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Adsorption and desorption of L-lysine onto synthesized apatitic calcium phosphates

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Abstract

This study describes the characteristics of the binding and release of the L-lysine by synthetic poorly crystalline apatitic calcium phosphate carried out in aqueous media. The adsorption proceeds rapidly at the initial stages; the maximum adsorbed amount is reached after five hours. However the rate of release of L-lysine bound to Calcium deficient apatites (CDA) is relatively slow. The saturated-adsorbed or released amount of L-lysine decreased with the increase of the content of carbonate. The carbonation rate of the synthetic apatites and the release of L-lysine can be related to the maturation processes. The influence on the release process of different parameters such as concentrations of calcium, phosphate and solution/solid ratio has been investigated. The process is notably influenced by adding calcium or phosphate to the system but, while calcium ions inhibit the release of CDA-bound L-lysine, phosphate ions increase it. The greatest release occurred when dilution ratio is increased. The adsorption experiments showed the considerable adsorption of L-lysine in the cationic form onto CDA takes place, suggesting that electrostatic interactions are dominant between the groups -COO^- of L-lysine and calcium Ca^{2+} ions of the CDA.

1. Introduction

Calcium phosphate apatites have received considerable attention for their use as biomaterials [1-2]. They exhibit several advantages, such as bone substitutes in the biomedical applications due to their biocompatibility, low density, chemical stability, high wear resistance, excellent osteoconductivity [3-4]. On the surface of these apatites, it's formed a hydrated layer contains mostly divalent ions such as Ca^{2+}, HPO_4^{2-} and CO_3^{2-} [5]. The high reactivity of biological and synthetic poorly crystalline apatite is thought to be directly linked to interactions of this structured layer with the surrounding body fluids [6].

Biomolecule/biomaterial interactions is an actual gaining-ground research field, many studies have been conducted on the interaction amino acids with calcium phosphates [7-10]. Other studies have been reported on the adsorption and release of various bioactive molecules in calcium phosphates [11-14].

Many ionic substitutions are possible in phosphate apatitic octocalcium (OCPa) of composition: $Ca_8(PO_4)_{3.5}(HPO_4)_{2.5}OH_{0.5}$. The substitution of carbonate ions (CO_3^{2-}) by hydrogenophosphate ions (HPO_4^{2-}) (type B substitution) allows a continuous variation of the Ca/P atomic ratio between 1.33 and 1.67 [15]. This leads to CDA: $Ca_{(8+0.57x)}[PO_4]_{(3.5+1.64x)}[HPO_4]_{2.5\,(1-x)}[CO_3]_{0.86x}OH_{0.5\,(1-x)}$, with $0 \leq x \leq 1$.

CDA closely resembles bone mineral in composition and structure. The difference between CDA and bone mineral lies in the impurity content. Bone mineral is poorly crystalline apatite and contains water and metallic ions [16–17]. Neglecting the metallic impurities, the average composition of bone mineral is indicated by the following chemical formula: $Ca_{8.3}(HPO_4,CO_3)_{1.7}(PO_4)_{4.3}(CO_3,OH)_{0.3}$ corresponding to CDA [18]. The carbonate group substitutes mainly for phosphate groups (type B substitution) and minorly for hydroxyl groups (type A substitution) in bone mineral. Moreover, bone mineral contains labile carbonate and phosphate and/or hydrogenphosphate groups with a non-apatitic environment [19]. These ions may be primarily located on the surface of the crystals.

The carbonate ion causes a decrease in crystallinity of CDA [20], which is considered to be related to biocompatibility. These ions also have an important role in terms of clinical potential in orthopedics and dentistry [21]. However, the adsorption properties and structure of CDA surface containing bicarbonate ions have been unknown. Proteins are intriguing adsorbates because they play variously important roles in a lot of biological function and phenomena. Furthermore, adsorption of proteins on solid materials has been one of hot and challenging topics due to application to drug delivery system [22]. The surface of CDA has the calcium and phosphor sites, the former is considered to be a selective adsorption site for carboxyl and phosphate ions, and the latter for amino groups [9].

Amino acid adsorption is not necessarily a model for protein adsorption; their behavior can be quite different. Amino acids have the C-terminal (-COOH) and the N-terminal (-NH_2), which have different chemical natures, and therefore, examining interaction properties of amino acids on carbonate-containing CDA can give us important information for modification effect of carbonate ion on the surface of CDA. The nature of the interaction between the amino acid and the CDA (e.g. a surface complex between COO^- and Ca^{2+}) surface may be different a different sites.

In this study, we report the binding and release characteristics of L-lysine by a slurry of CDA of Ca/P ratio between 1.33 and 1.67. The composition of synthetic apatites and the composition of interaction medium were considered as an experimental variable in order to understand the interaction mechanism between the L-lysine residue and CDA.

2. Materials and methods

2.1. L-lysine

L-lysine is comprised of basic amino group, an acidic carboxyl group, and characteristic side chain. Depending on the degree of protonation of these functional groups ($NH_2 \leftrightarrow NH_3^+$, $COOH \leftrightarrow COO^-$), the net charge of L-lysine changes greatly (positive, neutral or negative) as a function of pH in an aqueous solution (*Fig. 1*) [23], it is positively charged at physiological pH=7.4. The characteristics and properties of this amino acid have been reported previously in ref. [9, 24]. Lysine solutions were prepared by dissolving L-lysine powder (min. 98% TLC; Sigma-Aldrich) in deionized water.

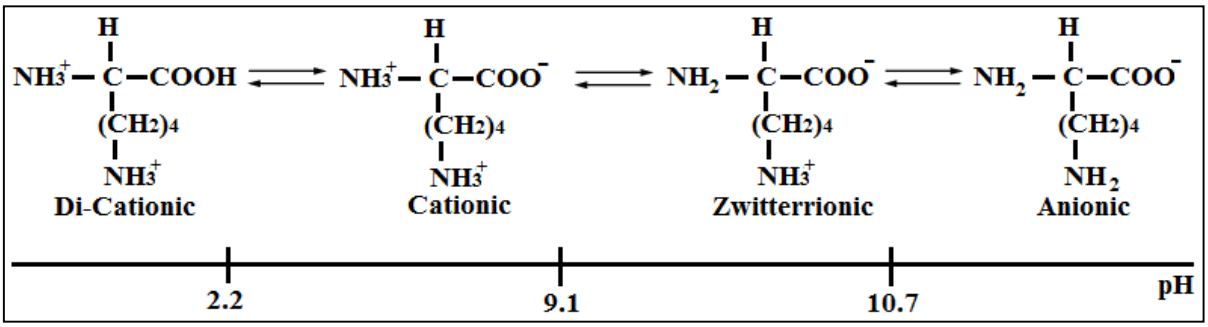

Fig. 1. L-lysine forms versus pH of the aqueous solution.

2.2. Calcium phosphate apatites

The apatite samples were synthesized by simple co-precipitation in a water–ethanol (50% –50%) at 37◦C [15]. The method is based by mixing 30 mmol of nitrate calcium Ca $(NO_3)_2{\cdot}4H_2O$ and 30 mmol of diammoniumhydrogenphosphate $(NH_4)_2HPO_4$ and ammonium carbonate $(NH_4)_2CO_3$.

The apatite samples prepared were characterized by scanning electron microscopy (SEM) (JSM- 6060LV, JEOL Ltd, Japan), X-ray powder diffraction (XRD) pattern (X'Pert PRO (Germany) -CuKα), the IR spectra of the specimens have been obtained on a 89 VERTEX 70/70 V FT-IR spectrometers (Bruker Optics) from KBr pellets (1.5 mg/300 mg). The specific surface area of the samples was determined according to the Brunauer, Emett and Teller (BET) method, using nitrogen N_2 adsorption. The calcium content was determined by complexometry with ethylene-diamine-tetraacetic acid (EDTA). UV-Vis spectroscopy of phospho-vanado-molybdic acid was used to evaluate the phosphate concentration, wavelenght used to measure the absorbance of the phosphate is λ = 460 nm and Volumetric method (ISO 10693:1995) for carbonate contents [25]. The synthetic apatites formed with Ca/P atomic ratios 1.33; 1.40; 1.48 and 1.67 have BET specific surface areas 58, 89, 95 and 108m^2/g, respectively (*table 1*).

The composition of the synthetic apatite was modified in the presence of CO_3^{2-} ions. Compared to the OCPa, we noted that the increase of $\%CO_3^{2-}$ increases Ca^{2+} ion content and decreases HPO_4^{2-} ion content (*table 1*). The Ca/P ratio increases linearly versus carbonate rate indicating the non-stoichiometry of synthetic apatites.

The chemical formulas of calcium phosphate apatite are grouped in *table 2*, they can be determined from the general formula: $Ca_{(8+0.57x)}[PO_4]_{(3.5+1.64x)}[HPO_4]_{2.5(1-x)}[CO_3]_{0.86x}OH_{0.5(1-x)}$ [15].

Table 1. Chemical composition of synthetic calcium phosphates in carbonate-containing

% (CO_3)	Molar ratio CO_3/ PO_4	Ca (mol)	P (mol)	Ca/P	Specific surface (m^2/g)
0	0/30	0.792	0.595	1.33	58
20	6/24	0.821	0.586	1.40	89
30	9/21	0.837	0.565	1.48	95
40	12/18	0.852	0.510	1.67	108

Table 2. Chemical formulas of apatitic calcium phosphates in $0 \leq x \leq 1$

Ca/P	x	Chemical formula
1.33	0	$Ca_8(PO_4)_{3.5}(HPO_4)_{2.5}OH_{0.5}$
1.40	0.225	$Ca_{8.13}(PO_4)_{3.87}(HPO_4)_{1.94}(CO_3)_{0.19}OH_{0.39}$
1.48	0.48	$Ca_{8.27}(PO_4)_{4.29}(HPO_4)_{1.3}(CO_3)_{0.41}OH_{0.26}$
1.67	1	$Ca_{8.57}(PO_4)_{5.14}(CO_3)_{0.86}$

Previous studies [9, 24] have shown in SEM images and XRD patterns that the synthetic calcium phosphate with Ca/P ratio between 1.33 and 1.67 are poor crystalline structure.

The IR spectra of synthetic apatites are dominated mainly by bands characteristic of apatitic phosphates and water molecules (*Fig.2*); the peaks at 472, 565, 603, 962 and 1038 cm^{-1} are due to the PO_4^{3-} groups. The band at 875 cm^{-1} was assigned to the HPO_4^{2-} and CO_3^{2-} common groups, which is slightly more intense for most carbonated apatite of Ca/P = 1.67 [26]. The bands at 634 and 3571 cm^{-1} belong to the stretching vibrations of hydroxyl OH groups [27], they become less intense when Ca/P increases and disappears for the most carbonated apatite of Ca/P = 1.67. The band observed at 1093 cm^{-1} is due to the phosphate stretching vibration; it is disappeared for the most carbonated apatite. The infrared peaks at 1638 and 3000–3430 cm^{-1} are due to the adsorption water [26]. Moreover, the IR spectra of apatites of Ca/P 1.40, 1.48 and 1.67 reveals the presence of adsorption peaks at 1415, 1455, and 1470 and at 1500, 1545cm^{-1} , which are consistent with carbonate type B (phosphate site) and type A (hydroxyl site) substituted [28-29]. This finding is in accordance with that reported in literature for the mineral phase of bone [18].

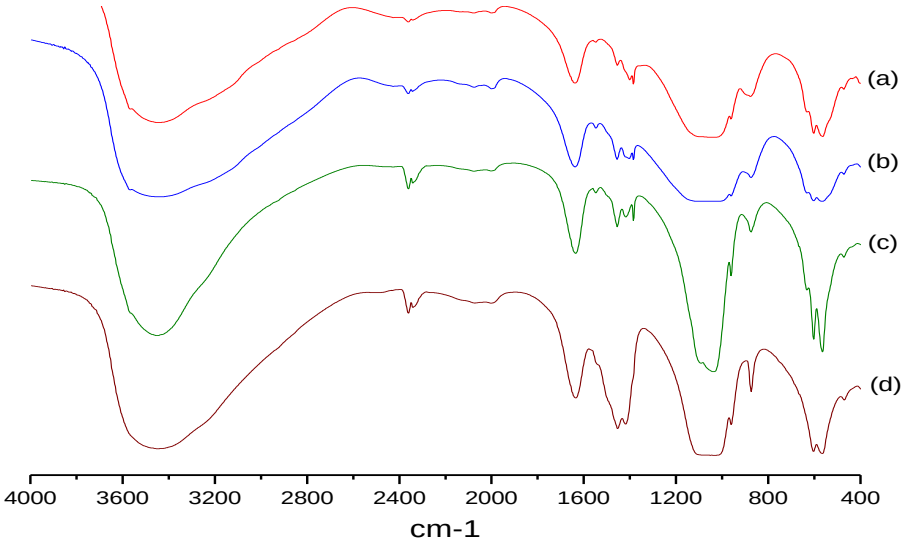

Fig. 2. IR spectra of synthetic calcium phosphate apatites of Ca/P: (a) 1.33; (b) 1.40; (c) 1.48 and (d) 1.67.

The characterization techniques indicate that the calcium phosphates prepared have a Ca/P ratio between 1.33 and 1.67; they are poorly crystalline apatites, deficient in Ca^{2+} ions in which CO_3^{2-} ions substitute by HPO_4^{2-} ions, similar to the mineral matrix of calcified tissues.

3. Results

3.1. Adsorption

3.1.1. Study of the solution

Kinetic studies of L-lysine 1 mmol/l is carried out in vitro following an experimental protocol; contact time (10–1440 min), at 37 ◦C and neutral solution pH by using the poorly crystalline CDA. The adsorption kinetics, Q_{ads} versus time, is represented in *Fig.3*. It is seen that adsorption proceeds rapidly during the first minutes, but the plateau or adsorption equilibrium is reached after approximately five hours from the start of the experiments. The chemical composition of apatites has an influence on adsorption; the high amounts adsorbed per surface unit at saturation are obtained for compounds containing the more HPO_4^{2-} ions of Ca/P=1.33. We calculated the adsorption rate (%ads) at 18h, the result obtained showed that the adsorption rate of L-lysine increases by increasing the % CO_3 in CDA; it passes from 95.82% for apatite not containing CO_3^{2-} ions (Ca/P=1.33) to 99.57% for apatite with a maximum carbonate content (Ca/P=1.67). The pH measured of all supernatants solutions after adsorption is between 5.21 and 7.02.

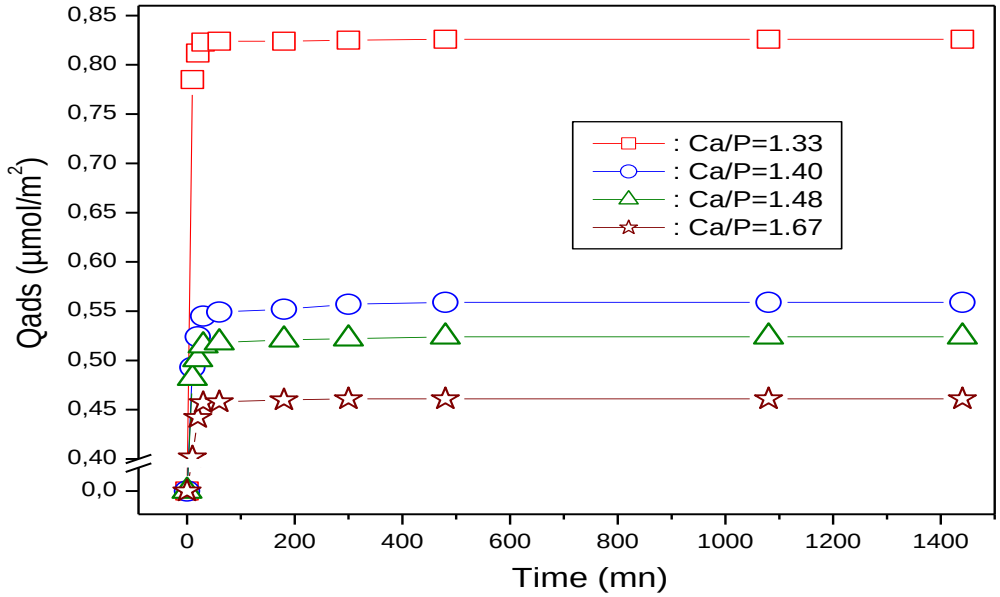

Fig. 3. Adsorption of L-lysine onto CDA of Ca/P: (a) 1.33; (b) 1.40; (c) 1.48 and (d) 1.67 versus time at 37 °C

3.1.2. Study of the solid

The infrared spectra recorded for the samples before and after adsorption are illustrated in *figures 4, 5, 6 and 7*. The incubation of apatites with L-lysine solution induced a change in the infrared spectra. After adsorption, the intensity of the band at 1638 cm^{-1} may be due to symmetric vibration of water molecules was increased slightly. This variation of intensity may be due to groups COO^- and NH_3^+ of amino acids fixed on the surface of the apatite [26].

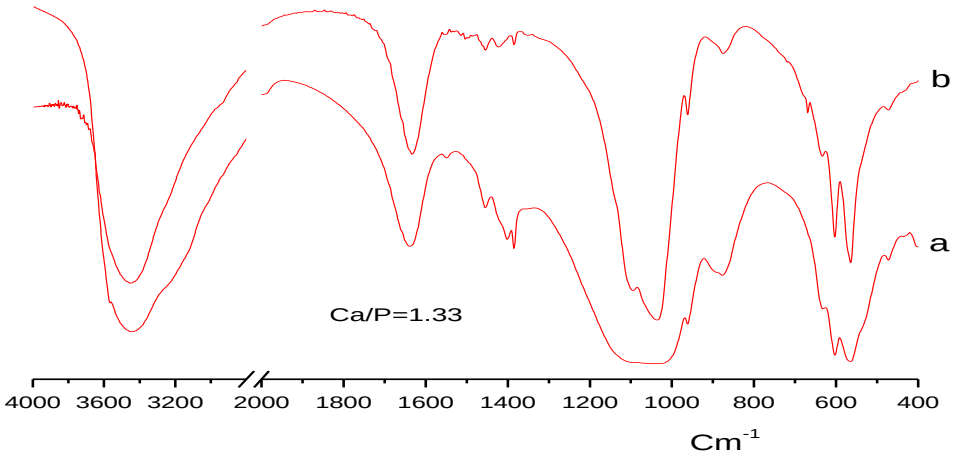

Fig. 4. FTIR spectra of apatite (OCPa) of Ca/P 1.33: (a) before, (b) after adsorption in 1 mM l-lysine at 37°C (400–4000 cm^{-1})

It appears, in the 1300–1600 cm−1 region, the bands of low intensities at 1384, 1412, 1458, and 1455 cm^{-1} are due to the carboxyl group COO^- [26, 30]. These bands unobserved for apatite with a maximum carbonate content (Ca/P=1.67). In the 718 –740 cm−1 domain, the bands of low intensities are assigned to $–CH_2–$ groups [31]. The enlargement of the band of H_2O molecules at 3450 cm−1 may be due to the stretching vibrations of NH_3^+ group of L-lysine [30]. It can also be seen from *Fig.4* that the spectrum of apatite not containing carbonate ions (Ca/P=1.33) treated with L-lysine solution generated additional bands of low intensity at 1505 and 1512 cm−1 which are related to symmetric vibrations of NH_3^+ groups [30]. However, the presence of carbonates ions in the apatites of Ca/p=1.40, 1.48 and 1.67 does not permit observe these bands (*Fig. 5, 6 and 7*).

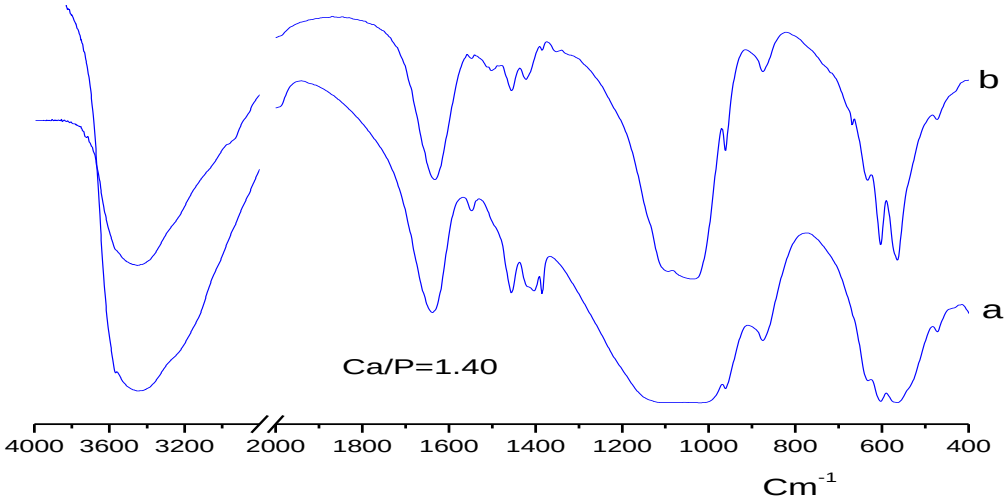

Fig. 5. FTIR spectra of apatite of Ca/P 1.40: (a) before, (b) after adsorption in 1 mM of l-lysine at 37°C (400–4000 cm^{-1}).

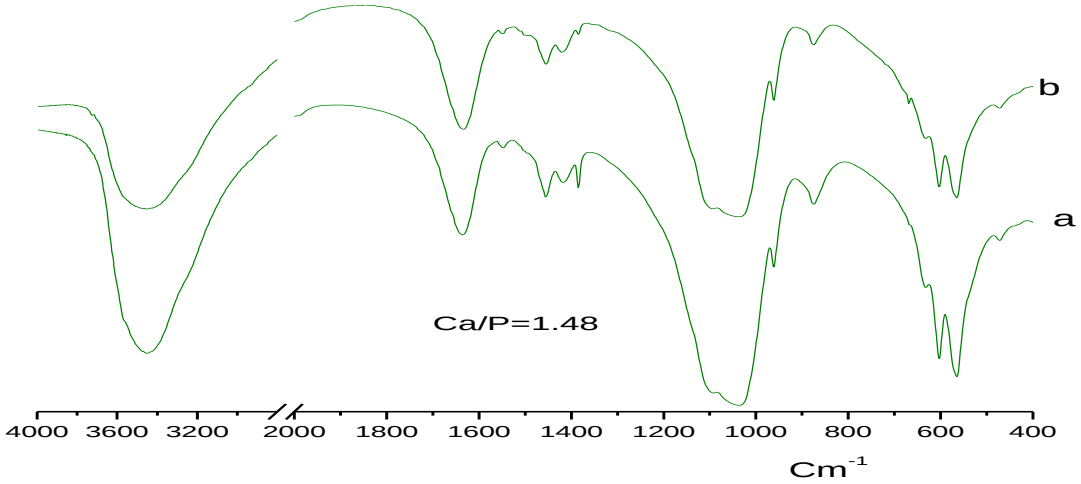

Fig. 6. FTIR spectra of apatite of Ca/P 1.48: (a) before, (b) after adsorption in 1 mM of l-lysine at 37°C (400–4000 cm^{-1}).

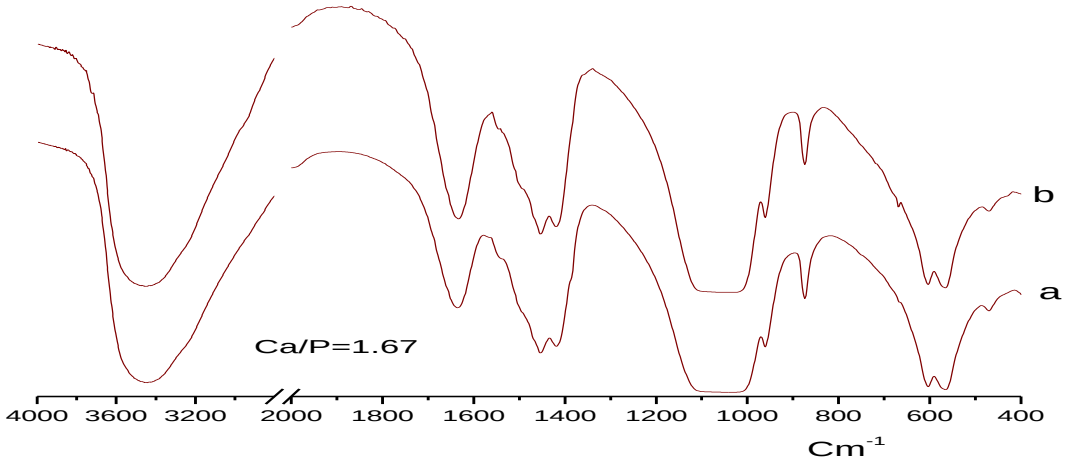

Fig. 7. FTIR spectra of apatite of Ca/P 1.67: (a) before, (b) after adsorption in 1 mM l-lysine at 37°C (400–4000 cm^{-1})

3.2. Release

3.2.1. Release kinetic

The release of CDA-bound L-lysine was studied as a function of time (0.25–10 days) in deionised water. The kinetic profiles (*Fig.8*) indicate a slow spontaneous release of L-lysine under the conditions examined (pH (initial) ~7; solution/solid ratio 5%; 37°C). The release rate is increased with time, it is more important for apatite rich in HPO_4^{2-} ions and not containing CO_3^{2-} ions. Approximately 12.5% of the L-lysine bound by the OCPa was released after 10 days of equilibration. Release of the bound L-lysine molecules was, however, reduced by increasing the CO_3^{2-} ions in calcium phosphates; L-lysine binding to a CDA which contained initially 20%, 30% and 40% of CO_3^{2-} (by molar), respectively, were shown to release about 9.86%, 9.32% and 5.1% after 10 days of equilibration. Results show that the in vitro release of CDA-bound L-lysine is dependent on the composition of the CDA-L-lysine complex.

The pH of the supernatant solutions after L-lysine release decrease until the fifth day and then varies in function of time, the values are between 4.7 and 7.4. In this interval, the lysine is released in the cationic form.

The chemical composition, determined from calcium, phosphate and carbonate analyses, of CDA-L-lysine after release of L-lysine versus time is given in *table3*. The carbonate/ phosphate (C/P) ratio increases in CDA-L-lysine which containing initially 20%, 30% and 40% of CO_3^{2-} ions during maturation, indicating the progressive of CO_3^{2-} ions. As the CO_3^{2-} ions are essentially substituted for HPO_4^{2-} ions, the Ca/P ratio does not account for the variations in the stoichiometry of CDA-L-lysine [32]. The cation/anion (Ca/P+C) ratio remains relatively constant, indicating that the number of calcium vacancies in the lattice does not decrease significantly during maturation. As the CO_3^{2-} ion content increases during maturation, the evolution of the Ca/P+C ratio suggests that the phosphate content decreases. The Ca/P report remains constant regardless of apatite composition after the release of lysine. This result suggests that interaction phenomenon between apatites and lysine can reduce or block the evolution of these apatites.

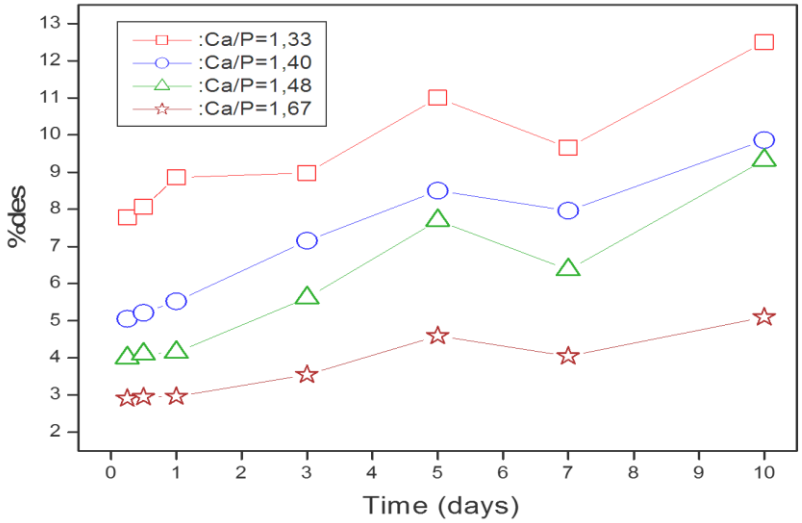

Fig. 8. Release profile of L-lysine from apatitic calcium-phosphates versus time at 37°C

Table 3. Chemical composition (atomic ratios) of CDA-L-lysine after release of L-lysine

Time	Ca/P=1,33			Ca/P=1,40			Ca/P=1,48			Ca/P=1,67		
(days)	Ca/P	Ca/P+C	C/P	Ca/P	Ca/P+C	C/P	Ca/P	Ca/P+C	C/P	Ca/P	Ca/P+C	C/P
0	1,40	1,40	0	1,43	1.35	0.059	1,54	1.37	0.124	1,68	1.42	0.183
1	1,36	1,36	0	1,43	1.34	0.075	1,54	1.36	0.132	1,66	1.40	0.186
3	1,38	1,38	0	1,44	1.34	0.075	1,55	1.34	0.157	1,67	1.39	0.200
5	1,38	1,38	0	1,45	1.33	0.090	1,55	1.34	0.157	1,66	1.40	0.187
7	1,40	1,40	0	1,45	1.33	0.090	1,56	1.34	0.164	1,67	1.38	0.210
10	1,40	1,40	0	1,45	1.32	0.098	1,56	1.34	0.164	1,67	1.38	0.210

Ca, calcium; C, carbonate; P, phosphate

3.2.2. Influence of hydrogen phosphate and calcium ions

The release of CDA-bound L-lysine as a function of $CaCl_2$ and Na_2HPO_4 concentration was carried out on samples of CDA–L-lysine complexes formed by equilibration of CDA and L-lysine with an initial concentration of L-lysine of 1mM in 5% solution/solid ratio at 37°C as previously described. The L-lysine–CDA complexes formed

were re-suspended, dispersed and equilibrated with electrolytes solutions of molar concentrations varying from 0 to 15 mM. In each case the equilibration time was 5 days after which the supernatants and re-suspended were separated by filtration. The L-lysine concentrations in all of the supernatants collected were measured after release.

As can be seen from *Fig.9*, the release of L-lysine bound to CDA is highly dependent on the presence of both hydrogen phosphate (*Fig. 9a*) and calcium (*Fig. 9b*) ions in the equilibrating solution, even at low concentrations. This observation clearly demonstrates that Ca^{2+} ions decrease the release of L-lysine by the CDA (*Fig.9b*). However the presence of HPO_4^{2-} ions in the equilibrating markedly increases the release of L-lysine (*Fig.9a*); 25.1% of L-lysine bound to OCPa is released in presence of 15mM of hydrogen phosphate.

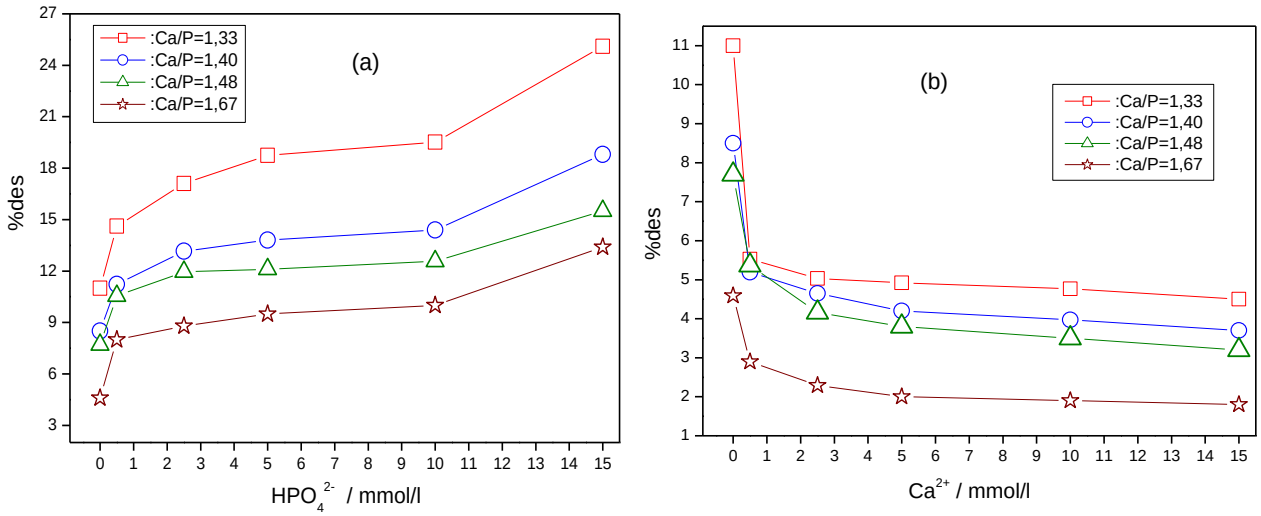

Fig. 9. Effect of HPO_4^{2-} (a) and Ca^{2+} (b) ions concentration on the L-lysine release from CDA.

3.2.3. Influence of Ra (ml/mg) ratio: volume / mass

The release of CDA-bound L-lysine as a function of dilution rate was carried out at 37°C, with the same batch of experiments as previously described. The range of solution/ solid ratio studied was 0.05 to 0.5 ml/mg. The release profiles of CDA are similar (*Fig.10*). The release rate of L-lysine molecules previously bound to CDA increases with increasing Ra (ml/mg) ratio, e.g. it increases from 11% (Ra=0.05 ml/mg) to 36.3% (Ra=0.5 ml/mg) for OCPa of Ca/P= 1.33 and it increases from 4.59% to 21.6% for apatite more carbonated of Ca/P=1.67.

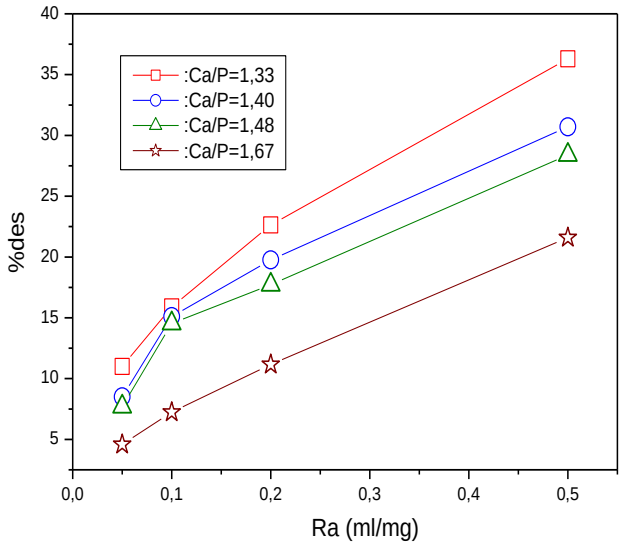

Fig. 10. Effect of ratio Ra (ml/mg) on the L-lysine release from CDA.

The chemical composition, determined from calcium and phosphate analyses, of supernatants and CDA-L-lysine after release of L-lysine as a function of Ra (ml/mg) ratio is given in *table 4 and 5*. These analyses indicated that, with the exception of apatite of Ca/P=1.67, the phosphate ions of CDA-L-lysine have a more pronounced release than the calcium ions (*table 4*). The release of phosphate ions increases with decreasing carbonate-containing solid CDA-L-lysine; it is more important for apatite not containing CO_3^{2-}ions (Ca/P=1.33). The Ca/P ratio

increases in supernatant with increasing dilution rate Ra (ml/mg). The Ca/P ratio of CDA-L-lysine after L-lysine release increases slightly and remains relatively constant beyond 0.2 ml/mg, indicating that the number of calcium vacancies in the CDA-L-lysine does not decrease significantly during dilution *(table 5*).

Table 4. Chemical composition of supernatants after release of L-lysine versus ratio Ra (ml/mg) at 5 days and 37°C

	Ca/P=1,33			Ca/P=1,40			Ca/P=1,48			Ca/P=1,67		
Ra (ml/mg)	Ca^{2+} mM	P mM	Ca/ P	Ca^{2+} mM	P mM	Ca/ P	Ca^{2+} mM	P mM	Ca/ P	Ca^{2+} mM	P mM	Ca/ P
0.05	0,92	2,90	0.32	0,57	1,55	0,37	0,77	0,90	0,80	0,83	-	-
0,10	0,85	1,81	0.47	0,55	1,14	0,48	0,70	0,80	0,87	0,76	-	-
0,20	0,72	1,20	0.60	0,50	0,88	0,57	0,60	0,67	0,90	0,68	-	-
0,50	0,6	0,87	0.69	0,44	0,70	0,63	0,54	0,56	0,96	0,57	-	-

Table5. Ca/P atomic ratios of CDA-L-lysine after release of L-lysine at 5 days and 37°C

Ra (ml/mg)	Ca/P=1,33	Ca/P=1,40	Ca/P=1,48	Ca/P=1,67
0.05	1,38	1,45	1,55	1,66
0,1	1,39	1,45	1,59	1,69
0,2	1,40	1,46	1,60	1,70
0.5	1,40	1,46	1,60	1,70

4. Discussion and conclusion

The solids prepared exhibit XRD patterns, SEM and FTIR spectra characteristic of poorly crystalline apatitic calcium phosphates corresponding to different stages of bone mineral evolution (maturation) with age. Chemical analyses showed that all the specimens Ca/P ratios were between 1.33 and 1.67. The interaction properties of L-lysine with the CDA samples of various carbonate contents were conditioned with maturation time, solution/solid ratio and changes in the content of mineral ions in solution.

The rapid adsorption kinetics shows the high reactivity of CDA surface with basic amino acid L-lysine; the adsorbed amount decreased with increase of the carbonate content, it is important for apatite not containing carbonate ions (OCPa). These ions which were substituted of the HPO_4^{2-} and OH^- groups (B and A-site) on the CDA surface cause a retardation of crystallographic growth of apatite. This result may suggest that the carbonate in biological hard tissues under physiological condition has a similar effect on adsorption of basic proteins or other biological substances.

The release kinetic of L-lysine bound to CDA is sufficiently slow, the chemical composition of CDA has an influence on the release; the large amount released is obtained for apatite containing more HPO_4^{2-} ion and less CO_3^{2-} ion. The release rate is increased with maturation time; it is not exceeded 11% for OCPa at 5days. The release process is favoured by dilution of the equilibrating solution, e.g. the release rate exceeded 36.3% for OCPa at 50% solution/solid rate but it is remains low for carbonated apatites. The greater release of CDA-bound L-lysine can be explained with release of $HPO_4^{2-}$and/or CO_3^{2-} ions from apatitic surfaces [17]. These observations suggest that these species compete with the L-lysine molecules for the same location on the apatite surfaces allowing a favorable L-lysine release.

Since the ionic species in solution such as Ca^{2+} and HPO_4^{2-}/PO_4^{3-} influence these surface equilibrium as well as the surface complexation, the net surface charge of the CDA will depend basically on the surface structure, pH and the concentration of the mentioned ions in solution. Phosphate anions in solution compete with carboxylate group of the L-lysine for adsorption on Ca sites. When phosphate is added, it adsorbs on Ca sites allowing a favorable L-lysine release through the carboxylate groups. On the other hand, when Ca^{2+} ions are added in solution, they adsorb on phosphate sites of the CDA surface causing an inhibited of L-lysine release.

The predominance kind of L-lysine–CDA interactions will depend on the L-lysine structure and the composition of the CDA. For these apatites, it seems reasonable that hydrophobic and covalent interactions can be discarded and that electrostatic interactions [7, 9] will take place. The L-lysine presents basic residue, whose ionization depends on the solution pH. The fact that the working pH values are lower to the isoelectric point pHi 9.74 of the L-lysine leads to net positive charge in the L-lysine. As reported in recent papers [33,34], the fixation of L-lysine (with a $(CH_2)_4NH_2$ side-chain) on the hydroxyapatite 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. In this case, spectroscopic studies on the adsorption of L-lysine on CDA surface suggested that L-lysine forms a rather weakly bound complex via coordination of the NH_3^+ and -COO^- groups. The presence of bands characteristic of -COO^- and the absence of bands attributed to -COOH (1700 cm^{-1}) indicates the cationic form of L-lysine. The adsorption is mainly due to the electrostatic interactions between the groups -COO^- of L-lysine and calcium Ca^{2+} ions of the CDA. L-Lysine is much more sensitive to lateral interactions in the presence of N atoms in the side chain.

Prospect

Results of the present paper are aimed to investigate the interaction of L-lysine which has basic side chain with apatitic calcium phosphates containing carbonate ions similar to the mineral matrix of calcified tissues 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 [35]; the study of interaction of the polar amino acid residues can help us to understand the properties of interaction of proteins that have polar side chains with the biological apatites. The release behavior of amino acids from the carbonate-containing apatites is necessary for practical views. The interaction process could give insights on how to prepare protein-apatites composites able to release the protein fraction at the implant site. Adsorption may be used to load the apatite crystals with selected active molecules in optimised and controlled ways. Moreover, the release process in vivo may occur by displacement of the agent by mineral ions or proteins from the body fluid, or alteration of the apatite.

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