



Novel biopolymer-coated hydroxyapatite foams for removing heavy-metals from polluted water

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ABSTRACT

3D-macroporous biopolymer-coated hydroxyapatite (HA) foams have been developed as potential devices for the treatment of lead, cadmium and copper contamination of consumable waters. These foams have exhibited a fast and effective ion metal immobilization into the HA structure after an *in vitro* treatment mimicking a serious water contamination case. To improve HA foam stability at contaminated aqueous solutions pH, as well as its handling and shape integrity the 3D-macroporous foams have been coated with biopolymers polycaprolactone (PCL) and gelatine cross-linked with glutaraldehyde (G/Glu). Metal ion immobilization tests have shown higher and fast heavy metals captured as function of hydrophilicity rate of biopolymer used. After an *in vitro* treatment, foam morphology integrity is guaranteed and the uptake of heavy metal ions rises up to 405 $\mu\text{mol/g}$ in the case of Pb^{2+} , 378 $\mu\text{mol/g}$ of Cu^{2+} and 316 $\mu\text{mol/g}$ of Cd^{2+} . These novel materials promise a feasible advance in development of new, easy to handle and low cost water purifying methods.

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1. Introduction

Due to the industrial waste of this modern society from mining, metallurgy, foundries, etc. as well as the increased use of toxic substances such as pesticides, heavy-metal intoxication has become an usual problem for population. [1] Also, the increasing development of catalytic processes and batteries has forced the arising of new factory protocols on waste materials and fumes. Moreover, exploitation of aquifer resources in underdeveloped areas, as for example in Asia and South America, deals with waters not suitable for consumption in their natural state [2].

As already well known, heavy metal absorption by the body can cause serious damages in vital organs that even could provoke their failure. Still nowadays, several studies have demonstrated the presence of these ions in consumable waters, in higher concentrations than the ones allowed by the organism [3,4].

Research for ensuring a more sustainable management of the environment and its resources is catalogued as one of the main technologies and services to be funded and studied, but until that happens, new technologies should be emerging for treating the consequences.

There are several patents and previous studies on purification treatments for waste and consumption waters describing ion immobilizers using new technologies as zeolites or phosphoric acid [5,6]. Also macroporous systems have been proposed as heavy metal water purifying materials [7] including hydroxyapatite (HA) systems, being efficient capturers of Cadmium (Cd) and Lead (Pb) among others [8]. In some of these systems, where the apatite is applied in the form of dissolutions/foams/powders/rocks, capturing results showed that immobilization takes place at different pHs, and their efficiency depends on the ion concentration, ion nature and temperature in soils and waters, for example [9–12]. It has also been reported that the use of HA/polyurethane composite as efficient lead removers [13]. The aim of all these phosphate systems is immobilizing metals through the formation of metal phosphates with reduced solubility in a wide range of environmental conditions.

Here we present novel 3D-macroporous biopolymer-coated HA foams as a potential alternative for the treatment of heavy-metal polluted waters. The proposed materials have recently proved viability and success to be used as a fast intoxication treatment after heavy metal ingestion, with the principal quality of maintaining their morphology after the digestion process leading to an easy expulsion by the body [14].

For the first time, these foams have been studied in terms of isotherm models and sorption kinetics analysis at neutral pH in order to consider their application as heavy metal removers from water. These foams could provide a new treatment with high effectiveness without high financial costs or the need of water

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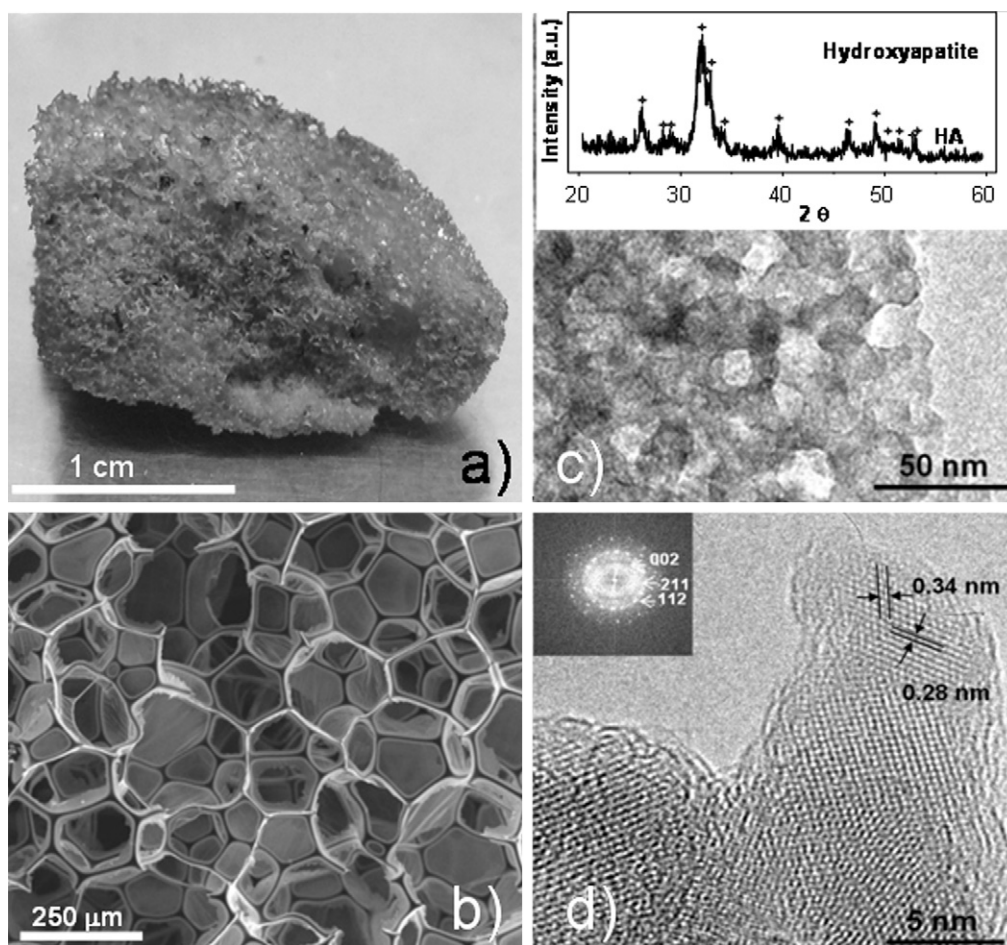


Fig. 1. (a) Digital photograph, (b) SEM micrograph, (c and inset) low magnification TEM image and XRD pattern, respectively and (d and inset) high resolution and its corresponding FT diffractogram. All correspond to a representative 1.2 G/Glu HA foam.

purification infrastructures. These features would be combined with easy logistics for treating consumable waters in underdeveloped areas, low degradability and a high efficiency in a wide range of pHs from acid to basic. Also, the easy way of removing the foams after the water treatments does not imply any filtration and is direct with the removal of the 3D foam from the treated water. For this study, Pb^{2+} , Cd^{2+} and Cu^{2+} have been chosen as typical heavy metals present in water contamination.

These foams are designed to exhibit a fast and effective ion metal immobilization into the reactive nanocrystalline HA structure and the polymer coating confers them protection avoiding foam's dissolution and disaggregation, maintaining its morphology and integrity through the purification process along the time.

2. Experimental

2.1. Biopolymer coated hydroxyapatite macroporous foams

Tridimensional macroporous HA foams have been synthesized using the sol-gel technique for preparing nanocrystalline HA, including a non-ionic surfactant in the synthesis, Pluronic F127 ($\text{EO}_{106}\text{PO}_{70}\text{EO}_{106}$), as macropore former in the accelerated evaporation induced self assembly (EISA) method. This process is described elsewhere [14].

Foams were obtained from a molar ratio $x = 11$ of F127:TIP (Triethylphosphite) as it was found previously that they were the most homogeneous with the highest pore volume, and coated with different biocompatible polymers approved by the US Food and Drug

Administration (FDA). HA foams were soaked in such biopolymer solutions, extracted and dried at room temperature. In one case, the coating solution was prepared from two different gelatine concentrations in water (1.2 and 2.4% (w/v)) crosslinked with glutaraldehyde to reduce the coating solubility in water [15,16], and in the second case, the coating was made of ϵ -polycaprolactone (PCL). In this case the pieces were soaked in a solution of 5% (w/v) of PCL in dichloromethane. The coating process is described elsewhere [14].

2.2. In vitro assays

In vitro heavy-metal immobilization tests were carried out in polluted media with pH 5. Dissolutions of $\text{Cu}(\text{NO}_3)_2$, $\text{Cd}(\text{NO}_3)_2$ and $\text{Pb}(\text{NO}_3)_2$ containing 300 ppm (1.5, 4.6 and 2.7 mmol/L of Pb^{2+} , Cu^{2+} and Cd^{2+} , respectively), were prepared in an aqueous 1% HNO_3 media (5 mM). Also, pH influence tests were performed for the case of Pb^{2+} contamination on the range of pH (2–6) adjusting pHs with HNO_3 1 M and NaOH 1 M in each case. In order to evaluate adsorption isotherms and kinetics studies, solutions of $\text{Pb}(\text{NO}_3)_2$ containing 2.5 and 4 mmol/L of Pb^{2+} were also prepared.

Samples were cut from the original specimen in small prisms of 20 mg (dimensions 7 mm \times 10 mm \times 5 mm) and immersed in 5 mL of each ion polluted water case. Immobilization assays were performed at 25 °C in continuous stirring for 30 min; 1, 2, 6 h; 1 and 4 days for all the cases.

The concentration of Ca^{2+} , Pb^{2+} , Cd^{2+} and Cu^{2+} ions was measured before and after the immobilization assay by inductively coupled plasma/optical emission spectrometry (ICP/OES) in a

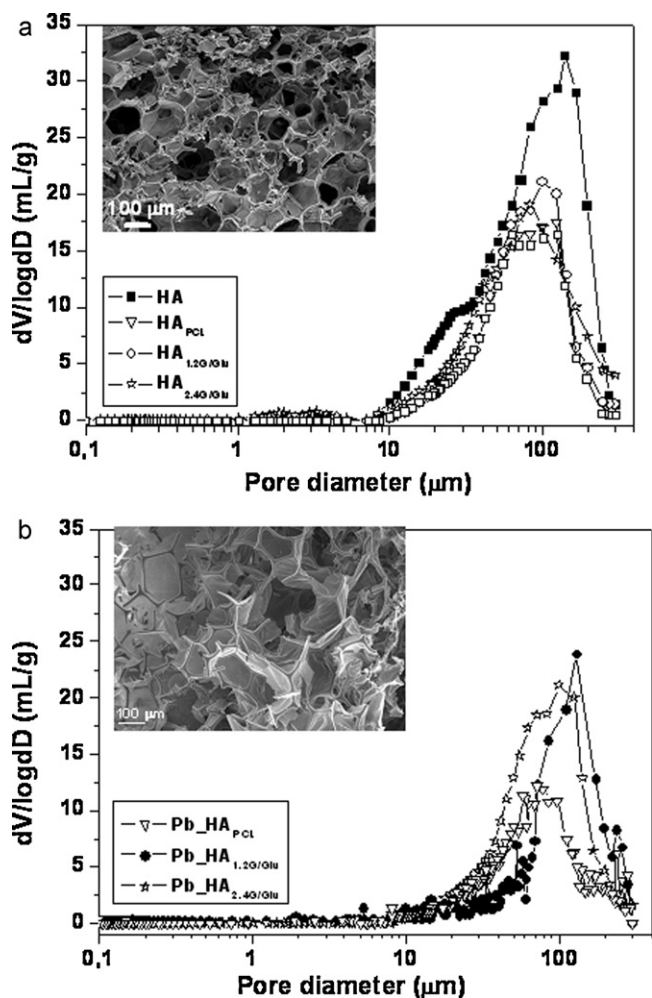


Fig. 2. (a) Pore size distributions by Hg intrusion corresponding to HA_{PCL} , $HA_{1.2G/Glu}$ and $HA_{2.4G/Glu}$ samples before immobilization tests. Inset: SEM micrographs corresponding to $HA_{1.2G/Glu}$ before immobilization test. (b) Pore size distributions by Hg intrusions after lead immobilization test. Inset: Representative SEM micrographs corresponding to foams after the immobilization test.

Perkin Elmer OPTIMA 3300 DV device, with detection limits of 1.38, 6.27, 8.89×10^{-3} and 2.8×10^{-2} $\mu\text{mol/L}$, respectively.

Each experiment has been carried out in four specimens split in two independent experiments.

2.3. Characterization

HA foams were characterized by X-ray diffraction (XRD) in a Philips X'Pert diffractometer using $\text{Cu K}\alpha$ radiation. Scanning electron microscopy (SEM) was performed in a JEOL JSM 6335F field emission scanning microscope. Transmission electron microscopy (TEM) was carried out using a JEOL 3000 FEG electron microscope operating at 300 kV (Cs 0.6 mm, resolution 1.7 Å) fitted with a double tilting goniometer stage ($\pm 45^\circ$) and with an ISIS Oxford LINK EDX analyzer. Elemental analysis was performed in a Perkin Elmer 2400 CHN and Analyzer Thermogravimetric (TG) analyses were carried out with a Perkin-Elmer Pyris Diamond TG/DTA instrument, between 30 and 900 °C in air at a flow rate of 100 ml/min and a heating rate of 10 °C/min. Hg porosimetry measurements were carried out in an AutoPore III porosimeter (Micromeritics Instrument Corporation, Norcross, GA, USA).

Textural properties were also evaluated by N_2 adsorption/desorption analyses performed at -196°C using a Micromeritics ASAP2020 porosimeter. In all cases, samples were degassed

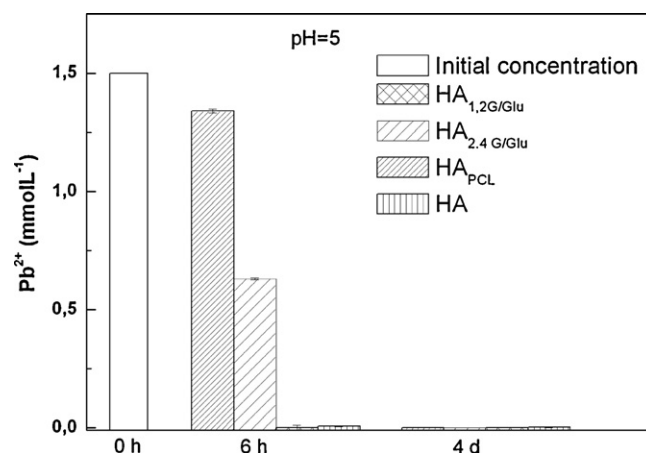


Fig. 3. Variation of Pb^{2+} concentration in the media as a function of incubation time and initial pH 5 corresponding to the HA, HA_{PCL} , $HA_{1.2G/Glu}$ and $HA_{2.4G/Glu}$ foams.

at 50 °C during 24 h under a vacuum lower than 10–5 Torr. Surface area was determined by the Brunauer–Emmet–Teller (BET) method [17].

3. Results and discussion

Obtained foams have variable dimensions depending on the amount of sol prepared and placed in the Petri dish or container to follow the EISA method. In our case, foams were obtained and cut into small pieces (20 mg) for their use in the different tests. For Fig. 1a, the foam has been dyed with brilliant blue for favouring the contrast. Foams have a macroporous structure in the range of 1–400 μm as it can be seen in Fig. 1a and b with a high volume and size of interconnected pores. As it can be seen in Fig. 1c (inset: XRD diffractogram) the analyzed phase corresponds to a pure HA phase of agglomerated crystallites forming a porous structure in the range of 10–15 nm (Fig. 1c), both pore and crystallite sizes. The TEM image of Fig. 1d and the FT diffractogram of the inset evidence the presence of halos at 0.27, 0.28 and 0.34 nm which could correspond to the 112, 211 and 002 reflections of the apatite-like phase. From EDX measurements combined in the TEM technique, a value of Ca/P ratio = 1.55 has been obtained describing a calcium deficient HA [18,19].

Hg porosimetry of the uncoated foam (named HA in Fig. 2a data) shows a total pore volume ranging from 1 to 300 μm (being 300 μm the maximum range measured in the Hg porosimeter), what was also seen by SEM images Fig. 1b. Surface area measured in the 10–300 μm range shows values of 1.6 m^2/g .

Foams were coated by immersion with the three different polymer solutions: polycaprolactone and with 1.2 and 2.4 wt% gelatine crosslinked with glutaraldehyde (0.05, w/v%), giving rise to the samples: HA_{PCL} , $HA_{1.2G/Glu}$ and $HA_{2.4G/Glu}$, respectively.

The amount of biopolymer adhered by adsorption into the foams surface was studied by thermogravimetric analysis giving values of adsorbed polymer of 40% in the PCL (HA_{PCL}) and 2.4 G/Glu ($HA_{2.4G/Glu}$) coated samples and 20% for the 1.2 G/Glu ($HA_{1.2G/Glu}$). Foams maintain their porous structure after the coating process with a slight decrease in the pore volume on the range 100–300 μm as can be seen on Fig. 2a and it is confirmed in the SEM image of Fig. 2a inset. The total porosity measured by Hg intrusion, of the three coated foams is approx. 70% and the specific surface area in the 10–300 μm range shows a slight decrease with values of 1.5, 1.0 and 0.8 m^2/g for the $HA_{1.2G/Glu}$, HA_{PCL} and $HA_{2.4G/Glu}$ samples, respectively.

The textural properties such as surface area and pore size were also analyzed for all the samples by means of N_2 adsorption

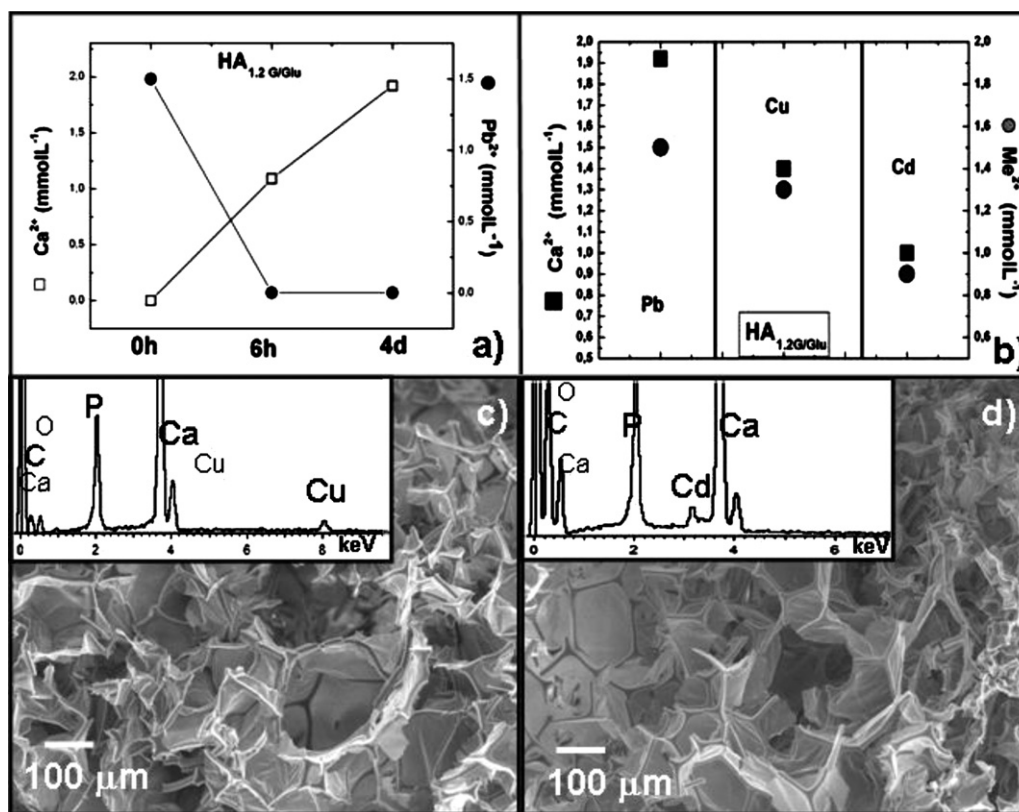


Fig. 4. (a) Variation of concentration (mmol/L) of Ca^{2+} and Pb^{2+} in the media as function of incubation time. (b) Initial concentration of each toxic metal in the media and the final calcium concentration in the media for each immobilization tests, (c and d) SEM micrographs and EDX analyses after immobilization test of copper and cadmium, respectively. All corresponding to a representative $\text{HA}_{1.2\text{G}/\text{Glu}}$ foam.

isotherms. The specific surface area in the 1–1000 nm range for the HA foam is 21 m^2/g while for the $\text{HA}_{1.2\text{G}/\text{Glu}}$, $\text{HA}_{2.4\text{G}/\text{Glu}}$ and HA_{PCL} foams shows a decrease with values of 16, 15 and 6.6 respectively. Pore sizes of media are approximately 21–14 nm for all the samples. Note that, although some pores become covered by polymer coating, polymer swelling allows this surface to remain available for ion capture.

3.1. *In vitro* assays

In order to evaluate the efficiency of these foams in an *in vitro* model mimicking a serious case of water contamination, biopolymer-coated foam samples of 20 mg were placed into the solutions following the method described above for immobilization tests.

The foams have been studied after the immobilization process, and it has been found that they maintain the morphology and all the percentage and range of porosity (Fig. 2b), unless for the case of the PCL coating, having a 60% decrease of total porosity. As it can be seen in SEM image of the inset (Fig. 2b) for the representative case of the most efficient foam coated with 1.2 G/Glu after Cu^{2+} capture. It demonstrates that polymer coating confers them protection avoiding foam's disaggregation as happens for the uncoated foam after the essay.

As the initial ion concentration in water before and after the foam uptake process is known, Fig. 3 shows the removal of ions from the water containing Pb^{2+} concentration at pH 5 directly related to the concentration of ions captured by the foam and removed from the solution. As it can be seen, effectiveness of capturing is different for each polymer coating. In the case of the non-coated HA and the $\text{HA}_{1.2\text{G}/\text{Glu}}$ foams, the complete Pb^{2+} uptake is faster and it is done in a shorter time compared to others that

can do it in a maximum of 4 days for the HA_{PCL} and $\text{HA}_{2.4\text{G}/\text{Glu}}$ foams.

These results are related with the different hydrophilic nature of the polymers. The fact that the PCL has lower hydrophilicity than the G/Glu polymer provokes less water uptake and therefore, less HA solubility [14]. This fact is directly related with the Pb^{2+} capturing process as it has been reported [20] that implies a partial-dissolution of HA and re-precipitation mechanism as a pyromorphite phase, $\text{Pb}_{10}(\text{PO}_4)_6(\text{OH})_2$ which is more stable than the HA phase [21,22]. It is reported also in literature that in the case of Cu^{2+} and Cd^{2+} uptake, the capture is performed by an ion exchange mechanism [23] and complexation mechanism [24]. Comparing Pb^{2+} , Cu^{2+} and Cd^{2+} absorption with the amount of Ca^{2+} released to the media in each case, the values almost follow a linear relationship. As it can be seen in Fig. 4a for the case of the most efficient foam (the $\text{HA}_{1.2\text{G}/\text{Glu}}$ sample), the amount of total Pb^{2+} captured is 1.5 mmol L^{-1} while the Ca^{2+} released in 4 days time to the media is of 1.9 mmol L^{-1} . This slightly higher value of Ca^{2+} than the uptaken lead means that apart from the capture mechanisms, there is also dissolution of HA that does not re-precipitate. It has been reported that for $\text{pHs} \geq 5$ the surface ion exchange mechanism/adsorption becomes important competing in the capture of lead [13]. Comparing the released Ca^{2+} with the Cd^{2+} and Cu^{2+} ions exchange (Fig. 4b), the captured concentration values are also nearly to 1:1 ratio.

To confirm the ion immobilization in the foams after the process, XRD spectra and TEM observations were performed in the dried foams. Fig. 5a shows the diffractogram of the foam after the capture of lead, showing the coexistence of the HA and pyromorphite phases also confirmed by TEM images (Fig. 5b and c). TEM images of the same foam revealing the change in morphology from both typical morphology from HA to pyromorphite crystals

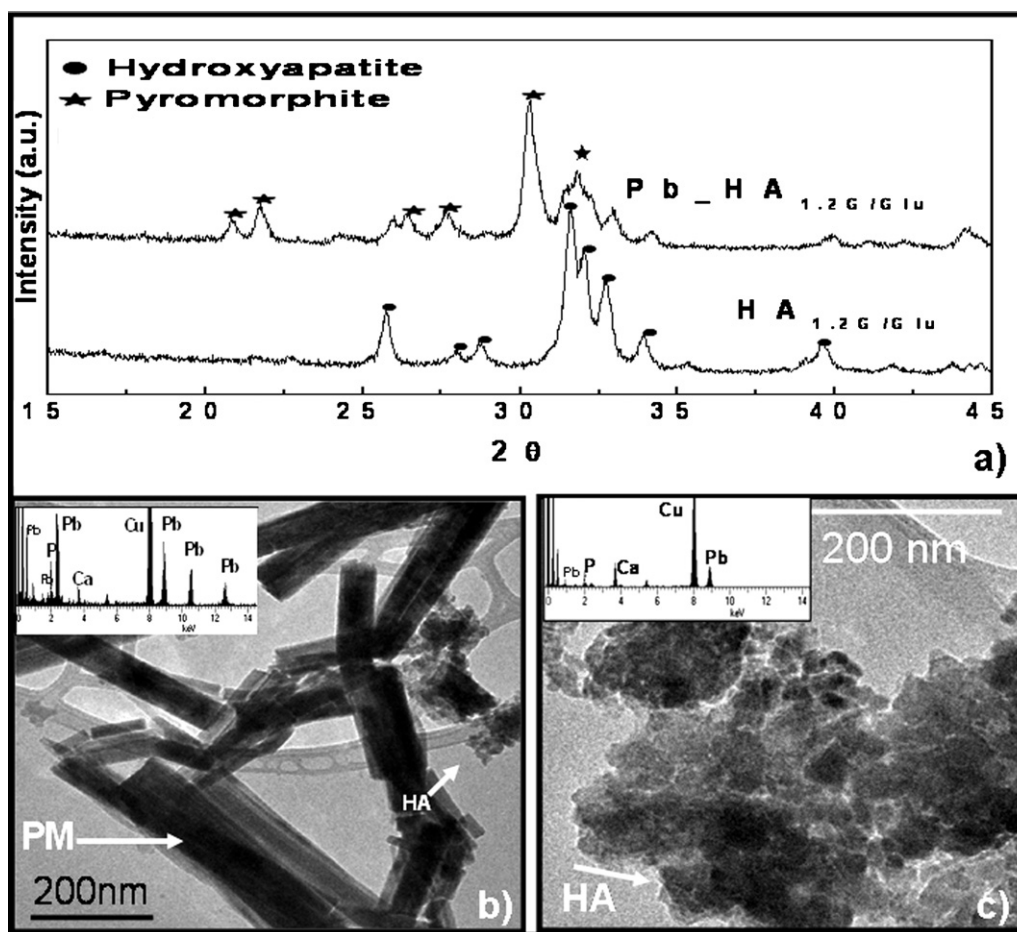


Fig. 5. XRD patterns corresponding to the $HA_{1.2G/Glu}$ before and after lead immobilization tests. The identified phases correspond to HA and (*) pyromorphite (PM). TEM images of $HA_{1.2G/Glu}$ after immobilization tests and their corresponding EDX analyses of different areas. Cu appearance in the EDX spectra is due to the copper TEM grid.

(signposted with PM). EDX performed in the HA phase grains detects a small amount of Pb^{2+} confirming the idea of another possible ion exchange mechanism in the lead uptake as it was mentioned before. In the cases of Cd^{2+} and Cu^{2+} , the X-ray diffraction patterns of dried foams do not show any modification in the crystalline phase after the immobilization tests (data not shown). However, the presence of Cu^{2+} and Cd^{2+} ions in the final HA macroporous structure have been confirmed by EDX (Fig. 4c and d).

3.1.1. Adsorption kinetic studies

The results of sorption studies, carried out as a function of contact time are presented in Fig. 6. The Pb^{2+} uptake presents rapid total ion sorption in the first hour followed by a constant plateau upon further increasing in the contact time. Nevertheless, Cd^{2+} and Cu^{2+} reach the plateau of absorption after 4 days and only perform partial adsorption of the ions present in the solution.

The uptake of heavy metal rises up to a $405 \mu\text{mol/g}$ of Pb^{2+} , $378 \mu\text{mol/g}$ of Cu^{2+} and $316 \mu\text{mol/g}$ of Cd^{2+} . As it has been reported, the different efficiency of the immobilization process is dependent on electronegativity and ionic radii of the metals. The higher electronegativity and ionic radii matching close to Ca^{2+} higher ion capture efficiency ($Pb^{2+} > Cd^{2+} > Cu^{2+}$) are [25,26] in good agreement with the presented data in Fig. 6.

To the best of our knowledge, higher values of lead uptake have been reported in 2010 by phosphate rocks at pH 5. In our case, foams are also efficient even at higher pHs [26] but also for phosphate rocks lower values have been reported for Pb^{2+} , Cd^{2+} and Cu^{2+} uptake in a similar range of pHs [26–28].

These results are another good advance from available solutions reported in literature concerning water purifying systems based on heavy metal absorption, reporting here a solution with higher uptake values [28,29].

Concerning the use of HA as immobilizers systems, excellent uptake values have been reported by Smiciklas et al. [30] regarding powder solutions of HA at different pHs in distilled water. In our case, despite the polymer coating retards the access of the cations to

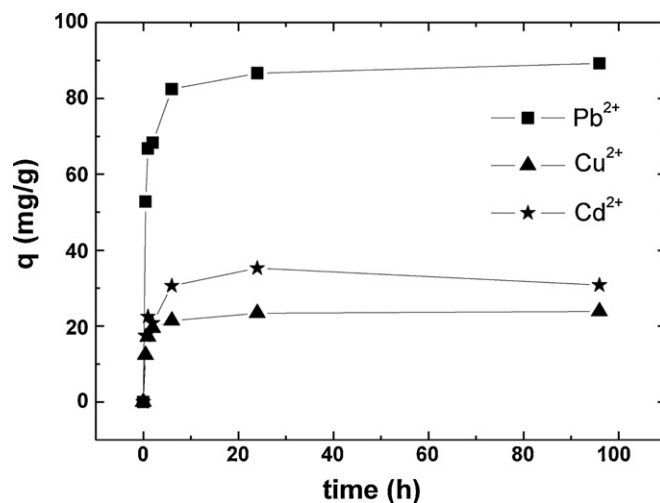


Fig. 6. Variation of sorbed amounts of lead cation on the $HA_{1.2G/Glu}$ with time.

Table 1
Kinetic models and statistical parameters at 25 °C and pH 5.

Kinetic model	Parameters $[\text{Pb}^{2+}]_0 = 1.44 \text{ mmol L}^{-1}$	
1st order equation	r^2	0.740
$-\ln(C/C_0) = K_1 t$	K_1	0.063
2nd order equation	r^2	0.953
$-\ln(1/C - 1/C_0) = K_2 t$	K_2	0.053
Pseudo-1st order equation	r^2	0.529
$\log(q_e - q) = \log q_e - (K'_1/2.303)t$	K'_1	0.021
Pseudo-2nd order equation	r^2	0.999
$t/q_t = 1/K'_2 q_e^2 + 1/q_e t$	K'_2	2.140

the HA foam surface, when the capture material is a macroporous system, the immobilization process is controlled by the diffusion process along the three-dimensional structure. The macroporosity provide a higher sorptive capacity giving rise to an increase in the mobility of the heavy metal ions and a decrease in the retarding forces acting on the diffusing ions [27].

Taking into account the high effectiveness on Pb^{2+} , and in order to determine the rate constants, the most widely used kinetics models in sorption processes have been applied to the Pb^{2+} experimental data on $\text{HA}_{1.2\text{G}/\text{Glu}}$ foam.

Kinetics models and other statistical parameters of experimental data obtained at 25 °C and pH 5 are shown in Table 1. As it can be seen, a good correlation coefficient was obtained for the pseudo-second order and second order kinetics model which indicates that the Pb^{2+} adsorption on the biopolymer coated foams follow its respective second order equations.

3.1.2. Effect of pH

In order to study the possible influence of pH in the sorption processes due to the influence of pyromorphite formation or changes in HA solubility [18], the sorption was studied under acidic and alkaline conditions. As it is shown in Fig. 7, the efficiency of Pb^{2+} capture by the foam at different pHs with a constant cation concentration in the initial solution, is decreasing with pH increase. This change on the capture efficiency could be explained by the change in HA solubility that is higher at pH 2 therefore, favouring the dissolution–reprecipitation mechanism while at higher pHs the immobilization could be attributed to surface adsorption [31,32].

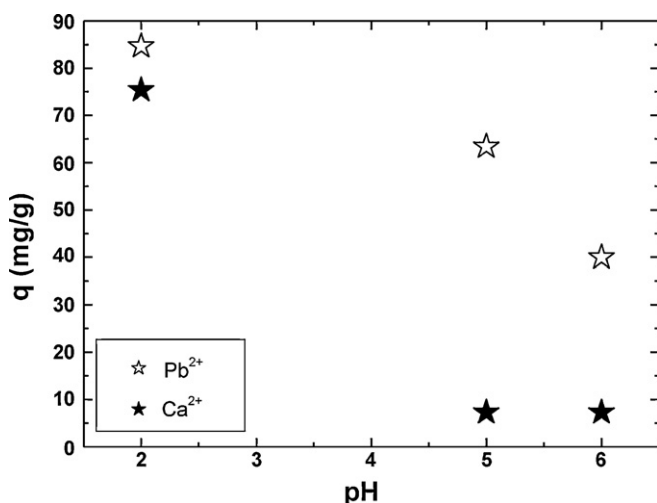


Fig. 7. The effect of pH on the amount of Pb^{2+} removed by the $\text{HA}_{1.2\text{G}/\text{Glu}}$ foam and Ca^{2+} released to the medium. Immersion time 1 h and initial Pb^{2+} concentration of 1.44 mmol L^{-1} .

3.1.3. Adsorption isotherms

Adsorption equilibrium data have been tested using Langmuir and Freundlich isotherms. Langmuir isotherm is expressed in the linear form as:

$$\frac{C_e}{q_e} = \frac{1}{K_e q_{\max}} + \frac{C_e}{q_{\max}} \quad (1)$$

where C_e (mg/L) is the equilibrium concentration, q_e (mg/g) the amount of adsorbed metal per amount of adsorbent, K_e the Langmuir equilibrium constant (L/mg), and q_{\max} (mg/g) is the amount of adsorbent corresponding to a monolayer adsorption on the surface. According to the experimental data, the linear plot of C_e/q_e against C_e gives a good linear fit for the coated foam as it is shown in Fig. 8a, yielding a q_{\max} of 84.3 mg/g and a K_e of 0.016 L/mg with a correlation coefficient r^2 of 0.994.

Freundlich isotherm describes a non-ideal sorption and its constants were determined using Eq. (2):

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (2)$$

As it can be seen in Fig. 8b, the plot of $\log q_e$ as a function of $\log C_e$ the description of the data following this model follows a worse scenario than the Langmuir isotherm model, giving values of Freundlich constant K_F of 4.12 mg/g, $1/n$ of 0.162, where the nondimensional parameter n can be regarded as reflecting the energetic heterogeneity of the binding sites of the sorbent, and with a correlation coefficient r^2 of 0.95.

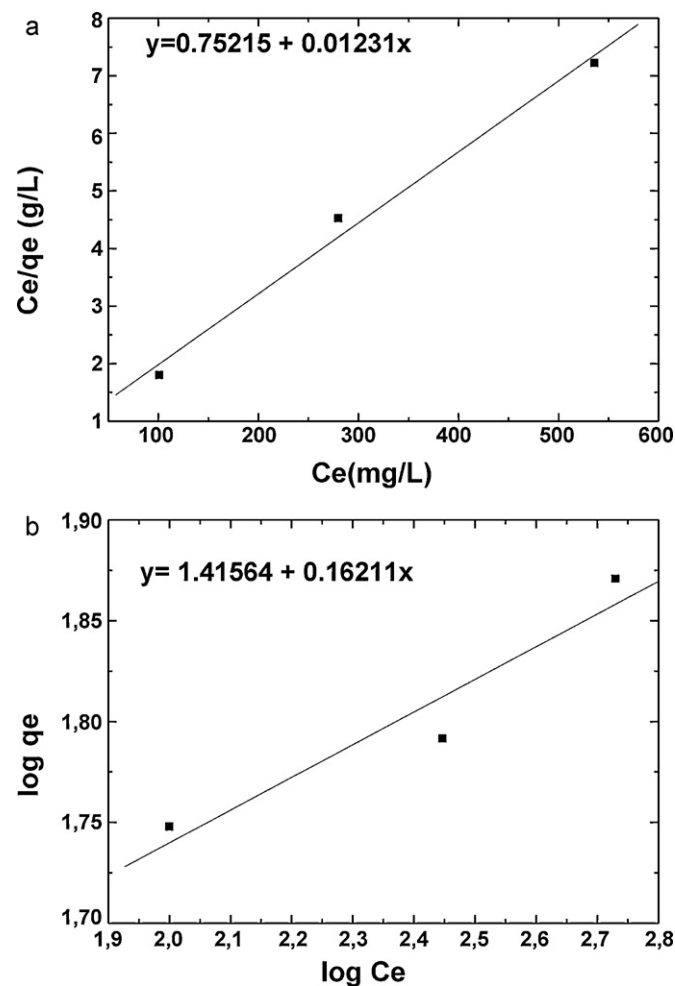


Fig. 8. (a) Langmuir isotherm plot for the adsorption of Pb ions by $\text{HA}_{1.2\text{G}/\text{Glu}}$ foam in an aqueous solution at pH 5. (b) Freundlich plot for the adsorption of $\text{HA}_{1.2\text{G}/\text{Glu}}$ foam in an aqueous solution at pH 5.

Consequently, the sorption metal ion is suggested to occur as an uptake on homogeneous surface by monolayer without interaction between adsorbed molecules.

4. Conclusions

HA biopolymers coated foams have been prepared and tested as heavy metal removers from consumption waters. HA foams have proved to be more efficient when coated with the 1.2 G/Glu coating capturing 405 $\mu\text{mol/g}$, 378 $\mu\text{mol/g}$ and 316 $\mu\text{mol/g}$ of Pb^{2+} , Cu^{2+} and Cd^{2+} ions, respectively, from severe water {AQ: for clarity} heavy metal polluted. After the immobilization process, foams can be removed easily from the purified solution maintaining the macroporous structure and the captured ions in their composition.

We consider this is an important progress for water treatments that could provide high effectiveness without high costs or without needing water purification infrastructures and easy logistics.

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