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ORIGINAL ARTICLE

Interaction of some essential amino acids with synthesized poorly crystalline hydroxyapatite

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Amino acid;
Medium composition;
Electrostatic interaction

Abstract This study focused on the release of two essential amino acids, L-lysine and DL-leucine, previously adsorbed onto poorly crystalline hydroxyapatite of Ca/P = 1.59, synthesis by precipitation methods. The composition of the calcium-deficient hydroxyapatite (CDHA) is chemically and structurally similar to the bone mineral. Their surface reactivity is indeed linked to the existence of hydrated surface particles (HPO_4^{2-} and Ca^{2+}). The adsorption kinetics is very fast while the release kinetics is relatively slow. The adsorption rate reached approximately 70%, but the release rate did not exceed 12%. The chemical composition of solution has an influence on the release processes. The presence of phosphate ions favored the release of amino acids, while the calcium ions inhibited it. Also, the release process is slightly influenced by Ra (ml/mg) ratio and incubation temperature of the medium. The charged $–COO^-$ and NH_3^+ of amino acids are the strongest groups that interact with the surface of hydroxyapatite, the adsorption is mainly due to the electrostatic interaction between the groups $–COO^-$ of amino acids and calcium Ca^{2+} ions of the hydroxyapatite. DL-Leucine (non-polar) and L-Lysine (polar–basic) interact with the hydroxyapatite surface in the zwitterionic and cationic forms, respectively. The study of interactions between amino acids and hydroxyapatite is carried out in vitro by using UV–vis and infrared spectroscopy IR techniques.

1. Introduction

The study of the interactions between biomolecules and biomaterials has received much attention in recent years because of the potentiality of nanotechnology applied to biotechnological processes and in biomedical applications (De et al., 2008; Gray, 2004; Vallet-Regí et al., 2004; Vo-Dinh, 2007). The adsorption and release at the apatite-solution interface are the result of the various interactions between and within the system components which include the solid surface, the adsorbate, the solvent and other solutes present. Among various biomaterials, calcium hydroxyapatite, has been widely considered as one of the most important inorganic materials for medical and dental applications such as dental implants, alveolar bridge augmentation, orthopaedics, maxillofacial surgery and drug delivery systems due to its biocompatibility, chemical and biological affinity with bone tissue (Burg et al.,

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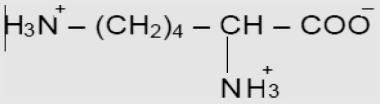
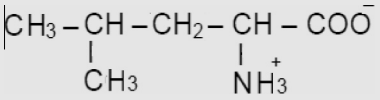
Table 1 Characteristics and properties of L-Lysine and DL-Leucine.

Amino acid	L-Lysine	DL-leucine
Chemical structure		
Molecular formula	$C_6H_{14}N_2O_2$	$C_6H_{13}NO_2$
Other names	2,6-diaminohexanoic	2-amino-4-méthylpentanoic
Hydrophobicity	Strongly basic and hydrophilic	Very hydrophobic (non polar)
Acidity (pK_a)	2,18 (carboxyl), 8,95 (amino), 10.53 (side chain)	2.36 (carboxyl), 9.60 (amino)
Molecular weight (g/mol)	146.19	131.17
Isoelectric point (pI)	9.74	6.04
Solubility in water at 25 °C (g/L)	1500	24

2000; Dash and Cudworth, 1998; Suchanek and Yoshimura, 1998; Zhou and Lee, 2011). Their availability structure, ionic exchange property, adsorption affinity, and their characteristic to establish bonds with organic molecules of different sizes have conferred to this material to be often used as a reference to study biomolecule/biocompatible surface interactions (Jones, 2001; Ratner et al., 2004). Multiple techniques have been used for the preparation of hydroxyapatite powders, as reviewed in several works (Aoki, 1991). Depending upon the technique, materials with various morphologies, stoichiometries, and levels of crystallinity can be obtained. In the present work, the poorly crystalline hydroxyapatite was prepared in an aqueous medium by rapid precipitation at room temperature and at neutral pH. The fixation/release process of low-crystalline calcium phosphate apatite is dependent on the composition of the hydrated surface layer and the surrounding environments (El Rhilassi et al., 2011, 2012a).

It is interesting to note that the composition of the phosphocalcic hydroxyapatite may vary in the domain in which the Ca/P atomic ratio is between 1.5 and 1.67. In fact, only the hydroxyapatite of ratio 1.67 is stoichiometric [$Ca_{10}(PO4)_6(OH)_2$], the other apatites are called calcium-deficient hydroxyapatites (CDHA). Several chemical formulas have been proposed for calcium-deficient hydroxyapatite (Dorozhkin, 2009; Elliott, 1994; Posner et al., 1960). An example of a proposed formula is: Ca_{10-x} $(PO_4)_{6-x}$ $(HPO_4)_x$ $(OH)_{2-x}$ $(0 < x < 1)$.

In the case $x = 1$ (the boundary condition with Ca/P = 1.5), the chemical formula of CDHA looks as follows: Ca_9 (HPO_4) $(PO_4)_5$ (OH).

The purpose of the present work is to study the adsorption and release process of amino acids L-lysine and DL-leucine onto poorly crystalline hydroxyapatite (CDHA) under physiological conditions. We propose to analyze the solution after release, and study the influence of calcium and phosphate ions,

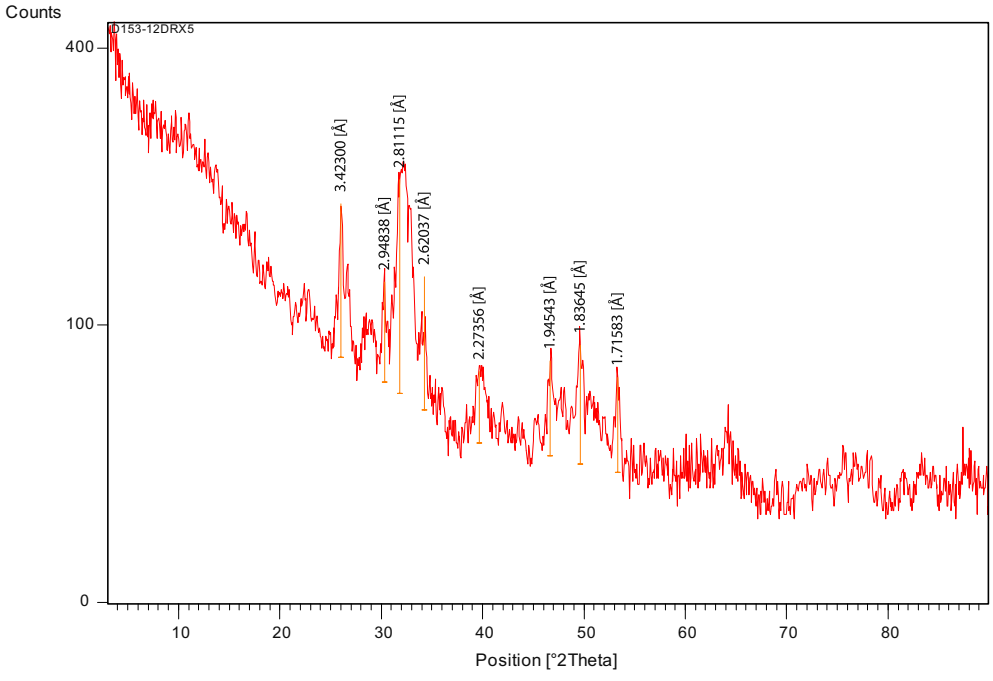


Figure 1 XRD patterns of poorly crystalline hydroxyapatite (CDHA).

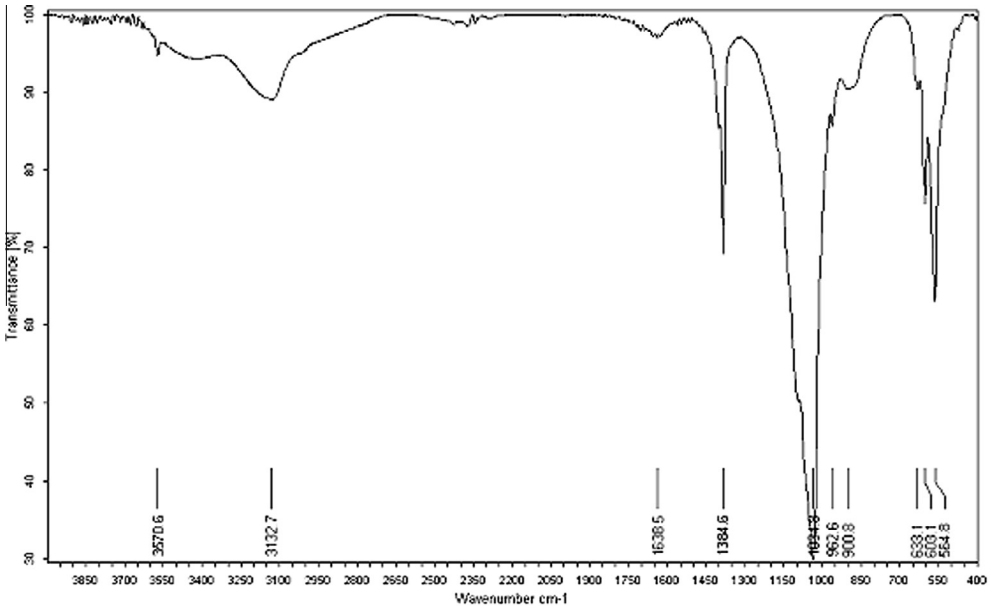


Figure 2 FTIR spectra of hydroxyapatite (CDHA) before adsorption of amino acids.

the ratio Ra (ml/mg) = volume solution/mass solid on the release process. The composition of the release medium is considered as an experimental variable in order to establish a relation between the release/fixation process and the surface characteristics of hydroxyapatite. It is important to know the side-chains and loads of amino acid residues involved in the interaction with hydroxyapatite surfaces.

2. Materials and methods

2.1. Adsorbates

The adsorbates that were chosen for this study are L-Lysine and DL-Leucine, they are two of nine essential amino acids which cannot be made by the human body and, therefore, must be obtained in the diet (Rose et al., 1951; Darmaun, 2008). Each of them represents about 8% of the amino acids of proteins in the human body. The characteristics and properties of these amino acids are illustrated in Table 1 (Dawson et al., 1959; Nagai and Carta, 2004).

2.2. Adsorbent

2.2.1. Synthesis of hydroxyapatite (CDHA)

The calcium hydroxyapatite powder was synthesized by precipitation according to the method described by Rey et al. (989). A calcium nitrate solution $Ca(NO_3)_2{\cdot}4H_2O$ (Riedel- de Haën - Germany) (35.4 g in 0.5 l of distilled water) was immediately poured at room temperature into a di-ammonium hydrogenphosphate $(NH_4)_2HPO_4$ (Riedel-de Haën- Germany) solution (34.8 g in 1 l of distilled water), the pH of the solution was adjusted to 7 by ammoniac solution. After stirring for 2 h at the speed of 250 trs/min, the suspension was filtered on a large Buchner funnel, washed at least three times in distilled water, dried at 70 °C for 48 h.

2.2.2. Characterization of the adsorbent

FTIR spectrum of the samples was characterized using VERTEX 70/70v FT-IR spectrometers. An X-ray powder diffraction (XRD) pattern was analyzed using X'Pert PRO (Germany) Xray diffractometer with Cu Ka radiation. Scanning electron microscopy (SEM) (JSM- 6060LV, JEOL Ltd, Japan) was used for three different magnifications x1000, x2000, and x4000.

The calcium content was determined by complexometry with ethylene-diamine-tetraacetic acid (EDTA) and the phosphate ion content by spectrophotometry of phospho-vanado-molybdic acid.

2.3. Experimental protocol

2.3.1. Adsorption

Adsorption was investigated in batch experiments by adding 10 ml of the amino acids L-lysine and DL-leucine (1 mmol/l) to a test tube containing 200 mg of powder hydroxyapatite (CDHA) of Ca/P ratio 1.59. After stirring for 1 min at the speed of 1000 trs/min, the mixture was placed in a thermostatic bath at physiological temperature 37 °C and neutral pH (~7) of different contact times. The quantity of adsorbed adsorbate Qads was calculated by measuring the concentration of the solution before and after adsorption using Eq. (1):

$$Q_{ads} = \frac{V(C_0 - C_{eq})}{m} \quad (1)$$

where Co and Ceq are respectively the initial and residual concentration of amino acid (μmol/l), V is the volume of solution (l) and m is the hydroxyapatite mass (g).

The adsorption rate has been calculated from the following Eq. (2):

$$\%ads = \frac{(C_0 - C_{eq})}{C_0} \times 100 \quad (2)$$

2.3.2. Release

Release studies of amino acids were investigated by batch experiments. A hydroxyapatite (CDHA) sample of 0.2 g previously adsorbed of the amino acid (freshly prepared and finely ground) was added in 10 ml of de-ionized water at neutral pH (~7). After stirring for one minute at the speed of 1000 trs/min, the mixture was placed in a thermostatic bath at the physiological temperature (37 °C) for a definite time. After treatment, the solid and solution were separated by fritted glass (Frit Glass N°4).

The effect of ratio Ra (ml/mg) = volume solution/mass solid was examined by using the same procedure. The mass of hydroxyapatite is fixed at 0.2 g, the volume of the solution is varied from 10 to 100 ml; Ra values obtained are between 0.05 and 0.5 ml/mg.

The quantity released Q_{des} (mg/g) was determined by using Eq. (3):

$$Q_{des} = C_{des} \times \frac{V}{m} \quad (3)$$

Cdes is the equilibrium concentration of the amino acid released in solution (mg/ml).

The released rate of amino acid has been calculated from the following Eq. (4):

$$\%des = \frac{Q_{des}}{Q_{ads}} \times 100 \quad (4)$$

The supernatants obtained were examined by measuring the pH and determining the equilibrium concentration using the UV–vis spectrophotometer at 570 nm. The solid was dried in an oven at 80 °C for 24 h.

3. Results

3.1. Adsorbent characterization

In order to characterize hydroxyapatite (CDHA), XRD and FTIR analyses were carried out on the synthesized CDHA samples. Fig. 1 shows the XRD patterns of synthesized hydroxyapatite (CDHA); no crystalline phase was detected, the precipitate yielded broad and overlapping reflections, indicating its low crystallinity; reflections characteristic of poorly crystalline hydroxyapatite similar to bone mineral.

Fig. 2 represents FTIR spectra of the sample before adsorption of amino acid. The bands at 3571 and 633 cm^{-1} belong to the stretching vibrations of hydroxyl –OH (Berry and Badiel, 1966). The bands at 1034, and 962 cm^{-1} are characteristic of the phosphate stretching vibration PO_4^{3-}, and the bands observed at 600,564, and 474 cm^{-1} are due to the phosphate bending vibration PO_4^{3-}. The broad peaks at 1638 cm^{-1} and 3132 cm^{-1} are due to adsorbed water. The band at 900 and

Table 2 Chemical analysis of the hydroxyapatite synthesized.

Ca (mol)	P (mol)	Ca/P
0.817	0.514	1.59

Table 3 Quantity adsorbed of L-lysine and DL-leucine versus time by hydroxyapatite (CDHA) ($C_0 = 10^{-3}$ mol/l).

Time (h)	L-Lysine		DL-leucine	
	Qads (μmol/g)	pH	Qads (μmol/g)	pH
0	0	7	0	7
0.25	33,925	5,57	28,945	5,59
0.5	34,185	5,52	29,525	5,57
1	34,225	5,47	30,053	5,49
3	34,225	5,45	30,165	5,46
5	34,225	5,37	30,165	5,43
8	34,225	5,34	30,165	5,37
18	34,226	5,30	30,165	5,34
24	34,225	5,29	30,165	5,33

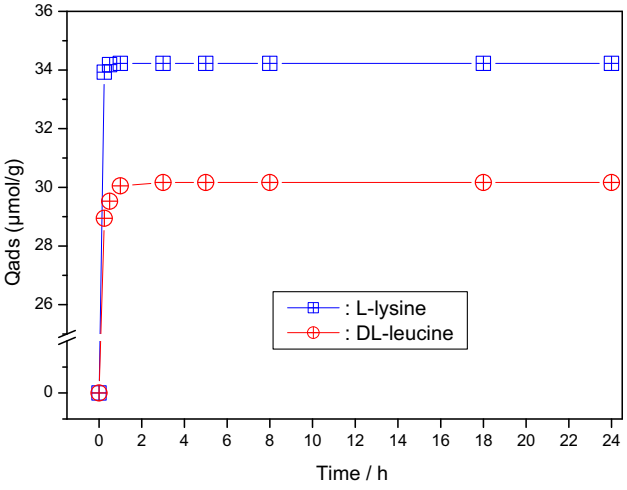


Figure 4 Adsorption kinetics of L-lysine and DL-leucine on hydroxyapatite (CDHA).

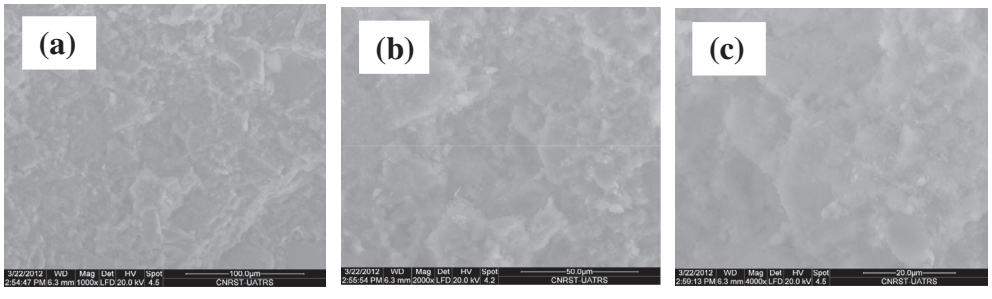


Figure 3 SEM micrographs of poorly crystalline hydroxyapatite: X 1000 (100 μm) (a), X 2000 (50 μm) (b) and X 4000 (20 μm) (c).

the band of low intensity at 2984 cm^{-1} are assigned to the HPO_4^{2-} groups as previously reported in the literature (Gibson et al., 1999; Nakamoto, 1986); the presence of these species indicates the non-stoichiometry of the hydroxyapatite. The characteristics of this poorly crystalline hydroxyapatite are similar to that of precipitated calcium phosphates of Ca/P between 1.33 and 1.67 previously investigated in our work (El Rhilassi et al., 2011).

The SEM (Fig. 3) of synthesized hydroxyapatite (CDHA) shows the irregularly shaped particles of different sizes, which demonstrates the poor crystalline structure of the calcium hydroxyapatite synthesized.

The Ca/P molar ratio of the precipitate is 1.59, indicating the formation of calcium-deficient hydroxyapatite (CDHA) (Table 2). The specific surface area of the precipitate was determined according to the Brunauer, Emett and Teller (BET) method using nitrogen adsorption. It is about 137 m^2/g

3.2. Study of adsorption

The result of kinetics adsorption of amino acids L-lysine and DL-leucine (1 mmol/l) by hydroxyapatite (CDHA) for contact time 15 min to 24 h is reported in Table 3. It shows that the adsorption kinetics is very fast, 30 min is adequate to reach equilibrium for L-lysine and 60 min for la DL-leucine (Fig. 4). There is no significant increase in the rate of adsorption for a contact time superior about 60 min (Table 3). This result is in accordance with the previous work (El Rhilassi et al., 2012a). The highest quantity adsorbed is obtained for L-lysine; polar amino acid.

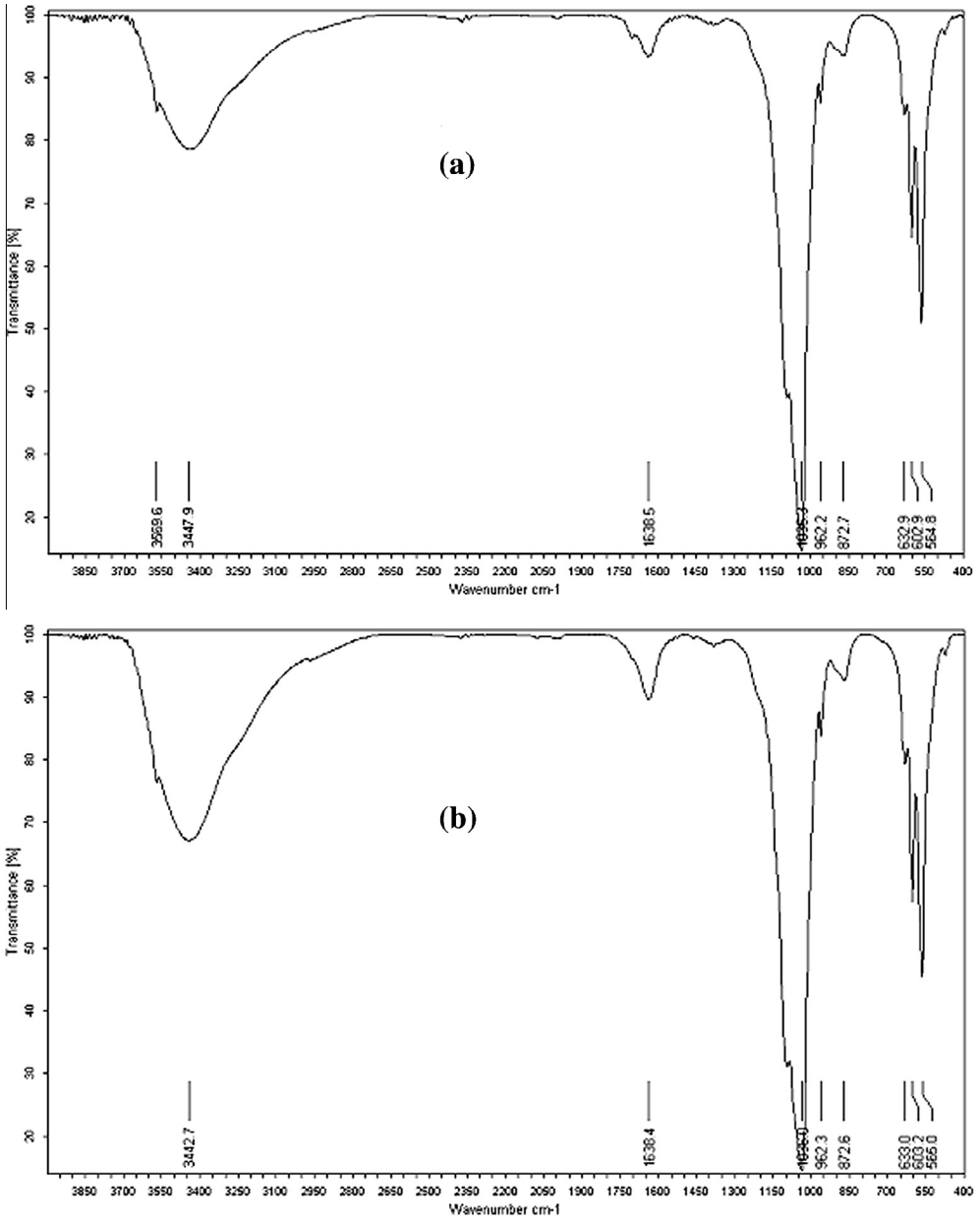


Figure 5 FTIR spectra of hydroxyapatite (CDHA) of Ca/P 1.59: (a) after adsorption in 10 mM of L-lysine, and (b) after adsorption in 10 mM of DL-leucine.

We calculated the adsorption rate of each amino acid (%ads); we found that the adsorption rate of L-lysine 68.45% is less than that of DL-leucine 60.33%.

The pH of the solution after adsorption varies slightly versus time; it passes from 5.6 to 5.3 (Table 3). It is less than the isoelectric point (pI) of each amino acid (Table 1).

Figs. 5, 2a and b represents FTIR spectra of the samples of calcium-deficient hydroxyapatite (CDHA) of Ca/P = 1.59 after adsorption in 10 mmol/l of L-lysine and DL leucine. The enlargement and displacement of the band characteristic of water molecules at 3132 (Fig. 2) to 3447 cm^{-1} (Fig. 5, 2a), and to 3442 cm^{-1} (Fig. 5, 2b) may be due to the stretching vibrations of NH_3^+ group of amino acids (Berry and Badiel, 1966; Nakamoto, 1986). Moreover, there is an increase in the intensity of $-COO^-$ band at 1638 cm^{-1} which may be due to amino acid adsorption.

Table 4 Rate of L-lysine and DL-leucine released from hdroxyapatite (CDHA) of Ca/P = 1.59 versus time.

Time (days)	L-lysine %des	DL-leucine %des
0	0	0
0,5	9	9,7
1	9,35	10,6
3	9,98	11,8
5	10,2	11,92
7	9,7	10,5
10	9,3	9,77
15	9,2	9,4

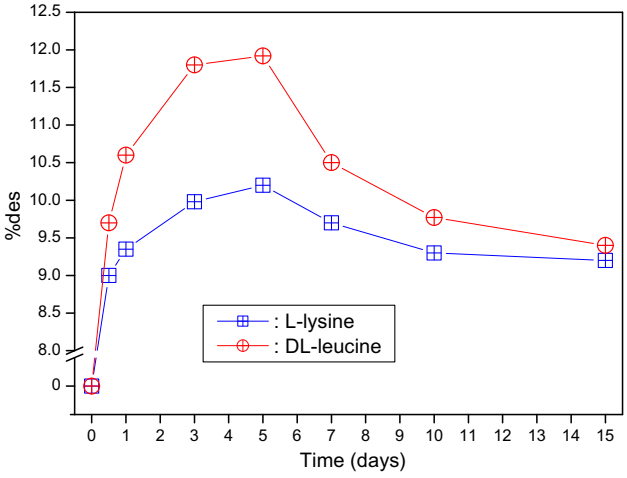


Figure 6 Release kinetics of amino acids from hydroxyapatite (CDHA).

3.3. Study of release

3.3.1. Kinetic of release

The aim of this work was to investigate, in vitro, the kinetics of release of amino acids L-lysine and DL-leucine which are previously adsorbed by poorly crystalline hydroxyapatite for contact times: 0.5 to 15 days (Table 4). The results obtained show that the release profiles corresponding to these two amino acids are similar (Fig. 6). There is an increase followed by a decrease beyond 5 days; the kinetics of release is slow. We show that the release rate (%des) of DL-leucine (non polar amino acid) is slightly superior to that obtained for the L-lysine. At 5 days (maximum release time), the release rate is about 11.92% for DL-leucine and 10.2% for L-lysine.

3.3.2. Chemical analysis

The results of chemical analysis and pH of the solution after release of L-lysine and DL leucine from hydroxyapatite (CDHA) are reported in Table 5. We observed a release of calcium and phosphate ions and a slight decrease in pH. The solution becomes more acidic versus time, the pH values are between 4.95 and 6.02. The maximum release rate of amino acids is observed at pH 5.5.

The concentration of the phosphate ion in solution increases with time and takes the maximum value after 5 days of contact. It is more important for the DL-leucine; amino acid has the highest rate released. However, the concentration of calcium ion decreases until the fifth day, and then increases slightly beyond this period. It is more important for L-lysine.

The value of Ca/P ratio of solution after release is higher for L-lysine than that of DL-leucine. This can be explained by the more pronounced release of phosphate ions for DL-leucine than for L-lysine (Table 4).

3.3.3. Effect of ratio Ra (ml/mg) on the release

The result experimental of ratio Ra (ml/mg) = volume solution/mass solid is given in Table 6. The release profiles corresponding to L-lysine and DL-leucine are similar (Fig. 7); the release rate (%des) increases slightly versus ratio Ra (ml/mg), e.g. for DL-leucine, it passes from 11.92% to 13.02% when Ra passes from 0.05 to 0.5 ml/mg. One also notes that the change in the pH of solution can be linked to the release of phosphate ions and amino acid.

Table 5 Chemical analysis of supernatant solution after release of amino acids versus time.

Time (days)	L-Lysine				DL-leucine			
	pH	Ca^{2+} mM	P mM	Ca/P	pH	Ca^{2+} mM	P mM	Ca/P
0.5	5.96	0.93	0.50	1.86	6.02	0.76	0.59	1.29
1	5.86	0.91	0.53	1.72	5.90	0.74	0.61	1.21
3	5.66	0.84	0.57	1.47	5.73	0.73	0.68	1.07
5	5.46	0.79	0.65	1.22	5.50	0.72	0.69	1.04
7	5.40	0.80	0.62	1.29	5.47	0.73	0.68	1.07
10	5.29	0.82	0.60	1.37	5.35	0.75	0.67	1.12
15	4.95	0.84	0.58	1.45	5.02	0.76	0.62	1.23

Table 6 pH of the supernatant solution and release rate (%des) of amino acids by hydroxyapatite (CDHA) at 5 days.

R (ml/mg)	L-Lysine		DL-leucine	
	pH	%des	pH	%des
0.05	5.4	10.2	5.3	11.92
0.1	5.54	10.54	5.45	12.21
0.2	5.79	11.05	5.74	12.64
0.5	6.16	11.54	6	13.02

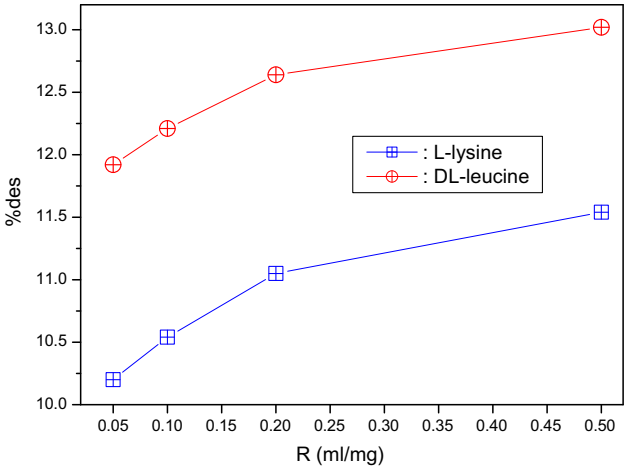


Figure 7 Effect of ratio Ra (ml/mg) on the release rate (%des) of amino acids by hydroxyapatite (CDHA) at 5 days.

3.3.4. Effect of ions on the release

We studied the effect of Ca^{2+} and HPO_4^{2-} ions on the release process of amino acids from Hydroxyapatite (CDHA). The release experiments were examined by varying the concentration of the electrolytes solution $CaCl_2$ and an equimolar mixture of KH_2PO_4/K_2HPO_4 0.5–10 mmol/l. Fig. 8 shows that the release of amino acids was influenced by the addition of these ions. Indeed, the addition of Ca^{2+} ions decreases slightly the release rate (Fig. 8a). While the addition of HPO_4^{2-} ions increases it (Fig. 8b), this increase is becoming increasingly important than the content of PO_4^{3-} ions that is higher.

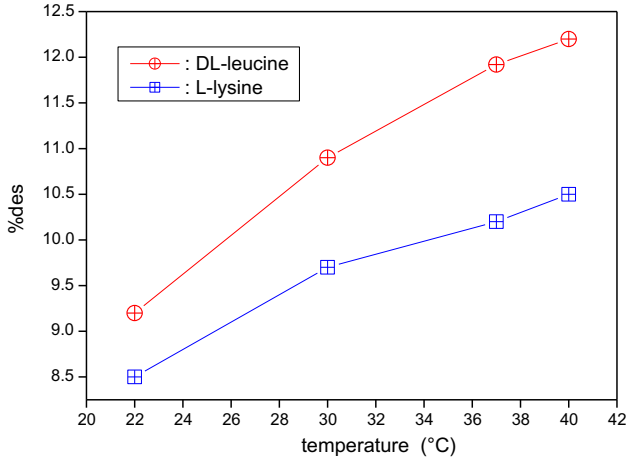


Figure 9 Effect of temperature on the release rate (%des) of amino acids by hydroxyapatite (CDHA) at 5 days.

3.3.5. Effect of temperature on the release

In the present work, we intend to study the temperature effect on the release of L-lysine and DL-leucine by hydroxyapatite (CDHA) which previously adsorbed these amino acids. The release experiments were performed for incubation period equal to 5 days and in a temperature range from 22 to 40 °C. Fig. 9 shows that the increase of temperature increases slightly the release rate (% des). Indeed, by increasing from 22 to 40 °C, the release rate increased from 9.2 to 12.2% for DL-leucine and from 8.5 to 10.5% for L-lysine.

4. Discussion and conclusion

In this study, the results of chemical analysis and characterization techniques (SEM, XRD and FT-IR) showed that the hydroxyapatite is calcium deficient and is poorly crystalline,

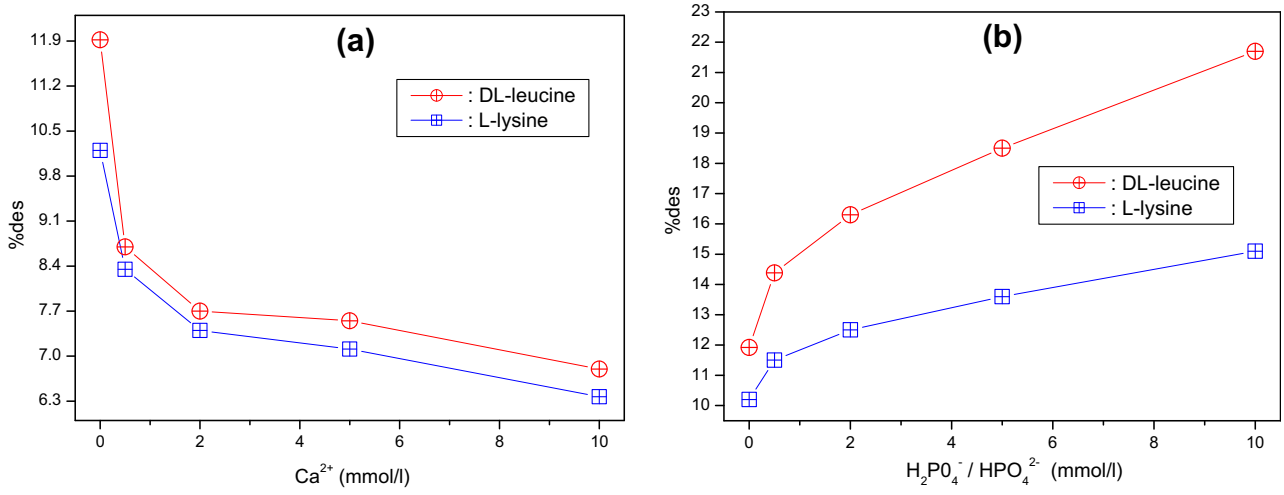


Figure 8 Effect of calcium (a) and phosphate (b) ion concentration on the release rate (%des) of amino acids by hydroxyapatite (CDHA) at 5 days.

similar to the mineral matrix of calcified tissues. Its Ca/P ratio was 1.59, which is lower than that of stoichiometric hydroxyapatite (1.67). This finding is in agreement with the presence of hydrogenophosphate groups as observed in the IR spectra. The specific surface area of the synthetic apatite was 137 m^2/g. This calcium deficient hydroxyapatite (CDHA) can have the chemical formula: $\mathbf{Ca_{9.54}\ (PO_4)_{5.54}\ (HPO_4)_{0.46}\ (OH)_{1.54}}$ ($x = 0.46$ for Ca/P = 1.59).

This work investigates the adsorption and release of two amino acids: L-lysine and DL-leucine by poorly crystalline hydroxyapatite (CDHA) of Ca/P = 1.59.

The kinetic adsorption is very rapid, the fixation reaction occurred after only 15 min of incubation. These results are in accordance to those obtained elsewhere (El Rhilassi et al., 2011, 2012a). This proves the high reactivity of hydroxyapatite surface with the surrounding environments such as molecules of biological interest.

FT-IR spectroscopy confirms the adsorption of amino acids; it can provide information about fixing $-COO^-$ and NH_3^+ groups onto the hydroxyapatite surface and can suggest the form of amino acid fixed. Indeed, the presence of bands characteristic of $-COO^-$ groups and the absence of bands attributed to –COOH groups (1700 cm^{-1}) indicate the cationic form of L-lysine (polar–basic) and zwitterion form of DL-leucine (non-polar). This shows that the adsorption is mainly due to the electrostatic interaction between the groups $-COO^-$ of amino acids and calcium Ca^{2+} ions of the hydroxyapatite. As reported in previous simulations (Almora-Barrios et al., 2009; Rimola et al., 2012, 2008), the fixation is due to the simultaneous presence of $-COO^-/Ca^{2+}$ electrostatic interactions and H-bonds between NH_3^+ protons and surface oxygen atoms of the PO_4 group. The common H_2N–CHR–COOH moiety shared by amino acids has been set up to interact with the surface of hydroxyapatite depending on the specific lateral chain (R: $(CH_3)_2CH$ and (CH2)4NH2 for Leucine and Lysine, respectively). In this case, DL-Leucine prefers to interact with the surface in its zwitterionic state, with the –COO– group interacting with Ca^{2+} ions and the NH^{3+} group making H-bonds with the oxygen surface atoms. L-Lysine is much more sensitive to lateral interactions in the presence of N atoms in the side chain.

The release kinetics is relatively slow. The release rate (%des) is low, it does not exceed 11.92%, even for DL-leucine, the amino acid is more released. While the adsorption rate is important, it reaches about 70%.

The release rate begins to decrease beyond five days (maximum release time); this suggests the existence of a re-adsorption reaction between the hydroxyapatite surface and the surrounding environment. This result is concordance with our previously published work (El Rhilassi et al., 2012b). The slightly acidic pH value noted of the supernatant solution after release may be due to a reaction exchange between amino acid previously fixed on poorly crystalline hydroxyapatite and labile ions (Ca^{2+} and HPO_4^{2-}) at the interface of hydrated surface particles. The binding and release of amino acids and ions at the interface between apatite and biological environments are crucial to determine how they perform *in vivo*.

Incubation of apatite after fixation of amino acids in the deionized water solution depends slightly on the effect of dilution. The rate of release increases only by 1.4% when Ra (ml/mg) is varied from 0.05 to 0.5. The variation in the pH of supernatant solution may be linked to the release of amino acid and also to the release of HPO_4^{2-} ions from the hydroxyapatite. A similar phenomenon is observed in the *in vivo* implantation of apatite materials where, in some cases, the pH of the surrounding environment of the implant can reach the value 3.7 (Amrah-Abouali et al., 1994).

We tried to investigate the effect of the nature of electrolyte used and ion concentration on the amino acid release. The release of amino acids was progressive by the addition of phosphate ions. The addition of 10 mmol/l of these ions leads to a release of 21.7% for DL-leucine and 15.1% for L-lysine. The opposite trend is observed by adding calcium ions. Indeed; the phosphate ions can compete, to a certain extent, with the amino acid molecules for the same location on the hydroxyapatite surface, the release of these ionic molecules was favored in the presence of excess phosphate. A similar trend was noted for the release of phosphoserine (as important actor in biomineralization processes) from poorly crystalline apatite of Ca/P ratio lower than 1.67 (Barroug et al., 2008). Nevertheless, the presence of calcium ions in solution inhibits the release process; they will bind to sites of phosphate apatite facilitating electrostatic interactions with negatively charged groups and suggesting a re-adsorption reaction.

On the other hand, the release process is weakly influenced by the temperature. There has been a slight increase of about 3% of amino acids released when the temperature increases from 22 to 40 °C.

The amino acids previously adsorbed on calcium-deficient hydroxyapatites (CDHA) can be released by a reverse ion exchange reaction involving functional groups of these amino acids and the ionic groups at the hydroxyapatite surface, due to the presence of the hydrated surface layers of hydroxapatite. Exchange reactions highlighted between hydroxyapatite surfaces similar to the bone mineral having previously fixed the amino acids and the surrounding environments, could be analogues to those involved in the regulation of bone metabolism.

The interaction of amino acids with calcium-deficient hydroxyapatites at near-physiological pH and temperature could be very important in the clinical relevance. Indeed, amino acids are necessary for protein synthesis and have various functions in the body (Akram et al., 2011); the study of adsorption and release of the amino acids can help us to understand the properties of interaction of proteins/peptides with the same apatites.

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