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Combined effect of magnesium and amino glutamic acid on the structure of hydroxyapatite prepared by hydrothermal method

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ABSTRACT

Magnesium modified calcium hydroxyapatite of different Mg/Ca compositions have been synthesized using the hydrothermal method in the presence of glutamic acid. The resulting materials have been characterised by X-ray powder diffraction, chemical analysis, IR Spectroscopy and Transmission Electron Microscopy (TEM). X-ray diffraction analysis shows that the resulting materials consist of a single phase having an apatitic structure. IR spectroscopy highlights the presence of carboxylic groups for the organic moieties grafted onto the apatitic surface. The surface properties of apatite samples are determined.

Keywords: hydrothermal method, apatite surface, glutamic acid, hybrid compound

1. Introduction

Among the minerals having an interest from an economic point of view, apatites, mostly hydroxy- (CaHAp) and fluoro-apatites (FAp), are of considerable interest in numerous research areas [1-3]. Apatites are used in several applications such as sorbents, catalysts and biomaterials [4, 5]. Apatites are component of bones and teeth [6, 7]. Apatites belong to the phosphate family of compounds, and are of general formula M₁₀(PO₄)₆Y₂ where M is a divalent cation: Ca, Sr, Ba, Pb…, and Y a hydroxyl (OH) or halide (F, Cl) [8, 9]. Apatites may contain several different substituents in their structure. This ability to trap substituents in their structures leads to the formation of total or
partial solid solutions. The substitution processes are controlled by the crystallographic rules related
mainly to: ionic radius, charge, electronegativity and polarisability [10, 11]. Previous works [12-14]
have shown that steric hindrance related to cations bigger in size than calcium ions, plays an important
role in the limitation of the cationic substitution processes. Magnesium is undoubtedly one of the most
important bivalent ions associated to biological apatites [15, 16]. It has been verified that in calcified
tissues, the amount of magnesium associated to the apatitic phase is higher at the beginning of the
calcification process and decreases on increasing calcification [17-19]. This interest is increasing
taking into consideration the ability of such mixed apatites to lead to hybrid organic-inorganic
materials by reaction with amino-acids [20-26]. The expected benefit from the introduction of an
amino-acid such as a glutamic acid in magnesium modified apatites is correlated with the ability of
magnesium to coordinate amino-acids more strongly that calcium ions do and consequently to bind a
greater proportion and more firmly proteins on their surfaces.

In this aim, we have carried out a structural, morphology and chemical investigation of the combined
effect of magnesium and amino glutamic acid on hydroxyapatite structure. We have also studied the
interaction between glutamic acid and the apatite surface. The results show that the acid forms a
complex on the apatite surface. As glutamic acid functionalization should potentially modify the
electrostatic interaction, the corresponding change in the surface charge of the powder was
monitored by zeta-potential measurements and [H\textsuperscript{+}] consumed as a function of the pH value.

2. Experimental and methods

2.1 Synthesis

The mixed Mg/CaHAp of general formula: Ca\(_{(10-x)}\)Mg\(_{x}\)(PO\(_4\)_6(OH)\(_2\) (x = 0, 0.5 and 1.0), named
Ca\(_{(10-x)}\)Mg\(_x\)HAp, have been synthesized using the hydrothermal method [27]. A demineralised water
solution (14 mL, 0.75 M) of a mixture of the two nitrates Ca(NO\(_3\))\(_2\).4H\(_2\)O and Mg(NO\(_3\))\(_2\).6H\(_2\)O in the
desired proportions is added to a (NH\(_4\))\(_2\)HPO\(_4\) water solution (25 mL, 0.25 M). The pH of the final
solution is adjusted to 10 by adding a NH\(_4\)OH solution (d = 0.89, Purity = 28%). The final solution is
transferred to an autoclave. The mixture is maintained at 120°C for 12 hours. After filtration and washing using hot demineralised water, the mineral is dried at 120°C overnight.

The hybrid materials were prepared according to the same experimental protocol, with addition of a quantity of organic reagent glutamic acid (GA) to the phosphate solution before pH adjustment [28]. The samples will be named as Ca$_{(10-x)}$Mg$_x$HAp-GA($n$), where $n$ is the value of the glutamic acid/(CaHAp) molar ratio ($n$= 10 and 20).

2.2 Powder characterization

$N_2$ adsorption-desorption isotherms were performed at 77 K using a Micromeritics ASAP 2000 instrument. The Brunauer–Emmett–Teller equation was used to calculate the specific surface area ($S_{BET}$). X-ray diffraction (XRD) analysis were carried out by means of a X’Pert Pro Panalytical X-pert diffractometer using Cu-Kα radiation ($\lambda$=1.5418 Å, with 0-0 geometry, equipped with an X’Celerator solid detector and a Ni filter). The 20 range was from 20 to 70° with a step size $\Delta$20=0.0167°. The experimental patterns were compared to standards compiled by the Joint Committee on Powder Diffraction and Standards (JCPDS cards) using the X’Pert High-Score Plus software [29]. The infrared (IR) adsorption analysis of the samples were obtained using a Spectrum Two 104462 IR spectrophotometer equipped with a diamond ATR setup in the range 4000-400 cm$^{-1}$. Nitrogen sorption isotherms for dried powders were recorded at 77K using a sorptometer EMS-53 and KELVIN 1040/1042 (Costech International). Points of Zero Charge (PZC) and Iso-Electric Point (IPE) of the samples are determined by zeta potential measurements, using a Malvern Nano ZS. Suspensions were prepared using NaCl (0.1M) as a background electrolyte with each powder using aqueous solutions, starting in an alkaline medium and stopping at pH = 4 under $N_2$ at 25°C [30]. The titrations were carried out on suspensions of different samples of apatite obtained by adding 0.15 g of apatite to 30 mL of electrolyte (NaCl) and then 1.5 mL of 0.1M NaOH. The titrant used is 0.1 M hydrochloric acid prepared from 1M HCl, at the same ionic strength as the electrolyte by the addition of NaCl. The phosphorus and calcium contents were obtained by ICP-OES on a Horiba Jobin Yvon modele activa.
The thermal analysis of the carbon was carried out using a SETARAM SETSYS 1750. Heating was performed in a platinum crucible in air flow at a rate of 10°C/min up to 800°C. For transmission electron microscopy (TEM) investigations, samples were prepared by dispersing the powders in a slurry of dry ethanol, deposited on a copper grid covered with a carbon thin film. High-resolution transmission electron microscopy (HRTEM) observations were performed on a JEOL JEM 2010 transmission electron microscope equipped with a LaB₆ filament and operating at 200 kV. The images were collected with a 4008 X 2672 pixels CCD camera (Gatan Orius SC1000). Circular dichroism (CD) experiments were performed at solid state using 5 mg of powder dispersed in nujol between NaCl pellets [31]. The measurements were performed by a TASCO J-815 spectropolarimeter. The scans were recorded from 190 to 300 nm wavelength with the following parameters: 0.5 data pitch, 2 nm bandwidth, 100 nm/min scanning speed, and are the result of 3 accumulations.

3. Results and discussion
3.1. Elemental analysis

The results of chemical analysis for mixed CaMgHAp with glutamic acid are reported in Table 1. The CaHAp sample shows a (Ca/P) molar ratio very close to the targeted stoichiometric value of 1.67. For the apatite series, the (Ca+Mg/P) ratio decreased from the starting CaHAp (1.68) to Ca₉Mg₁HAp-GA(20) (1.55). The presence of the organic anion in the precipitated material is attested and quantified by the total carbon analysis. We can note the increase in carbon amount with the concentration of Mg, this great affinity of glutamic acid to mixed CaMgHAp could be explained by the high electronegativity of magnesium (χ₉Ca = 1, χ₉Mg = 1.31) [32], in agreement with the results previously reported for CaCuHAp modified by polyaspartic acid [33] and CaZnHAp modified by tartric acid [34]. The larger absorption of carbon in the CaMgHAp-GA would explain their loss of stoichiometry. This indicates that our samples are indeed hydroxyapatite-glutamic acid composites. For the samples CaHAp, Ca₉.₅Mg₀.₅HAp and Ca₉Mg₁HAp the increase in carbon amount with the
concentration of Mg is explained by the disorder induced by the magnesium substitution promoting the incorporation of carbonates.

Table 1

Chemical composition (% weight ±0.02) of grafted mixed CaMgHAp

<table>
<thead>
<tr>
<th>Samples</th>
<th>% Ca</th>
<th>% Mg</th>
<th>% P</th>
<th>% C</th>
<th>(Ca + Mg)/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaHAp</td>
<td>39.31</td>
<td>-</td>
<td>18.09</td>
<td>0.12</td>
<td>1.68</td>
</tr>
<tr>
<td>Ca$<em>{0.5}$Mg$</em>{0.5}$HA</td>
<td>38.14</td>
<td>0.98</td>
<td>18.66</td>
<td>0.15</td>
<td>1.65</td>
</tr>
<tr>
<td>p Ca$<em>{0}$Mg$</em>{1}$HAp</td>
<td>35.28</td>
<td>1.82</td>
<td>18.01</td>
<td>0.18</td>
<td>1.64</td>
</tr>
<tr>
<td>CaHAp-GA(10)</td>
<td>39.05</td>
<td>-</td>
<td>18.15</td>
<td>0.72</td>
<td>1.66</td>
</tr>
<tr>
<td>Ca$<em>{0.5}$Mg$</em>{0.5}$HAp-GA(10)</td>
<td>37.94</td>
<td>1.08</td>
<td>18.63</td>
<td>0.87</td>
<td>1.64</td>
</tr>
<tr>
<td>GA(10) Ca$<em>{0.5}$Mg$</em>{1}$HAp-GA(10)</td>
<td>34.05</td>
<td>1.89</td>
<td>17.98</td>
<td>1.12</td>
<td>1.59</td>
</tr>
<tr>
<td>CaHAp-GA(20)</td>
<td>38.97</td>
<td>-</td>
<td>18.23</td>
<td>1.25</td>
<td>1.65</td>
</tr>
<tr>
<td>Ca$<em>{0.5}$Mg$</em>{0.5}$HAp-GA(20)</td>
<td>37.23</td>
<td>1.13</td>
<td>18.58</td>
<td>1.49</td>
<td>1.63</td>
</tr>
<tr>
<td>GA(20) Ca$<em>{0.5}$Mg$</em>{1}$HAp-GA(20)</td>
<td>32.51</td>
<td>2.03</td>
<td>17.88</td>
<td>2.58</td>
<td>1.55</td>
</tr>
</tbody>
</table>

3.2. Thermal analysis

The (TG) curves of Ca$_{0}$Mg$_{1}$HAp, Ca$_{0}$Mg$_{1}$HAp-GA(10) and Ca$_{0}$Mg$_{1}$HAp-GA(20) samples are reported in figure 1. The thermal decomposition shows a first weight loss between 50°C and 200°C, assigned to the removal of physisorbed water. The second one, between 200°C and 500°C, corresponds to the elimination of the organic matter of glutamic acid. The weight loss associated with this second process allows evaluation of the relative amount of glutamic acid in the composite hybrids. The values obtained for carbon expressed as wt% of the solid product are reported in Table 2. The relative amount of glutamic acid increases with its increasing concentration in the reaction with CaHAp. This result is in agreement with the increase of the percentage of carbon determined by chemical analysis. Figure 2 reports the differential thermal analysis curves (DTA) of the samples. These curves display an unexpected endothermic effect associated with water desorption. This effect was already reported for Mg modified hydroxyapatites [35, 36]. It was assigned to the fact that heating in the presence of physisorbed water first induce a structuring effect. The latter may be related both to the surface relaxation at solid-water interface upon water release [37] and to
the polarization process of OH groups from the columns known to occur at 200°C that initiates the proton mobility inside the columns [38]. An exothermal effect is observed in the temperature range 200-500°C with a peak top at 300°C for Ca9Mg1HAp-GA(10) and Ca9Mg1HAp-GA(20) samples. This peak, which is absent in the DTA plot of non-modified Ca9Mg1HAp, corresponds to the combustion of the organic material. Furthermore, the intensity of these peaks increases with increasing grafted amount content. In fact, their presence confirms that the prepared samples correspond to hydroxyapatite-glutamic acid composites, similar to that previously found for hydroxyapatite modified with glycine and sarcosine acids [39].

![Graph showing weight loss vs. temperature for different samples](image)

**Table 2**

Results of TG analysis of ungrafted and grafted apatites.

<table>
<thead>
<tr>
<th>Samples</th>
<th>First loss (% wt): water (50-200°C)</th>
<th>Second loss(% wt): glutamic acid (200-500°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca9Mg1HAp</td>
<td>2.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Ca9Mg1HAp-GA(10)</td>
<td>3.4</td>
<td>4.6</td>
</tr>
<tr>
<td>Ca9Mg1HAp-GA(20)</td>
<td>4.8</td>
<td>6.7</td>
</tr>
</tbody>
</table>

3.3. Circular dichroism
In figure 3 (left) are presented the dichroic curves obtained in the range 300-190 nm for glutamic acid (a) and Ca$_9$Mg$_1$HAp-GA(20) (b) respectively. The two curves are very close to each other showing a negative maximum at 200 nm for GA and 230 nm for Ca$_9$Mg$_1$HAp-GA(20). The corresponding UV absorption curves on the right are also very similar. The negative effect at 200 nm is also observed in water solution for glutamic acid oligomers [40]. The 30 nm difference observed between the curves (a) and (b) can be related to the difference in ionisation of the carboxylic group of adsorbed or free glutamic acid.

![Graph of CD vs wavelength and UV absorption curves](image)

**Fig. 3.** (Left) Circular dichroism spectrum and (right) UV absorption curves of (a) glutamic acid and (b) Ca$_9$Mg$_1$HAp-GA(20).

### 3.4. Infrared investigation

The IR spectra recorded with or without glutamic acid onto the apatite surface are illustrated in figure 4. The vibrations in the range 1800-1200 cm$^{-1}$ are summarized in table 3. In particular, the bands observed at 1598, 1445 and 1261 cm$^{-1}$ (Fig. 4b, 4c), which are not present in the spectrum of Ca$_9$Mg$_1$HAp (Fig. 4a) can be attributed to sorbed glutamic acid [41]. The vibration located at 1635 cm$^{-1}$ is attributed to adsorbed water [42]. The higher water content in the presence of glutamic acid is consistent with the decrease in the ratio (Ca + Mg) / P. In order, to eliminate the hypothesis of a simple mechanical mixture between glutamic acid and apatite, further data have been recorded from such
mixture containing Ca₉Mg₁HAp and glutamic acid in the same relative amounts as those contained in the Ca₉Mg₁HAp-GA(20) sample. The FT-IR spectrum of this mixture, which is reported in Fig. 4d, displays a number of bands due to the two discrete components of the mixture, and indicates the absence of specific interaction between the carboxylic groups of the glutamic acid and the calcium ions of hydroxyapatite. The comparison with the spectra reported in Fig. 4b and 4c, which show also a small shift of the carboxylic stretching band to lower wave numbers, in agreement with an increase of the C-O bond length can be attributed to those of the organic moieties grafted on calcium or magnesium atoms on the surface of the hydroxyapatite, in agreement with the results previously reported for CaHAp modified by amino-acids [39, 43].

![FT-IR spectra](image)

**Fig. 4.** FT-IR spectra of: (a) Ca₉Mg₁HAp, (b) Ca₉Mg₁HAp-GA(10), (c) Ca₉Mg₁HAp-GA(20), (d) Mixed Ca₉Mg₁HAp-glutamic acid and (e) glutamic acid.

**Table 3**

<table>
<thead>
<tr>
<th>Tentative assignments</th>
<th>Wave number (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic acid [41]</td>
<td>Ca₉Mg₁HAp-GA(10)</td>
</tr>
<tr>
<td>COO- asym.str.</td>
<td>1609</td>
</tr>
</tbody>
</table>
3.5 X-ray analysis

The X-ray powder diffractograms for CaHAp and Ca$_9$Mg$_1$HAp synthesized with or without the presence of glutamic acid are shown in figure 5. In table 4, we reported the size of the apatite crystallites induced by magnesium and glutamic acid for the reflections (002) and (310). For all samples, we observe a unique apatitic phase belonging to the P6$_3$/m space group (n° 9-432-ICDD-PDF). We could not prepare the grafted apatites Ca$_{8.5}$Mg$_{1.5}$HAp-GA(n), since all our preparations failed. Several authors have shown that the particular behavior of the Ca$_{8.5}$Mg$_{1.5}$HAp compound can therefore be explained by the fact that sample Ca$_{8.5}$Mg$_{1.5}$HAp is a pure non stoichiometric CaMgHAp which would turn into whitlockite under the effect of temperature and already made up of a mixture of phases CaMgHAp crystalline and amorphous whitlockite [Ca$_3$-$\gamma$Mg$_y$(HPO$_4$)$_z$(PO$_4$)$_2$3$z$/2], which is crystallized under the effect of heat treatment at 900 °C [44, 45].

Broadening of the diffraction lines increases with the concentration of magnesium and glutamic acid. The crystallite sizes were calculated from the broadening of the (0 0 2) and (3 1 0) using the Scherrer equation [46]:

$$ D \frac{K\lambda}{\beta_{1/2}\cos\theta} $$

Where $\theta$ is the diffraction angle, $\lambda$ the wavelength and $K$ a constant depending on the crystal (chosen as 0.9 for apatite crystallites) and $\beta_{1/2}$ is the line width at full width at half maximum (FWHM), of a given reflection. The line broadening of the (0 0 2) and (3 1 0) reflections was used to evaluate the crystallite size along the $c$-axis and along a direction perpendicular to it. The crystallinity ($X_c$) is defined as the fraction of the crystalline apatite phase in the investigated volume of powdered sample. An empirical relation between $X_c$ and $\beta_{1/2}$ was deduced, according to the following equation [47]: $X_c = [K_A/\beta_{1/2}]^3$. Where $K_A$ is a constant set at 0.24 and $\beta_{1/2}$ is the FWHM of the (0 0 2) reflection, the

| CH$_2$ str. | 1445 | 1442 | 1445 |
| Amide III | 1264 | 1263 | 1261 |

Asym.: Asymmetrical, str.: stretching
obtained values are reported in Table 4. The crystallite size and the crystallinity decrease with increasing magnesium and amino acid concentration. It can also be deduced that the crystallites are of nanometric sizes and the decrease is more important in the (3 1 0) than in the (0 0 2) direction. Such observation was earlier reported for other organic moieties grafted onto apatite surfaces and can be explained by a better interaction of the glutamic acid with faces parallel to the c axis [48, 33].

Fig. 5. X-ray diffractograms for CaHAp and Ca$_9$Mg$_1$HAp ungrafted and grafted.

The individual effect of magnesium and glutamic acid on the crystallite size can be observed in figure 6. The crystallite sizes $D_{(002)}$ and $D_{(310)}$ decrease slowly with the concentration of magnesium whereas the addition of glutamic acid induces a bigger change in the crystallinity. It would appear that the glutamic acid is the component most responsible for the loss of crystallinity which could be explained by the presence of the groups COO$^-$ on the surface of materials. We do not observe a change in position of the peaks between CaHAp and Ca$_9$Mg$_1$HAp diffractograms, here the main phenomenon is the broadening of the peaks, which has already been observed during the substitution of calcium by
another divalent ion. When glutamic acid is present, we observe no difference between Ca$_9$Mg$_1$HAp-GA(10) and Ca$_9$Mg$_1$HAp-GA(20) diffractograms. In contrast, peak shifts are observed compared to the CaHAp and Ca$_9$Mg$_1$HAp one, in particular for the (211) and (222) peaks. This phenomenon that we have already described was attributed to a better interaction of the glutamic acid with the faces parallel to the axis c during the process of crystalline growth [39].

Table 4: Evolution size of the apatite crystallites with magnesium and glutamic acid for the reflexion (002) and (310).

<table>
<thead>
<tr>
<th>Samples</th>
<th>$\beta$ (002)</th>
<th>$D_{(002)}$ (Å)</th>
<th>$\beta$ (310)</th>
<th>$D_{(310)}$ (Å)</th>
<th>Crystallinity ($X_C$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaHAp</td>
<td>0.191(1)</td>
<td>427</td>
<td>0.477(9)</td>
<td>177</td>
<td>1.981</td>
</tr>
<tr>
<td>Ca$<em>{9.5}$Mg$</em>{0.5}$HAp</td>
<td>0.207(2)</td>
<td>394</td>
<td>0.640(1)</td>
<td>132</td>
<td>1.554</td>
</tr>
<tr>
<td>Ca$_9$Mg$_1$HAp</td>
<td>0.264(2)</td>
<td>309</td>
<td>0.712(1)</td>
<td>119</td>
<td>0.749</td>
</tr>
<tr>
<td>CaHAp-GA(10)</td>
<td>0.230(4)</td>
<td>354</td>
<td>0.710(4)</td>
<td>119</td>
<td>1.042</td>
</tr>
<tr>
<td>Ca$<em>{9.5}$Mg$</em>{0.5}$HAp-GA(10)</td>
<td>0.283(4)</td>
<td>288</td>
<td>0.931(3)</td>
<td>91</td>
<td>0.607</td>
</tr>
<tr>
<td>Ca$_9$Mg$_1$HAp-GA(10)</td>
<td>0.364(5)</td>
<td>224</td>
<td>1.286(3)</td>
<td>66</td>
<td>0.285</td>
</tr>
<tr>
<td>CaHAp-GA(20)</td>
<td>0.233(1)</td>
<td>350</td>
<td>0.761(2)</td>
<td>111</td>
<td>1.029</td>
</tr>
<tr>
<td>Ca$<em>{9.5}$Mg$</em>{0.5}$HAp-GA(20)</td>
<td>0.322(3)</td>
<td>253</td>
<td>1.084(1)</td>
<td>75</td>
<td>0.413</td>
</tr>
<tr>
<td>Ca$_9$Mg$_1$HAp-GA(20)</td>
<td>0.389(2)</td>
<td>210</td>
<td>1.385(1)</td>
<td>61</td>
<td>0.234</td>
</tr>
</tbody>
</table>

![Graph showing the evolution of $D_{(002)}$ and $D_{(310)}$ with Mg content](image)
Fig. 6. Effect of the Mg and GA concentration on the crystallite size $D_{(002)}$ (A) and $D_{(310)}$ (B) relative to the CaHAp values taken as references.

3.6. TEM observations

Transmission electron microscopy (TEM) analysis micrographs of the samples are illustrated in figure 7A. From the photomicrographs, it can be seen that the size of precipitated apatite particles, prepared with and without glutamic acid, is on the nanometer scale. CaHAp is constituted of well dispersed plate-shaped crystals with an average size of about 40-150 nm long and about 30 nm wide. A small addition of magnesium induces a decrease of the size: 30-60 nm long and about 15-20 nm wide for the Ca$_{9.5}$Mg$_{0.5}$HAp. The presence of magnesium and glutamic acid in the start solution completely modifies the aspect. We obtain large bundles of CaMgHAp-GA fibers (300 nm length /80 nm width). The HRTEM images (Fig. 7B) reveal that these fibers are thin (15 nm wide) and stacked together to each other. The analysis of the FFT patterns show that the growth of the particles always occurs in the (002) direction, indicating that they are growing along the c axis direction such as the
pure CaHAp. The results of the TEM analysis confirm that the apatite structure is preserved with a
modification of crystal size in accordance with the analysis of XRD patterns.

Fig. 7A. TEM images (Scale bars=50 nm) of CaHAp, Ca$_{9.5}$Mg$_{0.5}$HAp, Ca$_9$Mg$_1$HAp-GA(10) and
Ca$_9$Mg$_1$HAp-GA(20).
3.7. Functionalization of CaHAp with glutamic acid

3.7.1. Speciation of the interface apatite-solution

The influence of the glutamic acid amount on the textural properties of CaHAp was examined (Table 5). We obtained 43 m$^2$/g for Ca$_9$Mg$_1$HAp and after functionalization 30 m$^2$/g and 24 m$^2$/g for Ca$_9$Mg$_1$HAp-GA(10) and Ca$_9$Mg$_1$HAp-GA(20), respectively. The decrease in the specific surface area of the apatite samples is related to the structural arrangement of the 2-aminopentanedioic acid on the surface of the solid. These results are in good agreement with those obtained by Oberto Da Silva et al. [49]. The initial pH$_i$ value measured in aqueous solutions increase with the glutamic acid ratio. We recorded 8.9 and 9.3 for Ca$_9$Mg$_1$HAp-GA(10) and Ca$_9$Mg$_1$HAp-GA(20) respectively against only 8.4 for Ca$_9$Mg$_1$HAp.

Table 5

Surface area and pH value of different apatite samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>$S_{BET}$ (m$^2$/g)</th>
<th>IEP</th>
<th>PZC</th>
<th>pH$_i$ in aqueous solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca$_9$Mg$_1$HAp</td>
<td>43</td>
<td>7.1</td>
<td>8.2</td>
<td>8.4</td>
</tr>
<tr>
<td>Ca$_9$Mg$_1$Hap-GA(10)</td>
<td>30</td>
<td>8.2</td>
<td>8.6</td>
<td>8.9</td>
</tr>
<tr>
<td>Ca$_9$Mg$_1$Hap-GA(20)</td>
<td>24</td>
<td>8.6</td>
<td>8.8</td>
<td>9.3</td>
</tr>
</tbody>
</table>

PZC: point zero charge is determined by an acid-basic titration equilibrated at 16h
IEP: Iso-electric point (mV) is determined by zeta-metry
The Zeta potential of different apatite samples is presented in figure 8. The values of Point of Zero Charge (PZC) and iso-electric point (IEP) were determined (Table 5). In a basic medium, the Ca$_9$Mg$_1$HAp sample is characterized by a highly negative zeta potential indicating a deficit of positive surface charge, a value lower than that of the apatites Ca$_9$Mg$_1$HAp-GA(10) and Ca$_9$Mg$_1$HAp-GA(20). This result indicates an increase in positive surface charge sites, on amino-acid grafted apatites [50].

Whereas the value of pH$_{IEP}$ is 7.1, the PZC for the Ca$_9$Mg$_1$HAp sample is around 8.2. The same value is found in the literature [51-54] (Figure 8(B)). This result can be explained by the presence of initial charge and specific adsorption at the surface of apatite. After glutamic acid functionalization, the Zeta-potential curve is shifted towards alkaline pH values, with pH$_{IEP}$ of 8.2 and 8.6 for Ca$_9$Mg$_1$HAp-GA(10) and Ca$_9$Mg$_1$HAp-GA(20), respectively. This result verified by measurement of PZC (from 8.6 to 8.8), confirms the functionalization of the surface of Ca$_9$Mg$_1$HAp by the glutamic acid.

The total number of protons consumed during titrations of different samples is determined using this equation:

$$[H^+]_s = \frac{1}{S_{exp}} \left\{ \left( \frac{C_a V_a - C_b V_b}{V} \right)_{\text{susp}} + \left( \frac{C_a V_a - C_b V_b}{V} \right)_{\text{blanc}} \right\} \frac{10^{-pH}}{\gamma_{H^+}} + \frac{10^{-(\text{Log}(K_e) - pH)}}{\gamma_{OH^-}}$$

S$_{exp}$: specific surface area (m$^2$/g) exposed in the suspension, V is total volume of solution; C$_a$ and C$_b$ are the HCl and NaOH concentration used for titration. K$_e$ is the dissociation constant of water. $\gamma(\text{H}^+)$ and $\gamma(\text{OH}^-)$ are the coefficient of the dissociation activity of H$^+$ and OH$^-$ calculated by Debye-Huckel relation. $\log(\gamma_I) = -(z_i)^2 D$

With $Z_i$ is the ion charge and $D = \frac{A\sqrt{I}}{1 + B0_{i}^{0}\sqrt{I}}$
I: Ionic strength of solution (mol/L), $A=0.507$, $B=0.328 \times 10^{-8}$ and $\alpha_i^0$ is the effective diameter (Fig. 8(B)). This quantity of protons ($H^+$) is deduced from the difference between the amount of ($H^+$) added to the suspension and the amount of free ($H^+$) in solution. This is calculated directly from the measured pH. The amount of the added protons is corrected by the amount of hydroxyl initially added to the suspension.

The proton quantity consumed by apatite samples [$H^+$] shows the same evolution: in an acid medium, [$H^+$] is between 9 and 12 $\mu$mol/m² (pH = 5) for Ca$_9$Mg$_1$HAp, Ca$_9$Mg$_1$HAp-GA(10) and Ca$_9$Mg$_1$HAp-GA(20), respectively. Then, it decreases regularly and becomes negative in a basic medium with the protons consumed in the order of -8 and -10 $\mu$mol/m² at pH = 11.
3.7.2. Proposed glutamic acid sorption mechanism

The IR spectra provide data about the ionization state of the carboxylate groups grafted onto the apatite surface. Indeed, the presence of bands characteristic of –COO\(^{-}\) groups and the absence of bands attributed to COOH groups (1700 cm\(^{-1}\)) indicate the carboxylate form. This shows that the interaction is mainly due to the electrostatic interaction between -COO\(^{-}\) groups of the glutamic acid and the calcium Ca\(^{2+}\)/Mg\(^{2+}\) ions of the hydroxyapatite. We cannot exclude also that interactions between COO\(^{-}\) and surface POH groups are possible. The fixation is due to the simultaneous presence of -COO-/Ca\(^{2+}\)/Mg\(^{2+}\) electrostatic interactions and H-bonds between NH\(_3^+\) protons and surface oxygen atoms of the PO\(_4\) group [55, 56].

The nature of interactions between the apatite surface and a glutamic acid depends on the pH value of the medium. Taking into consideration that the reaction is carried out at a pH above 9.5, we
can consider that in aqueous solution glutamic acid exists as a carboxylate ion, the amino group being neutral [57]. Under the same conditions, the apatite surface is considered as negatively charged, therefore some authors consider that the electrostatic interactions between the surface and the amino-acid are very weak [58, 59]. In the present study, glutamic molecules are present in the form of carboxylate ions, which can lead to calcium complexes that participate in the formation of the crystalline edifice. The observation of the IR spectra, showing characteristic vibrations of carboxylate salts, lead us to conclude that the amino-acid in this carboxylate ionic form exchanges an hydroxyl ion. Different model grafting mechanisms proposed based on the results obtained are shown in scheme 1.

**Scheme 1.** Formation of Ca carboxylate salt leading to the grafting of glutamic on the CaHAp surface.

**Conclusion**

In conclusion, we have successfully synthesized hydroxyapatite-glutamic composites of different glutamic acid content using the hydrothermal method. The presence of the glutamic acid and/or magnesium in the reaction solution does not change the apatite structure, but reduces the crystallinity and the crystallite sizes. According to IR spectroscopy, the new vibrations after adsorption can be attributed to those of the organic moieties grafted on calcium or magnesium atoms onto the surface of the apatite. TEM images confirm the reduction of crystallite sizes and indicate the change in its morphology.
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References


[29] PANalyticalX’Pert High Score Plus version 2.0.


