isoelectric point) as well as on the nature of the metal. In addition, the highly functionalised surface of Hap allows a facile anchoring on external surface of other materials, paving the way to the relatively easy production of Hap-containing composites. Thanks to these characteristics, no additional functionalization is required, thus reducing the cost and fabrication time of Hap-modified carbon paste electrodes. In line with these considerations, many researchers have attempted to exploit the sorption properties of Hap in sensing applications, usually compositing it with different kinds of mesoporous scaffolds as to maximize the exposed surface area. In the electrochemical literature, some examples of carbon-free electrodes[21] and carbon-based composite electrodes[22–24] containing Hap have been already reported for the determination of heavy-metal ions [Hg(II), Cd(II) and Pb(II)].

El Mhammedi et al.[23] demonstrated that the addition of 4 wt.% of Hap to a carbon-paste electrode (CPE) produced a sensor with improved performance compared to unmodified CPE in the determination of trace lead(II) by square-wave voltammetry, with a range for quantitative detection of lead from 2 nM to 0.24 μM. Beyond 4 wt.%, the voltametric response of the electrode decreased significantly, likely due to a decrease in the conductive area at the electrode surface. The concentration of Hap was a critical factor also in the production of hydroxyapatite-modified carbon ionic liquid electrodes for the simultaneous determination of ultra-trace lead and cadmium.[22] The sensor with 5 wt.% of Hap exhibited the best performance with a linear behavior in the range of 1 to 100 nM for lead and cadmium and detection limits of 0.2 nM for lead and 0.5 nM for cadmium. Not only the concentration, but also the morphology of Hap plays an important role. Chemically modified CPE doped by sea cucumber-like hydroxyapatite crystals had significantly lower linear ranges for Pb(II) and Cd(II) with a detection limit of 4.23 pM and 27 pM, respectively, and sensitivities about 10 and 100 times higher than those reported previously for HAp–CPEs.[25] In all cases, the proposed electrodes were invariably characterized by a very low amount of Hap (optimal content around 5 wt.%) due to the electrical insulating character of hydroxyapatite.

The goal of the present paper is to provide evidence that even neatly higher Hap content is allowed in the design of novel electrochemical sensors the monitoring of heavy metal cations in water. Looking at previous publications from some of us, suspensions of Hap-rich carbonaceous composites (Hap/C) powder, with a content of the carbonaceous scaffold ranging from 4 to 16 wt.%, have already demonstrated their capture ability towards polluting heavy metal cations [e.g., Pb(II), Cu(II) and Ni(II)] in virtue of the synergistically occurrence of an efficient adsorbent (Hap) well distributed over a high surface area scaffold (carbon).[26,27] In the present work, starting from this result, the full potentiality of the carbon-based scaffolds is further investigated by exploiting also its intrinsic electrical conductivity to develop an electrochemical probing material for the detection of two model metal contaminants, i.e., Pb(II) and Cu(II). As a result, adsorbent composites endowed with a sensing ability are prepared and characterized by combining electrical and electrochemical tests with compositional and morphological information. The composite formulations were specifically thought to improve the sustainable profile of the sensing material, as both Hap and the used mesoporous carbon may be sourced from biowastes.

Results and Discussion

Structural and morphological characterization of the Hap/C composites

The composition and textural properties of Hap/C_CMC composites, where CMC refers to a biomass-derived mesoporous carbon, have been recently presented in the literature.[26] Based on Thermogravimetric (TG) analyses, compositions (in wt.%) in line with those envisaged by synthesis were obtained (Table S1 of Supporting Information). Characterization by transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infrared (FT-IR) spectroscopy revealed that crystalline Hap and CMC moieties were perfectly dispersed onto each other throughout the whole CMC compositional range under investigation (Figure 1).[26] A different situation emerged from the here-reported characterization of the carbon nanofibers (CNF)-based composite where crystalline Hap nanoplatelets are present in isolated aggregates, not in complete contact with the carbonaceous scaffold (Figure 1). The reason behind
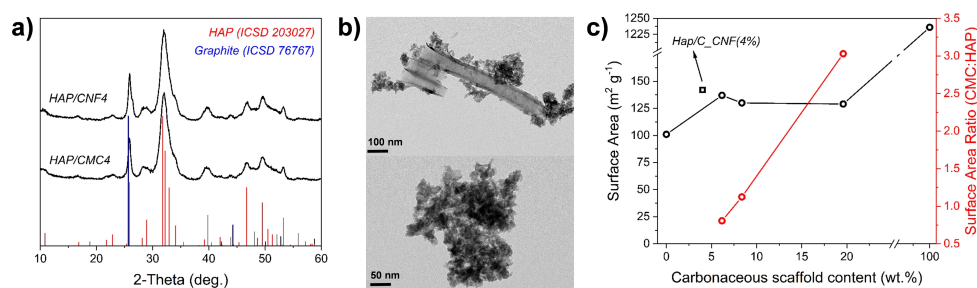

Figure 1. (a) XRD patterns and (b) BF-TEM images of Hap/C_CNF(4%) (top) and Hap/C_CMC(4%) (bottom). (c) Trends of surface area and CMC:HAP surface area ratio versus the scaffold content. Data in (a) and (c) adapted from Ferri et al.[26]

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these differences lays in the surface features of the two carbonaceous scaffolds. CMC exhibits large surface area and good pore volume (Table S1)[28] together with a highly functionalized surface,[29] all factors that concur in making it an ideal scaffold. Conversely, CNFs are characterized by low surface area (41 m^2 g^{-1}, Table S1) and highly graphitic and defect-free surfaces, features that however confer a remarkable conductivity.

Focusing on the morphology of the Hap/C_CMC series, it is interesting to notice that the surface area ratio between the two pristine moieties (CMC and Hap) spans from values that are lower than 1 to higher ones (Table S1 and Figure 1). In particular, although composites surface area is moderately influenced by the CMC content, a 1:1 surface area ratio between moieties is achieved for the composite with 8 wt.%. This feature should represent the ideal trade-off in terms of conductivity, Hap dispersion and exposure of the Hap functional groups to the surface.

Electrical conductivity of the Hap/C composite as foil

Thin foils of each Hap/C composite were prepared from the related powder through a rolling process, as described above. An eye investigation ensured the continuity of the foil, while scanning electron microscopy – energy dispersive X-ray spectroscopy (SEM-EDS) allowed to investigate the surface morphology and check the microscopic uniformity of the rolled foils (Figure S2). The solid-state charge transport mechanism of the composites was investigated by electrochemical impedance spectroscopy in a symmetric configuration (i.e., a foil sandwiched and pressed between two flat metal collectors). It is evident that the electric conductivity (Table S2) of the composites is strongly affected by the nature of the carbon scaffold, with CNF [Hap/C_CNF(4%)] exhibiting a conductivity that is 5-orders of magnitude higher than that of the corresponding CMC composite [Hap/C_CMC(4%)]. This is attributable i) to the intrinsically higher conductivity of CNF with respect to CMC (ca. 10^{12} μS m^{-1} and ca. 2·10^5 μS m^{-1}, respectively, for pressed tablets of the two materials)[30,31] and ii) to the different

morphology of the two kinds of composites. Indeed, the partial covering of the carbon fibers by the insulating Hap results in a higher contact area between adjacent conductive elements; on the contrary, the more uniform Hap layer grown during the co-precipitation with CMC should result in a lower amount of the sites of contact between adjacent conductive carbonaceous nuclei (Figure 1b). The two morphologies also affect the impedance spectra (Figure 2). The composite with CNF exhibited impedance spectra almost invariable in the selected frequency range (modulus around 80 Ω cm^2; phase around 0°), depicting an ohmic-like conductivity. On the contrary, the spectrum of the CMC-containing composite suggests a more complex and hindered charge transport mechanism, tentatively attributable to the occurrence of interfaces (i.e., capacitive phenomena) and grain boundary along the conductivity pathway.

Concerning the effect of the amount of the carbonaceous scaffold, an increase of the content of the conductive component results in a non-linear increase of the conductivity (Table S2). For the CMC-based series, the lower frequency impedance modulus ($|Z|_{0.1Hz}$) changes from around 90 nS m^{-1} to ca. 2 μS m^{-1} by increasing from 4 to 16 wt.% the amount of CMC. For sake of comparison, the composite with 96 wt.% of CMC exhibits a ca. 20 μS m^{-1} specific conductivity.

Intrinsic electrochemical features of the Hap/C composite electrodes

The brittleness of the composite foils did not allow their direct use as electrochemical sensors. Thus, for all the electrochemical tests, the composite was pasted on a strip of graphite foil acting as substrate and electron collector (Figure S1). A preliminary cyclic voltammetry investigation, performed in a 0.1 M KNO$_3$ blank solution, was aimed at elucidating the intrinsic electrochemical features of the composite electrodes as a function of the nature and amount of the carbon scaffold.

Cyclic voltammetry did not detect any faradaic electron transfer peaks (i.e., electroactive impurities and/or oxygenated carbon groups, if present, are below the detection limit), while
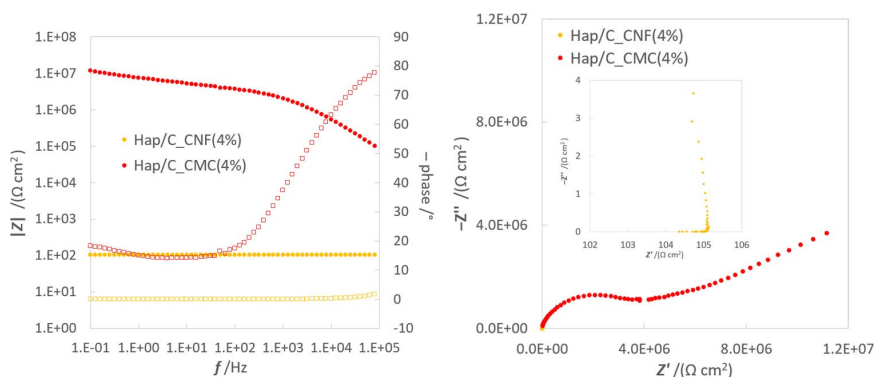

Figure 2. Bode plot (left) and Nyquist one (right) showing the charge transport features of the self-standing composite foils as a function of the nature of the carbon scaffold: carbon nanofiber (orange) and mesoporous carbon (red). For Bode plot: full dots refer to the values of the impedance modulus, empty squares refer to phase.

it showed a capacitive-like behavior (Figure 3 and Figure S3). The "rectangular shape" voltammograms extend for a potential window comparable to that of a graphite control electrode, sufficiently wide for the detection of the most common heavy metal cations by voltametric techniques. The capacitive current is a fruitful probe to establish the real surface area of an electrode, through an *in-situ* technique. In fact, the double layer capacitance (C_{dl}), extracted directly from the slope of a current vs. potential scan rate plot, or from the integrated charge, by cyclic voltammetry curves registered in a capacitive potential window, is proportional to the real surface area.[32,33] The double layer capacitance, and hence the electrochemically active surface area, decreases of around one order of magnitude by moving from CNF- to CMC-containing composites (Figure S3), notwithstanding the neatly higher physical surface area, as determined by Brunauer-Emmett-Teller (BET) method, of CMC scaffold with respect to CNF one (Table S1). This is in good qualitative agreement with the higher degree of coverage of CMC, with respect to CNF scaffold, by the electrical insulating hydroxyapatite (Figure 1 and Table S1). As a result, even if the CMC scaffold offers a higher surface area than CNF, its more uniform coverage by Hap results in a lower electrochemically active surface area.

By changing the relative amount of the carbonaceous scaffold, there is a more limited, but systematic increase of the voltammetric surface charge recorded for the CMC series that follows the percentage of carbonaceous scaffold in the composites (Figure 3, Figure S3 and Table S3). The operative

potential window of all high-content Hap composites (Figure S4) is wider than that of a composite with inverted amount of Hap and CMC (i.e., 96 wt.% CMC) and of an electrode assembled using only CMC (i.e., a Hap-free control electrode). This evidences the effect of the insulating Hap component on the electrochemical response of the composite electrodes.

Effect of the composition of the Hap/C composite for the detection of heavy metal cations

Looking at the lower cost, the improved sustainability (i.e., possibility to exploit bio-waste for the carbonaceous scaffold) and the better morphology of the resulting material, all further experiments were conducted only on CMC-containing composited. The performance of hydroxyapatite/CMC composite electrodes toward the detection of two model analytes, Cu^{2+} and Pb^{2+}, was investigated using the anodic stripping voltammetry (ASV) technique. The effect of the intrinsic adsorption ability of Hap towards metal cations on the electrochemical response of the composite sensors was assessed monitoring the peak current intensity of the ASV curves as a function of the exposure time to the analyte-containing solution, in absence of any external voltage bias (Figure 4 and Figure S5). It is evident that the composite electrodes response is strongly affected by the residence time. As a general trend, especially for lead, the maximum detected current density moves towards higher exposure time as the content of CMC in the composite
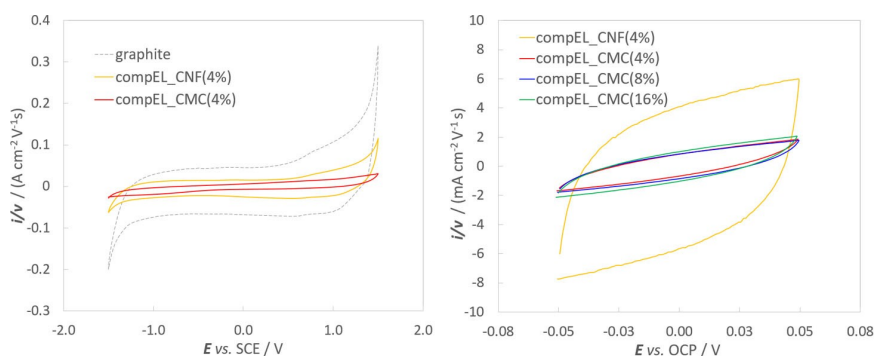

Figure 3. Left: full-window CV scans ($v = 50$ mV s^{-1}), recorded in a blank solution, of composite electrodes containing 4wt. % of CNF and CMC. For sake of comparison, CV traces of a graphite (current collector) is also reported in dashed grey line. Right: representative CV patterns recorded around OCP ($v = 50$ mV s^{-1}) for the CNF composite and the CMC series.
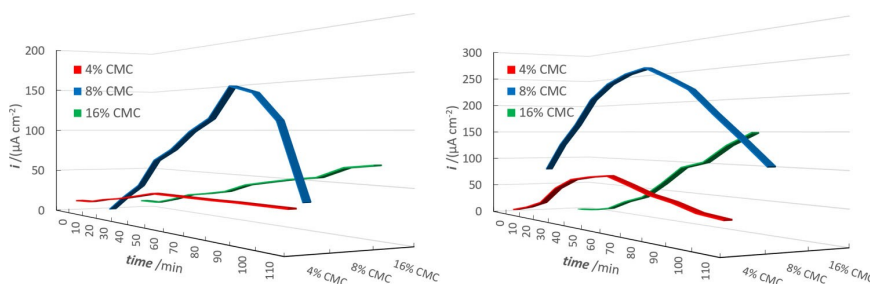

Figure 4. Average ASV peak current density for Cu^{2+} (left) and Pb^{2+} (right) as a function of the CMC content in the Hap-composite electrodes. Analyte concentration: 50 μM. Stripping conditions: accumulation at -1 V *vs.* saturated calomel electrode (SCE) for 60 seconds; unstirred solution.

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increases. This is in line with the progressive increase of the microporosity character of the composites by increasing the content of scaffold.[26] For both copper and lead cations, the best sensor performance (i.e., the maximum signal in the minimum exposure time) is obtained using compEL_CMC(8%) electrodes, the ones presenting an almost 1:1 area ratio among insulating and conductive material. The sensitivity improvement is even better if the current is normalized for the CMC mass content of each composite (Figure S6). As a control experiment, peak currents recorded with a pure CMC electrode are neatly lower, pointing to the crucial role of Hap (Figure S6).

A possible rationalization for the better performance of compEL_CMC(8%) is envisaged in the plausible mechanism of action of the composite sensors. To transduce the information collected by Hap (i.e., the receptor), the adsorbed cations must be sufficiently near to the electric conductive CMC surface (i.e., transducer) to be detected. Therefore, while the entire exposed surface of the Hap deposit is active in adsorbing cations from the solution, only a fraction of this surface (the inner layer, nearer to the CMC particles) is expected to be responsible for the electric signal generation. At the same time, a sufficient number of contact points (i.e., electrical contact area) between adjacent CMC particles is necessary to allow the mandatory electrical transduction of the sensor. The almost 1:1 ratio between the area of the two moieties (Table S1) endows compEL_CMC(8%) with the suitable electrical, morphological and compositional features to fulfil the above criteria.

Nonetheless, at higher residence times, the currents recorded with compEL_CMC(16%) electrodes are comparable or even higher than those recorded with the best performing compEL_CMC(8%), as the latter presents a typical "volcano shape" characterized by a relatively fast descending tail. After excluding that the characteristic hill-shaped behaviour of i vs. t plots for compEL_CMC(8%) is associated to any memory effect, resulting from the sequential analytical protocol adopted (Figure S7), the volcano shape can be reasonably attributed to the coexistence of two (or more) phenomena with opposite effects on the electrode response. Reasonably, the first is the cations adsorption onto Hap surface that, being proportional to time, accounts for the initial raise of the signal. The second phenomenon, more effective at higher exposure times, could be a progressive permanent immobilization of the metal cations (e.g., by exchange with Ca^{2+}, formation of new phases) that

would result no longer detectable by the CMC transducer. While no evidence of a structural modification of Hap charged with Cu^{2+} have been reported yet, it is known that Pb^{2+} interact with Hap by forming a new phase.[16]

As depicted in Figure 5, composite electrodes showed anodic voltammetric peaks, sharper for lead than for copper cations, suitable for analytical purposes. They are associated to the dissolution of the deposited zero-valence metal occurring at around -0.45 V vs. SCE and $+0.11$ V vs. SCE for lead and copper, respectively. The electrode prepared with 8 wt.% of CMC resulted the most performing one in term of sensitivity, with an average stripping oxidation peak intensity of ca. 250 µA cm^{-2} (after 40 minutes soaking at the Open Circuit Potential (OCP)) for a 50 µM Pb^{2+} solution and ca. 150 µA cm^{-2} (60 minutes) for a 50 µM Cu^{2+} solution. The different times to reach the maximum current is attributed to a different kinetics of the mechanisms involved in the adsorption and detection of the two cations, mechanism that seems to be different for the two analytes (cfr. following section). The current is actually proportional to the real surface area of an electrode, that is related to the geometrical one by means of a roughness factor.[32] Thus, the intrinsic sensibility of each composite electrode is obtained by normalizing the recorded current for the relative electrochemically active surface area (ECSA) determined for each type of composite electrode (Table S3). Square wave anodic stripping voltammetry (SW-ASV) patters normalized for the relative ECSA (Figure S8) evidence peak intensities for CompEL_CMC(8%) that is at least double than those of the other two composites. The better performance of CompEL_CMC(8%) is more evident if the peak current is normalized for the amount of carbon in the composite, reaching a value almost double with respect to 4 wt.% and more than ten-times higher than the sample with 16 wt.% (Figure S8). Looking at these results as a whole, neither the real surface area (electrochemical or physical one), nor the amount of conductive carbon can explain the best performance of CompEL_CMC(8%) in detecting the two heavy metal cations. Once again, experimental results suggest that the discriminating parameter among the CMC series could be envisaged in the ratio between carbon surface and Hap surface. A ca. 1:1 balancing exhibits the best sensing ability provided by the synergy between the Hap and CMC features, made possible by the surface morphology of the composite.
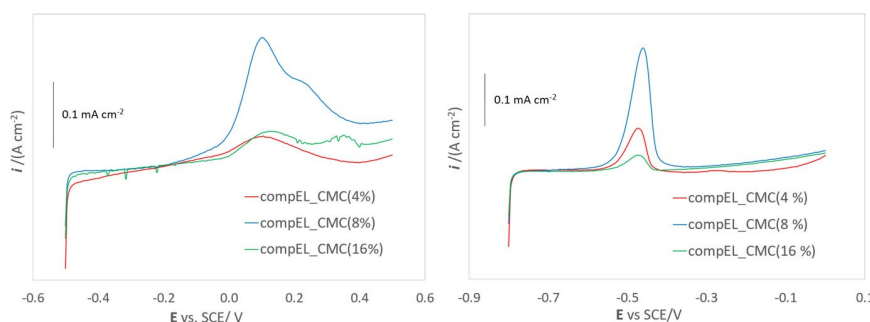

Figure 5. Synopsis of the most intense ASV peak for a 50 µM Cu^{2+} (left) and a 50 µM Pb^{2+} (right) solution, recorded after 40 and 60 minutes, respectively, of exposure at OCP of the electrode to the metal-containing solution. Stripping conditions: same of Figure 4.

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In order to compare the performance of the here reported carbon-modified hydroxyapatite paste electrodes with the Hap-modified carbon paste electrodes already reported in the literature (i.e., with few wt.% of Hap only),[22–24] control experiments were performed by using electrodes prepared with the composites made by 96 wt.% CMC and 92 wt.% CMC. The variation of the peak current intensities for the two selected analytes as a function of Hap/C mass ratio is reported in Figure 6.

Additional data are sorted in Table S4. Contrary to the expectations on the basis of the cited previous literature results,[22–24] the compEL_CMC(8%) electrode does provide electrochemical signals more intense than those obtained with the benchmark electrodes (96 wt.% and 92 wt.% CMC), that are in turns comparable to that obtained with only 16 wt.% CMC (the worst electrode of the high-content Hap series). For the interest of clarity, the whole current density vs. time plots for the best performing composite and an electrode with a Hap:carbon ratio comparable to that used in previous literature (control experiment), are gathered in Figure S9.

Interestingly, the neat variation in the electric conductivity of the composites (more than two order of magnitude) does not directly correlates with the sensing ability of the corresponding electrodes, with the most conductive CMC-doped composites (i.e., high content of CMC) exhibiting the worst current densities (Figure 6).

Calibration plots

In this section the correlation between the intensity of the analytical signal and the analyte concentration is investigated, focusing on the most performing composite electrode CompEL_CMC(8%). Considering the strong dependence of the signal from the exposure time to the analyte-containing solution, calibration plots were recorded at the resting potential and at constant residence time. To maximize the sensing performance for each metal cation the residence time resulting in the maximum detected current (Figure 4 and Figure S5) was selected. The results are reported in Figure 7, where the calibration plots are recorded with both the usual sequential protocol (one electrode-many detections) and the disposable one (one electrode-one detection). Independently by the adopted protocol, the sensor's response is not linear in the concentration range 1 μM–0.1 mM, with the signal reaching a maximum (almost constant) at intermediate concentrations. Once again, the shape of the calibration plots points to a not-innocent interaction between the metal ions and the Hap moieties of the composite electrode (cfr. previous section). Interestingly, such "self-quenching" is proportional to both residence time and concentration of the metal ions (Figure S10), with a different magnitude by changing the relative amount of the CMC and Hap (Figure S5).

Investigation on the interaction between metal cations and Hap/CMC composite

Plenty of literature reports describe the segregation capability of hydroxyapatite towards different transition metal cations.[20,34] Clues on the preservation of the up taking ability by the developed electrochemical sensors were obtained carrying out SW-ASV with a protocol denoted ex situ (Scheme S1). Even in the analyte-free solution, the peak currents show a comparable trend as a function of exposure time (Figure 8) pointing to the establishment of a strong interaction between the metal cations and the Hap/CMC composite, with the cations being retained even after the removal of the electrode by the soaking solution. The constantly higher current density detected with the in-situ protocol is tentatively related with the additive contribution of
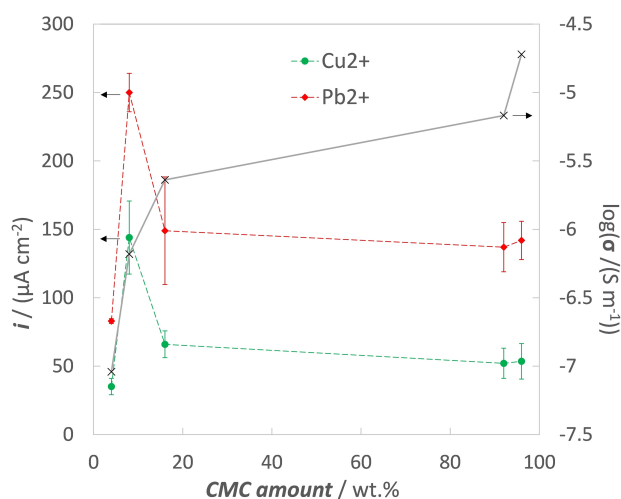

Figure 6. Influence of the CMC content (wt.%) on the maximum ASV peak current density of 50 μM Cu^{2+} (green) and Pb^{2+} (red). Stripping conditions as in Figure 4. For sake of comparison, electric conductivity of each composite is also reported (secondary Y-axis). Lines are just to guide the eyes from one hand to the other of the plot.
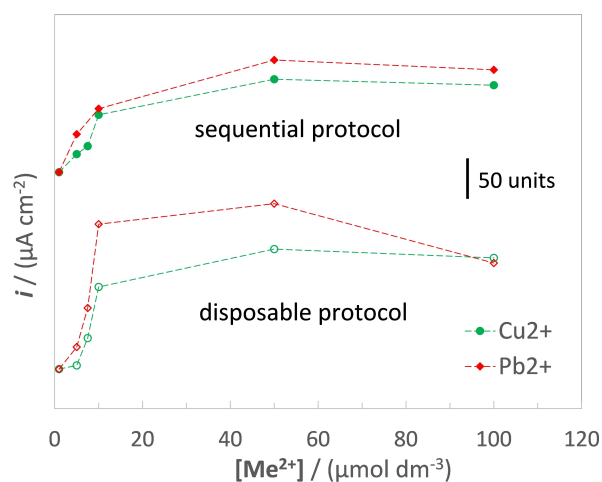

Figure 7. Comparison of the calibration plots for compEL_CMC(8%) electrodes obtained with two different protocols: same electrodes for all the concentrations (top) and one electrode for each concentration (bottom). Analytes: Cu^{2+} (green) and Pb^{2+} (red). Exposure time (at OCP): 60 and 50 minutes for copper and lead, respectively. Stripping conditions as in Figure 4.
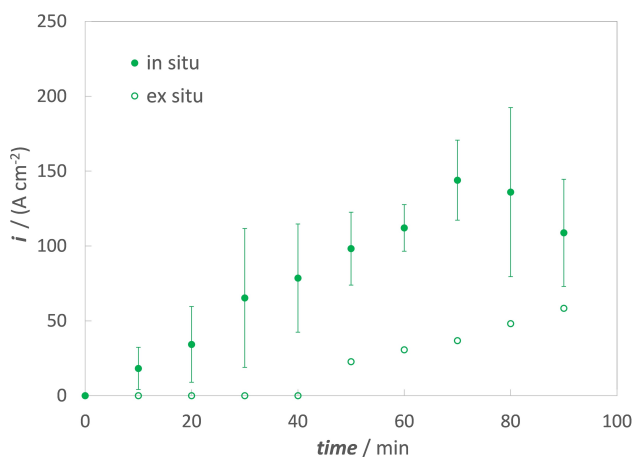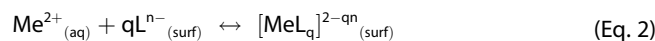
Figure 8. Comparison of in situ vs. ex situ protocol for compEL_CMC(8%) electrodes exposed to a 50 μM Cu^{2+} solution. In both protocols: exposure to analyte at OCP; stripping conditions as in Figure 4.

the dissolved ions in the solution permeated within the porous matrix of the composite electrode. These ions react (deposit and then re-dissolve) on the conductive portion of the composite surface contributing to generating the overall sensor output current. At the same time, the signal recorded with the ex-situ protocol confirms the electroactivity of the adsorbed/immobilized metal ions onto/into the composite matrix. The retained ions are still able to interact with the conductive component of the composite, thanks to a spatial proximity between Hap (adsorption center) and CMC (transducer center) and/or because of a heterogeneous equilibrium between the surface-confined metal species and the free-metal in the electrolytic solution. As a result, the detected currents in the in-situ protocol are likely the combination of a surface-confined contribution (ions adsorbed onto Hap, detectable via ex situ approach) and a solution contribution (ions directly reacting on the electrode), the latter accounting for up to 75% of the whole current signal.

A further step on was to clarify the mechanism of interaction between the metal cations and the composite. Indeed, concerning pure hydroxyapatite, literature has proposed different mechanism of segregation of heavy metal cations that spread from surface complexation to Hap dissolution and precipitation of a new phase.[20] The interaction mechanism between the Hap/CMC electrodes and the metal ions is here studied by taking inspiration from a well-known polarographic method, firstly proposed in 1940's by Lingane[35] and then further optimized in collaboration with Kolthoff,[36] developed to quantitatively study the complexation reaction between metal ions and interacting species (i.e., ligands). From the half-wave potential shift of the peak related to the reduction of the metal ion, plotted as a function of the concentration of the ligand, the coordination number and the stability constant of the *in situ* generated coordination compound can be argued.[36]

In the present study, the stripping reaction can be conveniently divided into two steps (Eqs. 1 and 2), as a mere artifice.

$$Me^0_{(aq)} \rightarrow Me^{2+}_{(aq)} + 2e^- \qquad (Eq. 1)$$

$$Me^{2+}_{(aq)} + qL^{n-}_{(surf)} \leftrightarrow [MeL_q]^{2-qn}_{(surf)} \qquad (Eq. 2)$$

Two important differences with the Lingane study hold:
i) monitoring of a stripping anodic peak (Eq. 1), instead of a cathodic signal;
ii) considering a heterogeneous equilibrium (Eq. 2) instead of a homogenous one, that is the formation (if any) of a surface-mediated metal-Hap species $[MeL_q]^{2-qn}$. In each test, the metal concentration is progressively increased while the number of the coordination sites available (i.e., the HAP/CMC electrode surface area) is kept constant.

Figure 9 depicts the results for the compEL_CMC(8%) electrode. A good linear correlation exists between the logarithmic concentration of the analyte and ΔE_p, that is the difference between the peak potential (E_p) of the stripped metal detected on the composite electrode and that recorded on the ideally non-interacting electrode surface (i.e., graphite). For both cations, an increase of their concentration in solution gives rise to a shift towards more positive potentials of the detected stripping anodic peak, which means closer to the E_p of the metal recorded on graphite.

Despite the Kolthoff and Lingane treatment cannot be rigorously applied, the regular shift in the peak potential points to some interesting statements:
- a reversible interaction between the composite surface and the stripped free metal ions occurs, whose equilibrium constant and stoichiometry affect intercept and slope of the regression line, respectively;
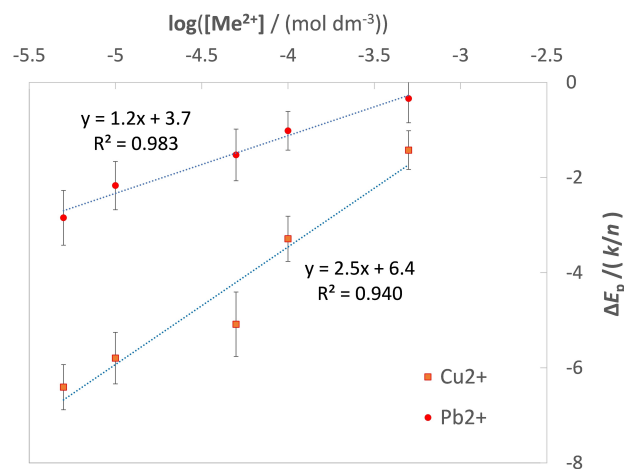

Figure 9. Variation of the peak potential (E_p) of the anodic stripping peak of Cu^{2+} and Pb^{2+} recorded for compEL_CMC(8%) electrodes by progressively increasing the concentration of the metal ion. K is equal to $RT/F = 0.059$ V, at 25 °C (R: ideal gas constant; T: temperature; F: Faraday constant); n stands for the moles of electrons exchanged per mole of reactant.

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– the interaction between ions and electrode surface determines a stabilization of the metal in the "surface-interacting" form $[MeL_q]^{2-qn}$ with respect to the free ions in solution;
– the interaction is controlled by the relative ratio between the surface concentration of the accessible interacting sites on the electrode and the concentration of the stripped metal ions, the latter being proportional to the concentration in the analyte solution. In particular, the higher the ligand:ion ratio, the lower value of the peak potential (cathodic shift).

The different slopes and intercept of the regression lines for the two metal ions suggest a different stoichiometry of the corresponding $[MeL_q]^{2-qn}$ species and a different thermodynamic affinity for the electrode surface, higher for Cu^{2+} than for Pb^{2+}.

Conclusions

The present work has developed high-content hydroxyapatite composite electrodes exploitable for the detection of heavy metal cations in water, representing a proof-of-concept against the paradigm that only a very small amount of Hap can be used in developing electrochemical sensors. The composite powders, obtained by a simple co-precipitation synthesis, merge a high adsorption capability towards inorganic species, offered by Hap (ranging from 84 to 96 wt.%), with a suitable electrical conductivity provided by a carbonaceous scaffold.

The nature of the carbonaceous scaffold, more than its relative amount, significantly affects the electrical conductivity of the Hap/C composite. At the same time, the carbon-based moiety affects the morphological features of the composite powder that, in turns, affect the electrochemical behavior of the derived composite electrodes. Contrary to the intrinsic higher physical surface area of CMC-containing composites (from BET), the related electrodes showed double layer capacitances lower than CNF ones as result of the more uniform coverage of the carbon moiety by the insulating Hap. Focusing on the cheaper and even more environmentally friendly mesoporous carbon than CNF, the present study shows that representative polluting metal cations (Cu^{2+} and Pb^{2+}) can be detected even with electrodes with a content of hydroxyapatite up to 96 wt.%. The best performance was obtained with compEL_CMC(8%) electrodes, characterized by an intermediate content of CMC that endows the material with a discrete electrical conductivity (ca. 1 $\mu S\,cm^{-1}$) and an optimal contact area between the carbonaceous matrix (transducer) and the hydroxyapatite precipitated on it (receptor). Interestingly, the analytical signal of both cations are amplified by ca. 65% with respect to a control experiment performed using a composite having a composition similar to that suggested by literature (e.g., 96 wt.% CMC and only 4 wt.% Hap).

The response of the composite electrodes is quite complex, with a multi-parametric dependence that includes at least the amount of carbonaceous scaffold, the residence time (at rest potential) in the solution containing the analyte and the concentration of the analyte itself. With compEL_CMC(8%), SW-ASV peaks suitable for analytical purposes have been detected

for Cu^{2+} and Pb^{2+} ions in a concentration range from 5 to 100 μM. While the sensibility is sufficient for identify copper contamination (20.5 μM, by United States Environmental Protection Agency), it is not satisfying for lead (48 nM).[37,38]

The sensibility of the here reported Hap/C composite electrodes towards trace metal elements is lower with respect to the state-of-the-art metal electrodes (like Hg and Bi) and to the performance reported by other Authors for Hap-doped carbon paste electrodes.[22,23] Nonetheless, the partial loss in terms of quantification limits can be partially counterbalanced by the almost unique ability to sequester the metal ions to be quantified thanks to the very high hydroxyapatite content. This can open the way to the development of two-in-one materials (e.g., self-sensing filters/membranes) that combine the always-active remediation action to the on-demand detection activity.

Experimental Section

HAP Synthesis

Calcium nitrate tetrahydrate [$Ca(NO_3)_2\cdot4H_2O$, > 99.0% purity, Merck ACS] and diammonium hydrogen phosphate [$(NH_4)_2HPO_4$, > 98.0% purity, Sigma-Aldrich] aqueous solutions have been used as precursors for both pristine HAP and HAP-based composites. Aqueous ammonium hydroxide (NH_4OH 28–30 wt%, Fluka) has been used for pH adjustment. Two different carbonaceous scaffolds have been implemented in Hap compositing, namely carbon nanofibers (CNF; PR24-HHT, Pyrograf) and a biomass-derived mesoporous carbon (herein after referred to as CMC) synthesised in Nanjing University laboratories according to previously reported procedures.[28] Unless otherwise stated, all solutions were prepared with MilliQ water (18.2 MΩ, Millipore).

Synthesis of Hap/C composites

Synthesis of high-content hydroxyapatite carbon composites have been carried out according to previous reports.[26] Composites are coded Hap/C_XXX(Y%), with XXX and Y standing for the carbonaceous scaffold (CNF or CMC) and its weight percentage, respectively. Briefly, a weighed amount of carbonaceous scaffold (0.3–1.5 g), based on its attended weight percentage in the final composite, were dispersed by ultrasonication for 15 minutes in an appropriate volume of MilliQ water (10–40 mL depending on the scaffold nature, porosity and hydrophobicity). The suspension was then mixed with 100 mL of a 0.400 M $(NH_4)_2HPO_4$ solution in a three-necked flask equipped with a mechanical stirrer. The slurry was stirred at 200 rpm for 5 minutes and the pH of the solution adjusted to ca. 9.7 (±0.2) by addition of ca. 10 mL of NH_4OH solution. Hap precipitation was carried out adding dropwise (1.65 mL min^{-1}, peristaltic pump) 100 mL of a 0.668 M $Ca(NO_3)_2\cdot4H_2O$ solution (Ca/P atomic ratio equal to 1.67, stoichiometric Hap) at room temperature, under mechanical stirring (200 rpm). The pH of the solution was kept constant at 9.7 by dosed NH_4OH additions throughout the precipitation. After completing the addition of the $Ca(NO_3)_2\cdot4H_2O$ solution, the suspension was filtered, washed until reaching neutrality of rinsing water and then dried (16 h at 50°C under vacuum followed by 8 h at 120°C under air). Hap/C composite with a scaffold content of ca. 96 wt.% was synthesized following the same route but increasing the carbonaceous scaffold mass in the slurry. Likewise, pristine Hap has been synthesized in the absence of the carbonaceous scaffold.

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Electrode crafting

A polytetrafluoroethylene (PTFE) suspension (60 wt.% dispersion in water; Sigma Aldrich) was used as binder in combination with Hap/C powder for crafting the composite electrodes. Graphite foil (99.8%; 0.130 mm thick; Alfa Aesar) was used as the support for the composite foil and as electron collector of the resulting electrode. Conductive glue (E-dug EB-012; Ladd Research Industries Inc.) was used to glue the self-standing composite foil to the underlying graphite strip and to assure the electric contact between the two layers. Before using, the glue was diluted with MilliQ water as recommended by the seller. Electrodes made of the Hap/C composites [herein coded as "CompEL_XXX(Y%)"] were prepared by gluing a thin self-standing film of the Hap/C composites, acting as the sensing component, to a thin foil of graphite, acting as the current collector. The self-standing film [herein coded as "Hap/C_XXX(Y%)", with XXX and Y retaining the same meaning above] of the composites were prepared as follows. The desired Hap/C composite powder (or pure CMC carbon powder for control experiments) was firstly finely grounded in a mortar. Then, 155 mg of the powder was suspended into 10 mL of absolute ethanol and sonicated for 2 minutes. After adding 15 μL of a PTFE suspension (corresponding to a PTFE around 8 wt.% with respect to the total amount of the resulting composite), the mixture was further sonicated for 10 minutes. The solution was then transferred on a heating plate (ca. 100 °C) to evaporate the solvent. During the process, the mixture was gently shaken. When the solid was completely dried, we have collected the HAP/C:PTFE blend paste and rolled it with a rolling pin for several times, adding few drops of ethanol to avoid cracks formation. Eventually, the resulting thin film was dried in oven at 70 °C for 3 hours and stored in lab in closed plastic small bags until usage. A 0.5×0.5 cm^2 square was cut from the main foil (ca. 100 μm thick) and glued on a strip of graphite (ca. 0.5 cm $\times$ 5.0 cm, 100 μm thick). The assembled electrode was then placed in oven at 80 °C for 30 minutes pressed by a 5 kg weight to foster the adhesion between the two layers. Before using the electrode, the excess of graphite strip was carefully covered using a polyimide tape (Kapton®) in order to leave in contact with the solution only the 0.5×0.5 cm^2 area of the HAP/C composite (Figure S1 of Supporting Information).

Structural and morphological characterization of the composites

Hap/C_CNF(4%) composite has been structurally and morphologically characterized through nitrogen adsorption-desorption isotherms (N_2 ads/des) and scanning transmission electron microscopy (STEM). Operative details are reported in a previous work.[27] A complete structural, morphological, and surface characterization of the other Hap/CMC composites has already been reported by Ferri et al.[26] Scanning electron microscopy equipped with electron microprobe (SEM-EDS; JSM-IT500LV, Jeol Spa Italia) was used for investigating the morphology and the elemental distribution of self-standing foils of CNF- and CMC-containing composites.

Characterization of the composite foils and electrochemical tests

The electrical and electrochemical characterization of the composite foils and of the assembled composite electrodes were carried out using an Interface 1000 (Gamry; USA) potentiostat/galvanostat run with Gamry Framework software.

Electrical conductivity

The electrical features of each self-standing composite foil were investigated by recording the impedance spectrum of a properly sized sample sandwiched between two stainless steel disks acting as electron collectors. A clamp was used to ensure the application of a comparable pressure among the different samples. The electrochemical impedance spectroscopy was carried out in potentiostatic mode by setting the following parameters: 60-logarithmically distributed frequencies in the range 100 kHz–0.1 Hz, 0 V as bias potential, 15 mV as amplitude of the sinusoidal perturbing voltage signal.

Electrochemical investigation

All the electrochemical investigation was carried out in a customized glass mini-cell filled with 3 mL of the working solution (blank or analyte-containing) deaerated by bubbling N_2 gas for few minutes before starting the acquisition and keeping the gas flux in the headspace during the acquisition. Measurements were carried out in a classical 3-electrode configuration with the crafted electrode as working electrode (geometrical area 0.25 cm^2), a platinum coiled wire as counter electrode and a saturated calomel electrode (SCE) as reference. The latter was inserted into a glass jacket, filled with the blank solution, ending with a ceramic porous frit that assures the electric contact between the SCE and the working solution. Due to the high conductivity of the electrolyte and the low current densities circulating between electrodes, no compensation of the ohmic drop was done. All electrochemical tests were performed in solutions prepared with MilliQ water and 0.1 M potassium nitrate (KNO_3, $\geq 99\%$; Fluka) as supporting electrolyte. Metal analytes were spiked in the blank solution using mother solutions prepared with MilliQ water. The following metal salts were used without further purification: copper(II) nitrate [Cu-$(NO_3)_2 \cdot 3H_2O$, $\geq 99\%$; Fluka], lead(II) nitrate [Pb$(NO_3)_2$, Sigma Aldrich].

The electrochemical features of the composite electrodes were evaluated by both cyclic voltammetry (CV) and square wave anodic stripping voltammetry (SW-ASV). For the capacity study of the composite electrodes, CVs were recorded in a blank solution, the potential being scanned in the range -1.5 V to $+1.5$ V vs SCE, starting from 0 V vs SCE and moving firstly in the anodic direction.[32,33] For the SW-ASV electroanalytical protocol used to assess the ability of the composite electrodes toward μM concentrations of target heavy metal cations, the following protocol was used. After a variable equilibration time at open circuit potential, the working electrode was polarized at -1.0 V vs SCE for 60 seconds to allow the electrodeposition of any metal cation present in solution. After this deposition stage (unstirred solution), the potential was scanned from -1.0 V to $+0.5$ V vs SCE by applying a SWV potential waveform (50 Hz frequency, 50 mV pulse size, integration period 20 ms, scan rate 50 mV/s) and by recording the resulting current signal. If not otherwise stated, the stripping step was performed in the same solution containing the analyte (in situ protocol, Scheme S1 of Supporting Information). For specific purposes, an ex-situ protocol was adopted, with the stripping step being performed in an analyte-free solution (Scheme S1). In transferring the electrode from the soaking to the measurement solution (with and without metal ions, respectively), the electrode was thoroughly rinsed with distilled water as to eliminate any non-chemically adsorbed metal cation.

Moreover, SW-ASV investigation was carried out according to two different operative modes, named disposable (one electrode-one detection) and sequential (one electrode-many detections). Unless otherwise specified, the default protocol was the sequential one

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that implies the use of the same electrode for recording signals at different exposure time or at different concentration of the analyte.

Attempts to investigate the interaction between Hap/CMC electrode and the metal ions has been done by applying a modified version of the Kolthoff and Lingane method.[36] Subsequent SW-ASV scans were recorded for a given composite electrode, by changing the concentration for the metal analyte in solution. The potential shift of the anodic peak, with respect to that recorded on a graphite electrode taken as reference value, was plotted as a function of the metal ion concentration. The slope of the resulting regression line was taken as an index of the affinity between the metal and the Hap/CMC surface.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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RESEARCH ARTICLE

Sensing heavy metal cations: The great performance of nanostructured hydroxyapatite (Hap) in adsorbing heavy metal cations with the good electrical conductivity of high-surface carbon materials is combined for the crafting of high-content hydroxyapatite electrodes (Hap 84–96 wt.%) endowed with the synergistic capability of detection and adsorption of heavy metal cations (Pb^{2+} and Cu^{2+}) by water solutions.
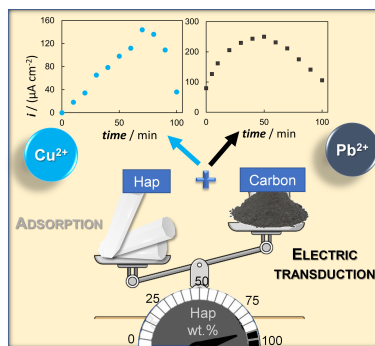
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High-Content Hydroxyapatite Carbon Composites for the Electrochemical Detection of Heavy Metal Cations in Water