# Exchange Reactions at Calcium Phosphates Surface and Applications to Biomaterials

Allal Barroug[1,a], Ahmed Legrouri[2,b] and Christian Rey[3,c]

[1]Laboratoire de Physico-Chimie des Matériaux et Environnement, PCMN-PI, Département de Chimie, Faculté des Sciences Semlalia, Université Cadi Ayyad, BP 2390, 40001 Marrakech, Maroc

[2]School of Science & Engineering, Al Akhawayn University, PO Box 1871, 53000 Ifrane, Maroc

[3]Laboratoire des Matériaux, Physico-Chimie des Phosphates, ENSIACET-INPT, CIRIMAT UMR CNRS 5085, 118 route de Narbonne, 31077 Toulouse, France

[a]a.barroug@ucam.ac.ma, [b]legrouri@aui.ma, [c]Christian.Rey@ensiacet.fr

**Keywords:** Adsorption, Biomaterials, Calcium phosphates, Exchange reactions, Release.

**Abstract**. The interaction of calcium phosphates with biological molecules under controlled conditions permits the formulation of meaningful conclusions concerning the driving forces. The uptake and release at the material-solution interface is 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. The understanding of adsorption and desorption mechanisms with respect to active molecules can be exploited for the development of drug delivery applications.

## Introduction

Calcium phosphate apatites have received a recurrent attention for the last twenty years mainly for their use as biomaterials related to compositional similarity with bone mineral and biological properties. These materials have found various applications especially as bone substitutes and have been used as drug delivery systems [1-4]. The binding and release of active molecules and ions at the interface between calcium phosphate minerals and biological environments are crucial to determine how they perform in vivo. We report here on the study of calcium phosphates interaction with phosphoserine (P-ser) and bovine serum albumin (BSA), two important actors in the biomineralization processes [5]. The composition of the adsorption medium was considered as an experimental variable in order to establish a quantitative relationship between the sorbent uptake – release and the surface characteristics of apatite crystals.

## Materials and methods

Several types of apatites were used: a well crystallized hydroxyapatite (HA) and biomimetic apatites corresponding to different stages of bone mineral evolution (maturation) with age. They were prepared by double decomposition according to methods previously described [3]. Different maturation stages were obtained by ageing of the precipitates at room temperature in mother solutions. The samples were removed by filtration, washed with de-ionized water and lyophilized overnight. The powders were characterized by X-ray diffraction, Fourier transform infrared spectroscopy, and chemical analyses. The surface area of the crystals was measured by nitrogen adsorption according to the BET method.

Adsorption experiments were performed using P-Ser and BSA in the presence of different electrolytes (de-ionized water, potassium nitrate and potassium nitrate containing calcium or phosphate). The solid was dispersed in the adsorption medium at room temperature. After an incubation time, the suspension was centrifuged and the supernatant obtained was assayed for BSA and P-Ser [6,7].

Desorption was examined considering changes in the bulk solution concentration. The supernatants obtained after centrifugation were diluted with a solution with the same electrolyte content as in adsorption tests but without adsorbate or replaced by the same volume of a solution

containing phosphate or carbonate. The pellets were re-dispersed and centrifuged again and the supernatants were thereafter examined for the presence of desorbed molecules.

**Results**

The solids prepared exhibit XRD patterns and FTIR spectra characteristic of poorly-crystalline (PCA) and well-crystalline (HA) apatitic calcium phosphates. Chemical analyses showed that all the specimens were calcium deficient (Ca/P ratio lower than 1.67). FTIR spectra Deconvolution of the PCA samples showed the presence of phosphates groups assigned to non-apatitic environments [8]. Maturation of the crystals was observed to cause a moderate improvement of the crystallinity index, a gradual decrease of the content of non-apatitic phosphates and cation vacancies in the lattice.

Despite the different characteristics exhibited by the adsorbates and the various solution compositions examined, the adsorption isotherms obtained were Langmuirian in shape (Fig. 1). The adsorption parameters, maximum amount loaded and affinity constant, are conditioned by the content of potential determining ions in solution (hydrogen, phosphate and calcium) and the surface composition of the nanocrystals.

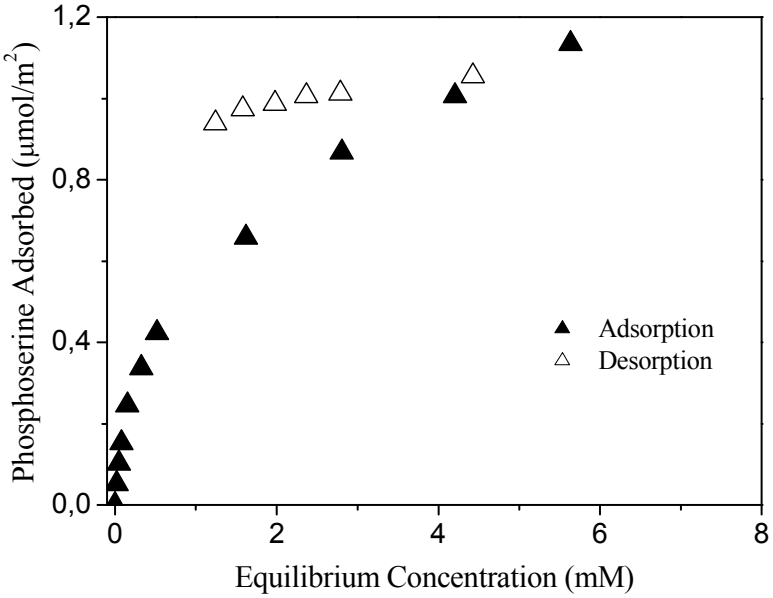


Fig. 1: Adsorption and desorption isotherms of phosphoserine on calcium phosphate

The influence of the solution composition on the adsorption of anionic molecules (amino acids, acidic proteins) on apatitic calcium phosphates shows a common behaviour. The isotherms obtained showed a distinct initial increase related to the affinity and adsorption plateau generally considered to represent the saturation of the adsorption sites. The maximum amount adsorbed decreases as the solution pH rises. Besides, the uptake in the range of concentration investigated was inhibited in presence of excess phosphate or carbonate, while a greater adsorption capacity occurred when calcium ions were added to the medium. However, an opposite tendency was observed with basic molecules such as lysozyme [9].

The P-Ser and BSA molecules bound to apatite crystals were not removable by dilution of the equilibrating solution. This phenomenon is well illustrated in Fig. 1 which revealed a difference between the adsorption and desorption branches for P-Ser. Similar trend was noted for the interaction of hydroxyapatite with acidic proteins such as catalase and succinylated lyzozyme [10,11]. Desorption can occur by changing the different solution parameters (pH, ionic strength, concentration of potential determining ions, etc). Indeed, the removal of P-Ser and BSA molecules was promoted by other surface active species such as phosphate and carbonate. Fig. 2 indicates that the extent of phosphoserine released markedly increased with increasing phosphate and carbonate

concentrations in solution. These observations suggest that these species compete, to a certain extent, with the adsorbate molecules for the same location on the mineral surface.

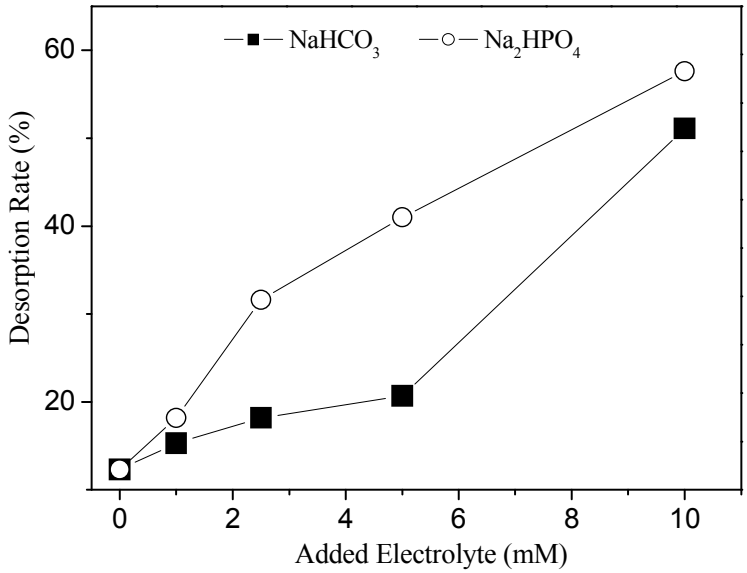


Fig. 2: Phosphoserine released versus phosphate and carbonate content

The hypothesis of an adsorption reaction involving ion replacement is further supported by the adsorption tests of P-Ser and BSA carried out on poorly crystalline samples at different stages of maturation. These samples exhibit on the crystals surface a hydrated layer with mobile bivalent ions (mainly $Ca^{2+}$ and $HPO_4^{2-}$) in non-apatitic environments. During maturation, the hydrated layer is progressively thinning as the more stable apatite domain increases. The freshly precipitated crystals, which are rich in non-apatitic environments, offer more ion exchange capabilities than the matured samples and the maximum amount adsorbed is larger than in more mature samples. This ion-exchange driven adsorption process would mainly be determined by the mobility of the ionic species at the mineral surface. Similar behaviour was obtained with heparin [12]. An example of the relationship between the surface content of non-apatitic, labile phosphate groups and the maximum amount adsorbed is presented in Fig. 3.

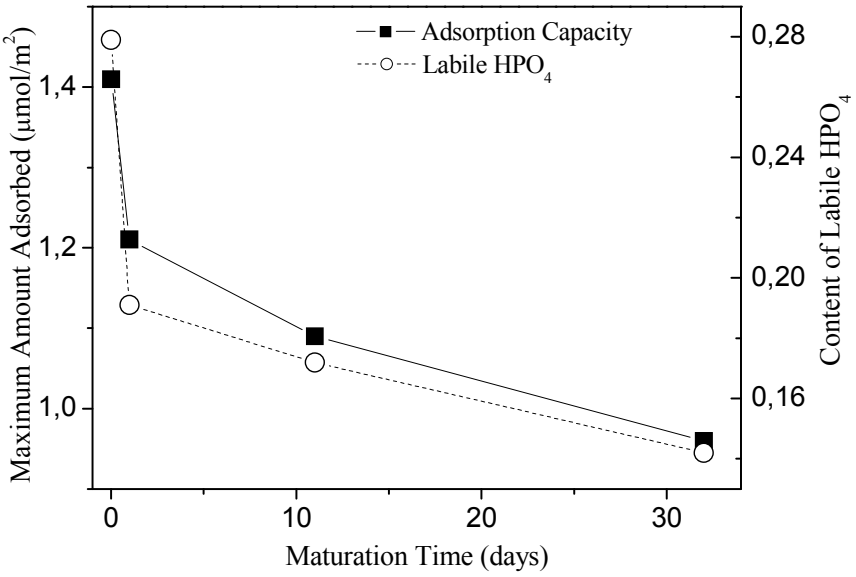


Fig. 3: Phosphoserine adsorbed versus content of non-apatitic, labile phosphate groups

## Discussion

The results reported in this study indicate that uptake and release of P-Ser and BSA molecules are conditioned by the changes in the content of different mineral ions in solution. The adsorption of these anionic molecules was inhibited in presence of excess phosphate or carbonate and favoured when calcium ions were added to the medium. Furthermore, the process was not reversible with respect to dilution. The adsorbed molecules can only be displaced by a reverse ion-exchange reaction. The main driving forces at the mineral interface seem to be an ion-exchange process involving the functional groups of the molecules and the ionic groups at the apatite surface. This is in line with the results reported in our previous studies [10,11], which revealed that interaction of proteins and apatite crystals, under conditions where they bear the same or opposite charges as evidenced by electrophoretic measurements, involves an exchange (incorporation or expulsion) with compensating low molecular weight ions. The correlation between the change of P-Ser adsorption behavior and the modification of surface composition of the nanocrystals upon maturation supports the hypothesis of an adsorption process driven by exchange reactions.

The present study stresses the fact that adsorption properties of calcium phosphates are essentially dependent on their surface characteristics, especially the development of the hydrated layer, and on the mineral ion composition of the adsorbate solution. Adsorption properties of apatites can strongly vary and data obtained on well crystallized samples cannot be compared to those obtained on nanocrystalline samples. The flexibility of surface and biological properties of nanocrystalline apatites suggests that these could be used as potential substrates for drug delivery. 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.

## Conclusion

The adsorption and release properties of apatite nanocrystals appear strongly dependent on the composition of the hydrated surface layer and the surrounding environments. The understanding of the interaction mechanisms and the control of the driving forces may provide fundamental tools for the development and application of delivery systems based on the adsorption of pharmaceuticals onto calcium phosphates.

## References


[1] S.J. Kalita, A. Bhardwaj and H.A. Bhatt: Mater. Sci. Eng. Vol. C27 (2007), p. 449.

[2] M.P.Ginebra, T. Traykova and J.A. Planell: J. Control. Release Vol. 113 (2006), p. 102.

[3] A. Barroug, L.T. Kuhn, L.C. Gerstenfeld and M.J. Glimcher: J. Orthop. Res. Vol. 22 (2004), p. 703.

[4] A.K. Jain and R. Panchagnula: Int. J. Pharm. Vol. 206 (2000), p. 1.

[5] M.J. Glimcher : Anat. Rec. Vol. 224 (1989), p. 139.

[6] L. Benaziz, A. Barroug, A. Legrouri, A. Lebugle and C. Rey: J. Colloid Interface Sci. Vol. 238 (2001), p. 48.

[7] S. Ouizat, A. Barroug, A. Legrouri and C. Rey: Mater. Res. Bull. Vol 34 (1999), p. 2279.

[8] C. Rey, M. Shimizu, B. Collins and M.J. Glimcher: Calcif. Tissue Int. Vol 36 (1984), p. 48.

[9] A. Barroug, J. Lemaitre and P.G. Rouxhet: Colloids Surf. Vol. 37 (1989), p. 339.

[10] A. Barroug, J. Fastrez, J. Lemaitre and P.G. Rouxhet: J. Colloid Interface Sci. Vol. 189 (1997), p. 37.

[11] A. Barroug, E. Lernoux, J. Lemaitre and P.G. Rouxhet: J. Colloid Interface Sci. Vol. 208 (1998), p. 147.

[12] A. Menbaoui, A. Barroug, A. Lebugle, C. Rey and A. Legrouri: *in preparation*

**Bioceramics 20**
doi:10.4028/www.scientific.net/KEM.361-363

**Exchange Reactions at Calcium Phosphates Surface and Applications to Biomaterials**
doi:10.4028/www.scientific.net/KEM.361-363.79