



HAL
open science

Adsorption on apatitic calcium phosphates for drug delivery: interaction with bisphosphonate molecules

Patricia Pascaud, Farid Errassifi, Fabien Brouillet, Stéphanie Sarda, Allal Barroug, Ahmed Legrouri, Christian Rey

► To cite this version:

Patricia Pascaud, Farid Errassifi, Fabien Brouillet, Stéphanie Sarda, Allal Barroug, et al.. Adsorption on apatitic calcium phosphates for drug delivery: interaction with bisphosphonate molecules. *Journal of Materials Science: Materials in Medicine*, 2014, vol. 25 (n° 10), pp. 2373-2381. 10.1007/s10856-014-5218-0 . hal-01250381

HAL Id: hal-01250381

<https://hal.science/hal-01250381v1>

Submitted on 4 Jan 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Open Archive TOULOUSE Archive Ouverte (OATAO)

OATAO is an open access repository that collects the work of Toulouse researchers and makes it freely available over the web where possible.

This is an author-deposited version published in : <http://oatao.univ-toulouse.fr/>
Eprints ID : 12074

To link to this article : DOI:10.1007/s10856-014-5218-0

URL : <http://dx.doi.org/10.1007/s10856-014-5218-0>

To cite this version :

Pascaud, Patricia and Errassifi, Farid and Brouillet, Fabien and Sarda, Stéphanie and Barroug, Allal and Legrouri, Ahmed and Rey, Christian *Adsorption on apatitic calcium phosphates for drug delivery: interaction with bisphosphonate molecules*. (2014) *Journal of Materials Science: Materials in Medicine*, vol. 25 (n° 10). pp. 2373-2381. ISSN 0957-4530

Any correspondence concerning this service should be sent to the repository administrator: staff-oatao@listes-diff.inp-toulouse.fr

Adsorption on apatitic calcium phosphates for drug delivery: interaction with bisphosphonate molecules

P. Pascaud · F. Errassifi · F. Brouillet ·
S. Sarda · A. Barroug · A. Legrouri ·
C. Rey

Abstract Bisphosphonates (BPs) are well established as an important class of drugs for the treatment and prevention of several bone disorders including osteoporosis. This work investigated the interaction of two bisphosphonates, risedronate and tiludronate, with several apatitic supports, a well-crystallised hydroxyapatite (HA) and nanocrystalline apatites with varying maturation times, chemical composition and surface characteristics. The purpose was to fully understand the adsorption mechanism and desorption process, by the evaluation of the effect of several physicochemical parameters (temperature, pH and concentration of calcium and phosphate ions). Whatever the nature of the BP and the structure and composition of the apatite, the adsorption of such anti-resorptive agents can be well described as an ion exchange-reaction between phosphates species on the apatitic surface and BP molecules in

solution. However, the parameters of adsorption can vary depending on the physicochemical conditions of the adsorption reaction. In addition, the structure and composition of the apatitic surface also influence the adsorption properties. Finally, BPs molecules are slowly released from apatitic supports, because most of the adsorbed molecules are irreversibly bound and not spontaneously released by dilution or simple washing. Moreover, similar to their adsorption, the release of bisphosphonates is strongly affected not only by the chemical properties of the molecule, but also by the chemical and structural characteristics of the apatitic substrates. The understanding of the adsorption and release processes provides fundamental tools for the development of drug delivery systems using apatite materials.

P. Pascaud · F. Errassifi · S. Sarda (✉)
CIRIMAT INPT-CNRS-UPS, Université de Toulouse,
Université Paul Sabatier, 31062 Toulouse, France
e-mail: stephanie.sarda@iut-tlse3.fr

F. Brouillet
CIRIMAT INPT-CNRS-UPS, Université de Toulouse, Faculté
des Sciences Pharmaceutiques, 31062 Toulouse, France

A. Barroug
Département de Chimie, LPCME-CNRST-URAC 20, Faculté
des Sciences Semlalia-Université Cadi Ayyad, Bd Prince My
Abdellah, BP 2390, 40001 Marrakech Islands, Morocco

A. Legrouri
School of Science & Engineering, Al Akhawayn University,
BP 104, 53000 Ifrane, Morocco

C. Rey
CIRIMAT INPT-CNRS-UPS, ENSIACET, Université Toulouse,
31030 Toulouse, France

1 Introduction

Bisphosphonates (BPs) are well established as an important class of drugs for the treatment and prevention of several bone disorders including osteoporosis. BPs are stable synthetic analogues of inorganic pyrophosphate characterised by a non-hydrolysable P-C-P structure with two phosphonate groups attached to the same carbon [1, 2]. Their mode of action comes from both physicochemical effects and biological activity: like pyrophosphate, BPs present a high affinity to adsorb onto bone mineral, and thereby prevent mineral dissolution and bone resorption, inhibiting osteoclasts activity. BPs have two additional substituents in their molecule attached to the central carbon, which may significantly affect both the mineral adsorption affinity and the cell activity: the ability of BPs to bind to bone mineral is increased by a functional group able to co-ordinate to calcium such as a hydroxyl group

(OH) [1, 2], and those containing a nitrogen atom within a heterocyclic ring (such as risedronate and zoledronate) have the most potent antiresorptive effect [3].

The classic BPs treatment is the systemic way by oral administration or intravenous injection. However, systemic use of BPs can result in undesirable side-effects like fever [4], ulcers [5, 6] or osteonecrosis of the jaw especially with intravenous injection [7]. Moreover, low bioavailability is commonly associated with oral administration [8]. In order to avoid these adverse effects and increase BPs bioavailability, the development of strategies for local administration of such antiosteoporotic drugs at bone sites exhibiting a risk of fracture becomes even more interesting. Taking into account the affinity of BPs for bone, calcium phosphate-based biomaterials appear to be appropriate carriers for such molecules. Few studies have focused on the development of bioactive implants based on calcium phosphates for BPs local release, such as ceramics [9, 10] or cements [11]. An interesting approach is based on the adsorption of such drugs on apatitic supports such as stoichiometric hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) [12]. Previous studies show that the presence of BPs species in solution participates in an ionic exchange process with active mineral ions on the apatite surface in which the apatitic crystals/BP association can be chemically controlled [13]. However, very few studies have investigated the release of BPs from nanocrystalline apatites (NCA) [14]. Compared to well crystallised hydroxyapatite (HA), NCA samples present a structured hydrated layer at the surface, which is responsible for a high superficial ionic mobility and represents an “important ion reservoir” allowing ionic exchange with the solution. This layer is related to the precipitation process at physiological pH and its structure as well its extension depend on the ageing time: the hydrated layer disappears when the ageing time (also referred to as maturation time) increases [15].

On the basis of these previous observations, this work investigated the interaction of two bisphosphonates, risedronate and tiludronate (Fig. 1), with several apatitic supports, a well-crystallised hydroxyapatite (HA) and biomimetic nanocrystalline apatites (NCA) with varying maturation times, leading to hydrated surface layers with different thicknesses and variable chemical compositions and Ca/(P+C) ratio, related to calcium deficiency as explained in earlier studies [16]. The purpose was to fully understand the adsorption mechanism and desorption process, by the evaluation of the effect of several physicochemical parameters (temperature, pH and concentration of calcium and phosphate ions). The data presented were obtained at different periods with BP samples obtained from different pharmaceutical laboratories, and some experiments are specific to one kind of BP due to the limited amount of drug we could obtain. Therefore, the

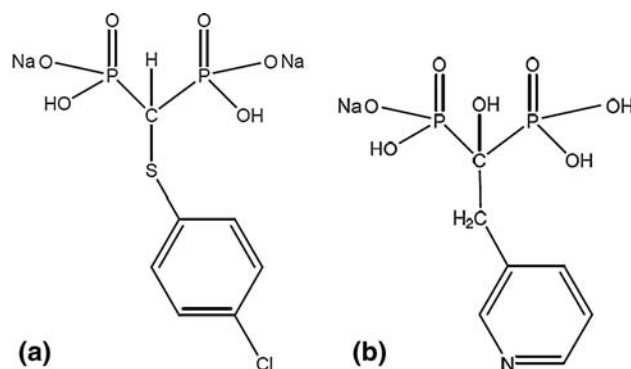


Fig. 1 Chemical structure of **a** tiludronate disodium salt and **b** risedronate monosodium salt

release kinetics were performed on tiludronate only. The understanding of the adsorption and release processes provides fundamental tools for the development of drug delivery systems using apatite materials.

2 Materials and methods

2.1 Materials

Well crystallised hydroxyapatite (HA) was synthesised by double decomposition between a phosphate salt solution ($(\text{NH}_4)_2\text{HPO}_4$ (433.3 mL, 1.54 M in deionised water) and a calcium salt solution $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (1,100 mL, 1 M in deionised water). The phosphate solution was added dropwise for about 3 h into the calcium solution maintained at 90 °C under continuous stirring [17]. The pH of the suspension was around 10. After filtration, the precipitate was washed and lyophilised. Then the powder was sieved (<125 μm) and stored in a freezer.

Nanocrystalline apatites (NCA) were synthesised by a double decomposition process at physiological pH, by pouring rapidly at ambient temperature a cationic solution containing calcium nitrate salt ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $C = 0.29$ M, 750 mL) into an anionic solution containing ammonium phosphate salt with an excess of phosphate ions for buffering the solution and sodium bicarbonate salt ($(\text{NH}_4)_2\text{HPO}_4$, $C = 0.60$ M, NaHCO_3 , C from 0 to 0.71 M, 1,500 mL) [18]. The suspension was left to mature at room temperature without stirring. Several NCAs were synthesised by varying the maturation times (from 0 to 30 days) and carbonate content during the synthesis. The precipitates obtained were quickly vacuum filtered, washed with deionised water and freeze-dried. The powders were then sieved (<125 μm) and stored in a freezer.

The calcium content of the synthesised solids was determined by complexometry with EDTA and the

phosphate content was obtained by UV spectrophotometry of phosphovanado molybdic acid complex [19]. The relative error on the level of calcium and phosphate was 0.5 %. The determination of the atomic Ca/P ratio of the precipitate had a relative error of 1 %. The level of carbonate ions was measured by a coulometry technique (UIC, Inc. CM 5014 coulometer with CM 5130 acidification unit). The specific surface area of the crystals was measured by nitrogen adsorption according to the BET method using a quantachrome instruments monosorb NOVA 1000. Powdered tiludronate (di-sodium((4-chlorophenyl)thio)methylene-bis-phosphonate) hemihydrate and risedronate (monosodium (1-hydroxy-2-(3-pyridinyl)ethylidene-bis-phosphonate) hemipentahydrate samples were a gift from Sanofi Aventis and Procter & Gamble, respectively (Fig. 1).

2.2 Adsorption experiments

The apatite sample (50 mg of HA or NCA) was dispersed in 5 mL of the adsorption medium, an aqueous solution of bisphosphonate (risedronate or tiludronate in 1 mM potassium chloride aqueous solution), in a polyethylene tube by sonication for few minutes. The suspensions obtained were incubated for 2 h without stirring and centrifuged for 20 min at 5,000 rpm. The supernatants were obtained by filtration through Millipore filters (pore size 0.2 μm) and analysed. Blanks containing only the adsorption solution incubated without solid were used as controls.

The adsorption experiments were performed in 1 mM potassium chloride aqueous solution. The tests were also carried out under different conditions to determine the effect of temperature (physiological temperature (37 ± 1 °C) and/or room temperature (25 ± 1 °C)), pH (by addition of HCl or KOH solution in the adsorption solution) and solution composition (by addition of calcium chloride (0–0.2 mM) or potassium phosphate (0–10 mM)).

2.3 Adsorption reversibility assays

Adsorption reactions, such as those involved in obtaining Langmuir adsorption isotherms, are supposed to be reversible, and changes in the equilibrium concentrations in solution (far enough from concentrations corresponding to the surface saturation (or plateau) of the isotherms) result in changes in adsorbed amounts. Desorption was examined at 25 and 37 °C with respect to dilution, and adsorption experiments were performed as described above using HA sample and risedronate (50 mg of solid dispersed in 1 mM potassium chloride solution containing risedronate). The suspensions obtained after adsorption were centrifuged, and portions of the supernatants were removed and replaced by the same volume of a solution (1 mM

potassium chloride solution without risedronate) previously equilibrated with HA powder, in order to prevent any dissolution of the substrate. The sediments were re-dispersed and incubated for 2 h to reach new adsorption equilibrium; the suspensions obtained were again centrifuged and the risedronate concentration determined in the supernatant solution.

The specific role of phosphate ions in the reversibility was also evaluated by a similar method using for dilution the same volume of 1 mM potassium chloride aqueous solution containing various amounts of phosphate salt (2.5, 12.5 and 25 mM of potassium phosphate).

2.4 Solution analysis

The risedronate and tiludronate concentrations in solution were determined using UV adsorption spectrophotometry at 262 and 265 nm, respectively (Hewlett Packard 8452A Diode Array Spectrophotometer). Phosphate concentration was determined by UV spectrophotometry of phosphovanado molybdic acid complex [19] and calcium content by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, Horiba Jobin Horiba).

2.5 Release kinetics

Tiludronate release profiles were obtained with a flow-through cells system (USP Apparatus 4 Sotax CE6, Sotax AG, Switzerland) with 12 mm cells (6 mL) and a piston pump (Sotax CY7, Sotax AG, Switzerland). In all experiments, laminar flow was used with a bed of 1 g of glass beads covered with 100 mg of NCA sample after tiludronate adsorption. The release tests were carried out at 37.0 ± 0.5 °C under sink conditions according to European Pharmacopoeia guidelines [20]. The dissolution medium used was deionised water pumped through the column at a flow rate of 15 mL/min. A closed system was used, recycling 50 mL of dissolution medium. Periodically, fractions of 5 mL were collected and tiludronate content was determined by UV spectroscopy at 265 nm (Hewlett Packard 8452A Diode Array Spectrophotometer). The same volume of dissolution medium was replaced back after each sampling in order to maintain constant volume and sink conditions. Each release study was performed in triplicate. The results are presented as kinetics cumulative of BP released (%) as a function of time:

$$\text{BP released}(\%) = 100 \times \frac{Q_r \text{ cumul}}{Q_{\text{ads}}} \quad (1)$$

where $Q_r \text{ cumul}$ is the cumulative concentration of BP released (mmol/L) and Q_{ads} the initial quantity of tiludronate adsorbed (mmol/L).

3 Results

3.1 Adsorption experiments

The results of chemical analysis and specific surface area of the adsorbents used in this study are shown in Table 1.

The evolution of the amount of risedronate and tiludronate adsorbed Q_{ads} ($\mu\text{mol}/\text{m}^2$) from the diluted solutions on several apatitic samples as a function of their remaining concentration in solution C_{eq} (mmol/L) is plotted in Fig. 2. The isotherms obtained are Langmuirian in shape, whatever the nature of the BPs and the apatitic support, as previously published [3, 12]. The adsorption plateaus were reached at relatively low bisphosphonate concentrations, indicating a high affinity of these molecules for apatitic surface. The parameters of the isotherms can be deduced from the Langmuir equation:

$$Q = \frac{KNC_e}{1 + (KC_e)} \quad (2)$$

by plotting C_e/Q versus C_e , where C_e (mmol/L) and Q ($\mu\text{mol}/\text{m}^2$) correspond to the stationary concentration in the solution and the amount adsorbed per unit area, respectively. The linear regression provides a way to determine the adsorption parameters: N is the maximum amount adsorbed of BP ($\mu\text{mol}/\text{m}^2$) and K the affinity constant of BP for the apatitic surface (L/mmol). As we can see in Fig. 2, the amount adsorbed at saturation on the HA specimen appears higher for risedronate than tiludronate and thus, the chemical structure of bisphosphonates influences their capacity of adsorption. Moreover, Fig. 2 illustrates the fact that the quantity of risedronate adsorbed at saturation on a well crystallised apatite (HA) appears always lower than that found for a nanocrystallised apatite (NCA). Figure 3 reports the variation of the adsorption parameters on several types of NCA crystals for tiludronate and risedronate. The data obtained evidence the influence of the nature of the apatitic surface on the values of the adsorption parameters: a decrease in the maximum amount adsorbed at saturation, N , with the maturation time and the development of the surface hydrated layer. For long

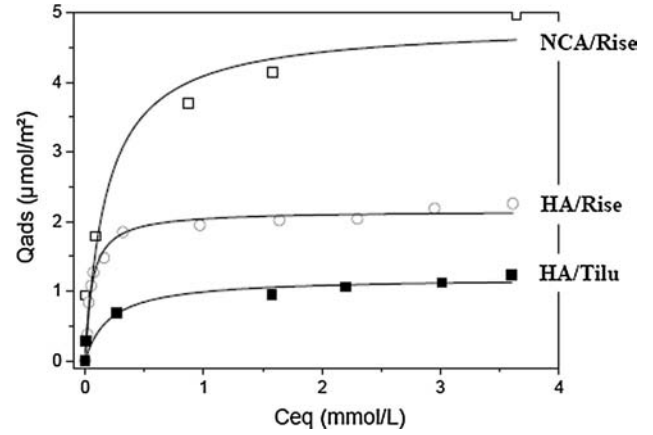


Fig. 2 Adsorption isotherms of tiludronate (Tilu) and risedronate (Rise) at 37 °C and pH 7.4 on HA and NCA (carbonated and matured for one day with $\text{Ca}/(\text{P}+\text{C}) = 1.38 \pm 0.03$). The data depend on the nature of the apatitic adsorbent surface and of the adsorbate molecule

maturation times, in the case of tiludronate, a strong difference in the values of affinity constant, K , is observed, related possibly to changes in chemical composition.

The effect of the solution composition on the adsorption was investigated in detail. The results obtained for risedronate adsorbed on HA for various solution compositions are presented in Table 2. Although the adsorption experiments were conducted under different conditions, the curves obtained always corresponded to Langmuir isotherms (data not shown). The uptake of risedronate by HA was dependent on the content of hydroxonium, phosphate and calcium ions in the solution. The variation of pH in the standard solutions at room temperature (Table 2a) showed that the apparent affinity constant (K) and the maximum amount adsorbed (N) decreased as the pH rose. As it can be seen, the amount loaded at pH 6.6 is about three times higher than the value obtained at pH 9.5. Furthermore, the influence of adding phosphate into the adsorption solution was examined (Table 2b). Increasing the phosphate concentration resulted in a decrease of the maximum amount adsorbed and the apparent affinity constant. By contrast, as the calcium concentration rose from 0.05 to 0.2 mM

Table 1 Main characteristics of the adsorbents used in this study

Adsorbent	CO_3 (wt%) (± 1 %)	Ca/P (± 1 %)	$\text{Ca}/(\text{P}+\text{C})$ (± 2 %)	Specific surface area (m^2/g) (± 5 %)
HA	–	1.64 ± 0.02	1.64 ± 0.03	59 ± 3
NCA matured for 1 day with carbonate	3.5 ± 0.1	1.53 ± 0.02	1.38 ± 0.03	96 ± 5
NCA matured for 7 days with carbonate	4.5 ± 0.1	1.59 ± 0.02	1.39 ± 0.03	201 ± 10
NCA matured for 1 month with carbonate	5.5 ± 0.1	1.62 ± 0.02	1.38 ± 0.03	190 ± 9
NCA matured for 1 month without carbonate	0.5 ± 0.1	1.61 ± 0.02	1.59 ± 0.03	154 ± 8
NCA non matured without carbonate	0.3 ± 0.1	1.40 ± 0.02	1.39 ± 0.03	193 ± 9

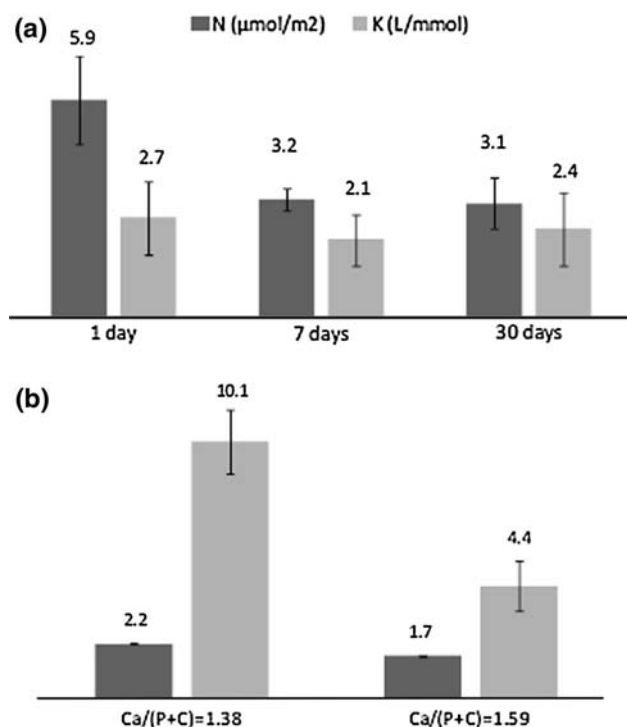


Fig. 3 **a** Adsorption parameters of risedronate for different maturation times of NCA. The Ca/(P+C) ratio remains constant in the solids and equal to 1.38 ($\pm 2\%$) indicating a similar amount of bivalent anions and calcium vacancies. The carbonate content varies from 3.5 wt% $\pm 1\%$ for one day to 5.5 wt% $\pm 1\%$ for 30 days of maturation state related to a progressive replacement of HPO_4^{2-} ions by carbonate ions during maturation. Most importantly the surface hydrated layer considerably decreased with the maturation time. The data indicate a decrease of the maximum amount adsorbed at saturation N with the maturation time. **b** Adsorption parameters of tiludronate on two NCA matured for one month with and without carbonate. The surface hydrated layer appears more important for the most carbonated sample. The data indicate a variation of N and K possibly related to the development of the surface hydrated layer and its composition [13, 16]

(Table 2c), a slight increase in the apparent affinity occurred, whereas no significant change was noted for the maximum amount of adsorbed risedronate. The range of calcium concentration investigated was lower than that of phosphate ions to prevent the formation of Ca-risedronate precipitate. These findings suggested that the content of mineral ions in the solution played an important role in the uptake of risedronate by apatite crystals, and that these species should be considered, to some extent, in the evaluation of the driving factors in the adsorption process.

3.2 Influence of adsorption on the solution composition

As previously published [13, 21], the analyses of the solutions after adsorption revealed that the binding of bisphosphonates molecules to the apatite surface affects the amount of ionic species in the solution. Figure 4 presents

Table 2 Influence of the solution composition on the adsorption parameters of risedronate by HA, i.e. maximum amount adsorbed (N) and apparent affinity constant (K): (a) influence of pH at 25 °C, (b) influence of phosphate concentration at pH 7.4 and 37 °C, (c) influence of calcium concentration at pH 7.4 and 37 °C

	N \pm Δ N (μmol/m ²)	K \pm Δ K L/mmol
(a) pH (25 °C) (± 0.05)		
6.60	2.3 \pm 0.1	53.3 \pm 15.7
7.50	1.6 \pm 0.1	7.6 \pm 1.2
9.50	0.8 \pm 0.1	4.9 \pm 1.0
(b) Phosphate added (37 °C) (mM)		
1.00	2.2 \pm 0.1	12.4 \pm 4.2
5.00	1.9 \pm 0.0	11.8 \pm 2.2
10.00	1.9 \pm 0.0	9.7 \pm 2.2
(c) Calcium added (37 °C) (mM)		
0.05	2.1 \pm 0.1	18.9 \pm 6.8
0.20	2.1 \pm 0.0	22.7 \pm 10.3

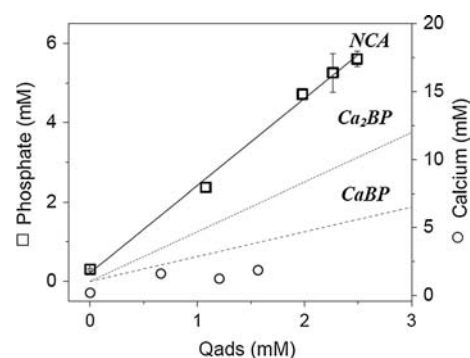


Fig. 4 Concentration of phosphate (Open square) and calcium (Open circle) ions released (mM) in solution as a function of the amount of tiludronate adsorbed on the solid (Q in mM). The adsorption was performed on NCA sample non-carbonated and matured for 1 month ($\text{Ca/P} = 1.61 \pm 0.02 = X$). The slope of the straight line was equal to 2.35 ± 0.16 . The dashed lines correspond to the variation of phosphates species in solution if the uptake of BP were related to the dissolution of the apatite and the precipitation of Ca_2BP (slope equal to $2/X$) or CaBP (slope equal to $1/X$) salts

the variation of calcium and phosphate ion concentrations in the solution as a function of tiludronate adsorbed on NCA sample (non carbonated and matured for 1 month, with $\text{Ca/P} = 1.61 \pm 0.02$). The amount of calcium content in the solution remained very low (<10 ppm considering a NCA sample, lower for HA), whereas the release of phosphate ions increased linearly with the amount of adsorbed tiludronate, with a slope close to 2.35 ± 0.16 . The uptake of tiludronate is proportional to the amount of phosphate ions released in the solution indicating that the main reaction is an ion-exchange process involving surface anions, whatever the apatitic surface, as previously

published [13, 22, 23]. However, the slope of the straight line is different depending on the calcium phosphate sample and the bisphosphonate used: a slope close to 1 for the adsorption of risedronate on HA and NCA [21], and from 1.2 to 2.3 for tiludronate on several nanocrystalline apatites [13]. Therefore, the number of phosphate groups released per single adsorbed molecule of tiludronate (corresponding to the slope) varies depending on the nature of the support and the chemical structure of the BPs.

3.3 Adsorption reversibility assays

Figure 5 presents the adsorption isotherms and the results of reversibility assays of risedronate adsorption performed at 25 and 37 °C. A simple comparison over the range of concentrations investigated indicated that the highest adsorption capacity was observed at 37 °C (Fig. 5, solid symbols). Thus, the amount of risedronate adsorbed reached $2.50 \mu\text{mol}/\text{m}^2$ at 37 °C vs. $1.56 \mu\text{mol}/\text{m}^2$ at 25 °C. The reversibility assays involve a decrease of the risedronate concentration in the solution by diluting with a KCl solution 1 mM (Fig. 5, open symbols). The dashed lines indicate that the decrease in the equilibrium concentration of given points from the isotherms did not change the values of the corresponding amounts adsorbed. This observation suggests that the adsorption process is irreversible with respect to dilution in the range of concentrations examined. A similar trend was noted for the two temperatures investigated (25 and 37 °C).

However, the desorption data present different results in the presence of phosphate ions (0–25 mM) in the solution (Fig. 6). The increase of phosphate species in solution involves the release of BPs molecules from the apatite surface by ion-exchange.

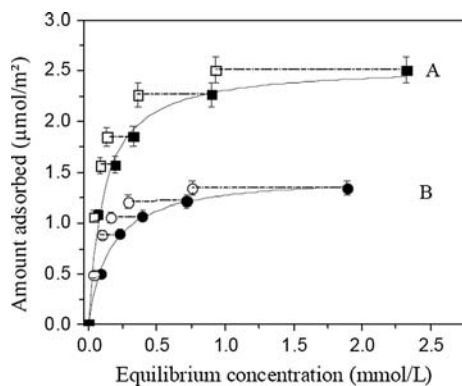


Fig. 5 Adsorption isotherms (solid symbols) and reversibility data (open symbols) of risedronate (pH 7.4) by HA from dilute aqueous solutions (0–3.8 mM) at **a** 37 °C and **b** 25 °C; the dashed lines indicate that no risedronate desorbs upon dilution

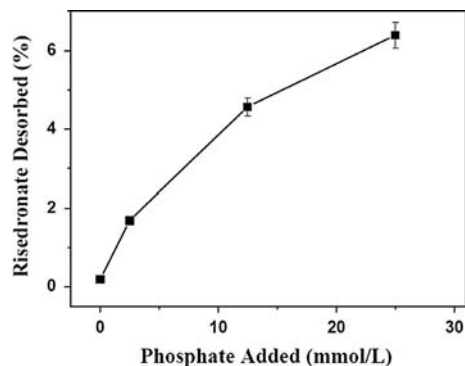


Fig. 6 Effect of added phosphate in solution of adsorption (mmol/L) on the release of risedronate (wt%) from HA at 25 °C and pH 7.4

3.4 Release kinetics

The results of the kinetics of release of tiludronate (%) from several NCAs with a $\text{Ca}/(\text{P}+\text{C})$ ratio constant (equal to 1.38 ± 0.03) are presented in Fig. 7. In all samples, the release was fast during the first hour and slowed down afterwards. The total amount released was significantly lower for matured (1 month) NCA than the non-matured NCA. However, the quantities of BPs released represented a minor fraction of the total amount adsorbed for all the samples: around 5–6 % of the initial quantity adsorbed for the non-matured NCA and around 3–4 % for the matured NCA.

4 Discussion

This paper illustrates the variability of the adsorption process for bisphosphonates on apatites but common

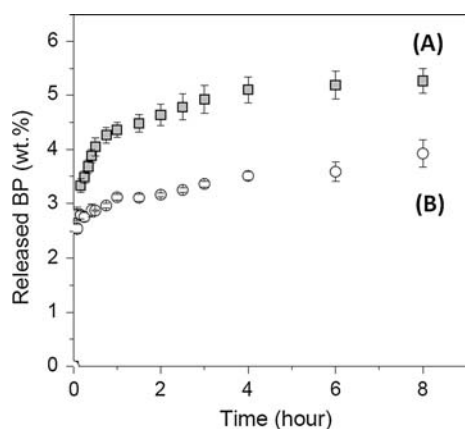


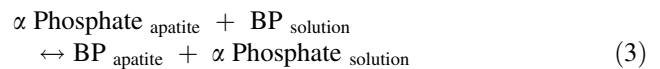
Fig. 7 Kinetics of release of tiludronate (%) from several NCA with a $\text{Ca}/(\text{P}+\text{C}) = 1.38 \pm 0.03$: **A** non-matured and non-carbonated (Filled square); **B** matured for one month and carbonated (Open circle). The amount of initial concentration of tiludronate on each NCA sample corresponds to the adsorption plateau of the Langmuir isotherm, i.e. $Q_{\text{ads}}(\text{A}) = 2.47 \text{ mM}$ and $Q_{\text{ads}}(\text{B}) = 1.99 \text{ mM}$

features have been observed. The adsorption of BPs molecules on apatites is well described by a Langmuir isotherm, as previously observed for bioactive molecules, such as drugs and proteins, strongly interacting with apatitic surface [24–26]. However, a major characteristic of this adsorption process is the absence of reversibility, which is in contrast with the Langmuir theory of adsorption. As often noticed for bioactive molecules on apatitic surfaces [12, 27], no desorption was observed by simple dilution of the solution or even upon washing of the samples, except for faint release effects possibly due to the dissolution of the apatite. However, the process was shown to be reversed by phosphate addition. This phenomenon is commonly observed in chromatography on apatite columns [28].

As for the interaction of organic molecules on calcium phosphate surfaces, an interesting aspect of the adsorption of bisphosphonate is that the parameters of adsorption can vary depending on the physicochemical conditions of the adsorption reaction. The results show that a decrease in pH, an increase in temperature or a decrease in the amount of phosphate in the solution induced an increase in the maximum amount adsorbed. The influence of temperature, at pH 7.4 for example, indicates a higher adsorption limit at physiological temperatures compared with room temperature. Indeed, the sharp rise of the adsorption curve for low concentrations at 37 °C is indicative of a greater affinity between risedronate and the apatite surface. The increase in the maximum amount of risedronate adsorbed with rising temperatures seems to indicate an increase in the amount of adsorption sites. A similar evolution of the adsorption parameters with temperature was noticed for the interaction of cisplatin, an anti-cancer drug [29], and pyrophosphate [30] with HA. Yin et al. [31] also showed that increasing the temperature resulted in an increase in the loading capacity of bovine serum albumin by HA. Similar tendencies have been reported for the effect of pH and other parameters on the adsorption of anionic species (proteins, citrates and amino acids) [25–27, 32]. These observations have received different interpretations including surface charge and/or surface energy variations, molecular conformation changes and speciation in the solution. In addition, the structure of the bisphosphonate molecule influences the adsorption parameters calculated from Langmuir isotherms, as observed for risedronate and tiludronate (Fig. 2). Nancollas et al. [3] explained the modification of HA crystal surfaces after adsorption of several BPs by differences in solid/liquid interfacial properties. The molecular charges related to the protonation of the BPs side-chain moieties may influence their mechanisms for binding.

However, regardless of the evolution of mineral species concentration in the adsorption solution, it seems that the binding properties cannot be explained based only on the

physical characteristics of the interfacial properties. The follow-up of the concentration of the mineral content of the solution (Fig. 4) indicates that the adsorption of BP molecules is associated with a chemical phenomenon: the release of phosphate ions, as previously published for risedronate on HA [21] and tiludronate on several NCAs [13], and by other authors concerning zoledronate adsorption on calcium phosphates [10]. This observation suggests that BPs molecules substituted for phosphate ions on the mineral surface and the adsorption of BPs can be well described by an ion-exchange process onto the apatitic support. The amount of phosphate ions displaced by one molecule of bisphosphonate can then be evaluated for all points of the isotherm, and a ratio of phosphate ions displaced per BP molecule adsorbed can be calculated. Although this ratio seems very close to 1 for risedronate adsorption, whatever the apatitic calcium phosphate support considered (HA or NCA) [12], the ratio for tiludronate is not equal to one or two and appears different as a function of the nature of the NCA [13]. Thus, the adsorption reaction can be better represented as an ion-exchange equilibrium at the surface of the apatite:

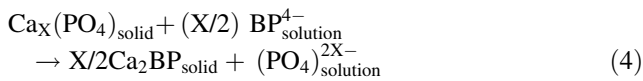


where “Phosphate” corresponds to phosphate species (PO_4^{3-} and/or HPO_4^{2-}). This representation is consistent with the observation of a Langmuir isotherm involving the concentration of phosphate in solution as developed in different reports [12, 13].

The description of the adsorption as an ion-exchange reaction explains the apparent irreversibility of the adsorption observed upon dilution. Because dilution with a solution in equilibrium with the apatite support and without BPs involves a reduction of both concentrations (concentration of BPs at equilibrium and phosphate concentration in solution) in equal proportion, the conditions of the equilibrium are approximately unchanged. The effect of phosphate addition on the displacement of adsorbed species is generally assigned to a competition for the occupancy of active adsorption sites on the mineral surface, in agreement with the ion exchange reaction proposed. Moreover, it has been found that the adsorption parameters N and K varied with the maturation time of the apatitic supports, related possibly to changes in chemical composition. In addition higher amounts of BPs adsorbed at saturation were obtained on NCA compared to well-crystallized HA, as observed in previous studies [13, 33]. Similar data were obtained for BSA adsorption [26] and phosphoserine [25] indicating an increase of the affinity constant and a decrease of the maximum amount adsorbed at saturation with the maturation time. These results can be explained by the presence of the hydrated layer rich in

labile species on the surface of NCA being able to exchange, and the influence of maturation times on the development of the surface hydrated layer and its composition. These data illustrate the variability of adsorption on apatite substrates and the need to define and characterize precisely the type of apatite used and its surface.

It is important to note that other phenomena could explain the release of mineral species in solution, such as the apatite dissolution and the formation of BP-calcium salt [10, 34]. The adsorption of BPs weakly influences the amount of calcium released in solution and most calcium ions remain attached to the solid phase, thus the formation of insoluble risedronate calcium salts must be considered as a competitive reaction to the adsorption. The reaction on the apatitic surface could then be written as a dissolution-precipitation phenomenon and BP-calcium salt formation, as proposed in a previous paper for Ca_2BP formation [13]:



where X represents the Ca/P ratio of the apatite support and (PO_4) the phosphate species (PO_4^{3-} and/or HPO_4^{2-}). In this case, the amount of phosphates released per BP molecule in solution would be equal to $2/X$, and $1/X$ for the formation of the CaBP salt. Figure 4 presents the results of phosphate concentration in solution versus the amount of tiludronate from soluble NCA with 1 month of maturation time and no carbonate content ($\text{Ca/P} = 1.61 \pm 0.02$). The slope of the straight line appears equal to 2.35 ± 0.16 , which would exclude the formation of a BP-calcium salt. Furthermore, no other crystalline calcium phase was detected by XRD and SEM (data not shown) and in addition the dissolution-precipitation process would not lead to a Langmuir-type adsorption equilibrium in contrast to the ion-exchange process [12].

The release kinetics of bisphosphonate molecules from apatitic samples has been also investigated. Generally, drugs and other biomolecules, such as growth factors, are incorporated into apatitic calcium phosphates by impregnation and drying, thus the binding properties are often unknown and the release rate uncontrolled [35, 36]. In contrast, adsorption on apatitic surfaces controls the amount of such molecules and the dose released. As observed in the current paper, bisphosphonate molecules are slowly released from the apatitic support, because most of the adsorbed molecules are irreversibly bound and not spontaneously released by dilution or simple washing as explained above [12]. They can only be displaced by a back-exchange process with mineral ions and/or soluble proteins from the solution, or dissolution of the support stimulated by external chemical factors such as pH. This characteristic has been observed for growth factors [35, 37]

and drugs [29, 38–40]. Moreover, similar to their adsorption, the release of bisphosphonates is not only strongly affected by the chemical properties of the molecule, but also by the chemical and structural characteristics of the apatitic substrates. As seen in Fig. 7, maturation stage and chemical composition of the apatitic supports affect the development and chemical composition of the surface hydrated layer, and thus the adsorption parameters and BPs release. Such properties have been used by several authors to control the release rate of proteins and drugs [14, 29, 37]. This behaviour could be useful to conceive “smart delivery systems” of bisphosphonates from apatitic biomaterials in vivo.

5 Conclusion

This work clarifies the interaction of two bisphosphonates, risedronate and tiludronate, with several apatitic supports, a well-crystallised hydroxyapatite (HA) and nanocrystalline apatites (NCAs). Whatever the nature of the BP and the structure and composition of the apatite, the adsorption of such anti-resorptive agents can be well described as an ion exchange-reaction between phosphates species on the apatitic surface and BP molecules in solution. This description explains the apparent irreversibility of the adsorption observed upon dilution. However the parameters of adsorption can vary depending on the physicochemical conditions of the adsorption reaction. The results show that a decrease in pH, an increase in temperature or a decrease in the amount of phosphate in the solution induces an increase in the maximum amount adsorbed. Moreover, the structure and composition of the apatitic surface also influence the adsorption properties as an increase of maturation time and changes in the chemical composition of the apatitic supports influence the adsorption parameters, related possibly to changes in development of the surface hydrated layer and its chemical composition. These data illustrate the variability of adsorption on apatite substrates and the need to define and characterize precisely the type of apatite used and its surface.

Finally, BPs molecules are slowly released from apatitic supports, because most of the adsorbed molecules are irreversibly bound and not spontaneously released by dilution or simple washing. Moreover, similar to their adsorption, the release of bisphosphonates is strongly affected not only by the chemical properties of the molecule, but also by the chemical and structural characteristics of the apatitics substrates. Such properties can be used to control the release rate of BP. This behaviour could be useful to conceive “smart delivery systems” of bisphosphonates from apatitic biomaterials in vivo.

Acknowledgments A part of this research was supported by the French-Moroccan Volubilis integrated action MA/05/122. The authors thank Procter & Gamble and Sanofi Aventis for the generous donation of risedronate and tiludronate samples respectively.

References

1. Rodan GA, Fleisch HA. Bisphosphonates: mechanisms of action. *J Clin Invest.* 1996;97(12):2692.
2. Russell RGG, et al. Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. *Osteoporos Int.* 2008;19(6):733–59.
3. Nancollas GH, et al. Novel insights into actions of bisphosphonates on bone: differences in interactions with hydroxyapatite. *Bone.* 2006;38(5):617–27.
4. Mönkkönen J, Similä J, Rogers MJ. Effects of tiludronate and ibandronate on the secretion of proinflammatory cytokines and nitric oxide from macrophages in vitro. *Life Sci.* 1998;62(8):PL95–PL102.
5. Elliott SN, et al. Alendronate induces gastric injury and delays ulcer healing in rodents. *Life Sci.* 1997;62(1):77–91.
6. Demerjian N, Bolla G, Spreux A. Severe oral ulcerations induced by alendronate. *Clin Rheumatol.* 1999;18(4):349–50.
7. Kos M, Luczak K. Bisphosphonates promote jaw osteonecrosis through facilitating bacterial colonisation. *Biosci Hypotheses.* 2009;2(1):34–6.
8. Hoffman A, et al. Mode of administration-dependent pharmacokinetics of bisphosphonates and bioavailability determination. *Int J Pharm.* 2001;220(1–2):1–11.
9. Denissen H, et al. Ceramic hydroxyapatite implants for the release of bisphosphonate. *Bone and Miner.* 1994;25(2):123–34.
10. Josse S, et al. Novel biomaterials for bisphosphonate delivery. *Biomaterials.* 2005;26(14):2073–80.
11. Panzavolta S, et al. Alendronate and Pamidronate calcium phosphate bone cements: setting properties and in vitro response of osteoblast and osteoclast cells. *J Inorg Biochem.* 2009;103(1):101–6.
12. Errassifi F, et al. Adsorption on apatitic calcium phosphates: application to drug delivery. In: Soc AC, Editor. 8th Pacific rim conference on ceramic and glass technology. Vancouver; 2010. p. 159–174.
13. Pascaud P, et al. Interaction between a bisphosphonate, tiludronate, and biomimetic nanocrystalline apatites. *Langmuir.* 2013;29:2224–32.
14. Palazzo B, et al. Biomimetic Hydroxyapatite-Drug Nanocrystals as Potential Bone Substitutes with Antitumor Drug Delivery Properties. *Adv Func Mater.* 2007;17(13):2180–8.
15. Rey C, et al. Nanocrystalline apatites in biological systems: characterisation, structure and properties. *Materialwiss Werkstofftech.* 2007;38(12):996–1002.
16. Eichert D, et al. Nanocrystalline apatite-based biomaterials: synthesis, processing and characterization. *Biomater Res Adv.* 2008. **93**.
17. Trombe JC. Contribution à l'étude de la décomposition et la réactivité de certaines apatites hydroxylées carbonatées ou fluorées alcalino-terreuses. 1972. Toulouse: UPS.
18. Rey C, et al. Maturation of poorly crystalline apatites: chemical and structural aspects in vivo and in vitro. *Cells Mater.* 1995;5(4):345–56.
19. Charlot G. Chimie analytique quantitative. Masson et Cie ed: Paris; 1974.
20. Europe Co. (Editor). European Pharmacopoeia 7th ed. Strasbourg: 2011.
21. Al-Kattan A, et al. Medical potentialities of biomimetic apatites through adsorption, ionic substitution, and mineral/organic associations: three illustrative examples. *Adv Eng Mater.* 2010;12(7):B224–33.
22. Errassifi F. Mécanismes d'adsorption du risedronate par des phosphates de calcium biologiques : applications aux biomatériaux. Cadi Ayyad University: Marrakech; 2011. p. 239.
23. Pascaud P, et al. Interaction between a bisphosphonate, tiludronate and nanocrystalline apatite: in vitro viability and proliferation of HOP and HBMSC cells. *Biomed Mater.* 2012;7(5):1–9.
24. Gómez-Morales J, et al. Progress on the preparation of nanocrystalline apatites and surface characterization: overview of fundamental and applied aspects. *Prog Cryst Growth Charact Mater.* 2013;59(1):1–46.
25. Benaziz L, et al. Adsorption of O-phospho-L-serine and L-serine onto poorly crystalline apatite. *J Colloid Interface Sci.* 2001;238(1):48–53.
26. Ouizat S, et al. Adsorption of bovine serum albumin on poorly crystalline apatite: influence of maturation. *Mater Res Bull.* 1999;34(14–15):2279–89.
27. Barroug A, et al. Adsorption of catalase on hydroxyapatite. *J Colloid Interface Sci.* 1998;208(1):147–52.
28. Gorbunoff MJ. The interaction of proteins with hydroxyapatite: II. Role of acidic and basic groups. *Anal Biochem.* 1984;136(2):433–9.
29. Barroug A, et al. Interactions of cisplatin with calcium phosphate nanoparticles: in vitro controlled adsorption and release. *J Orthop Res.* 2004;22(4):703–8.
30. Jung A, Bisaz S, Fleisch H. The binding of pyrophosphate and two diphosphonates by hydroxyapatite crystals. *Calcif Tissue Res.* 1973;11(4):269–80.
31. Yin G, et al. Impacts of the surface charge property on protein adsorption on hydroxyapatite. *Chem Eng J.* 2002;87(2):181–6.
32. López-Macipe A, et al. The role of pH in the adsorption of citrate ions on hydroxyapatite. *J Colloid Interface Sci.* 1998;200(1):114–20.
33. Errassifi F, et al. Infrared, Raman and NMR investigations of risedronate adsorption on nanocrystalline apatites. *J Colloid Interface Sci.* 2014;420:101–11.
34. Cukrowski I, et al. Modeling and spectroscopic studies of bisphosphonate-bone interactions. The Raman, NMR and crystallographic investigations of Ca-HEDP complexes. *Bone.* 2007;41(4):668–78.
35. Midy V, et al. Adsorption of vascular endothelial growth factor to two different apatitic materials and its release. *J Mater Sci Mater Med.* 2001;12(4):293–8.
36. Liu Y, et al. The influence of BMP-2 and its mode of delivery on the osteoconductivity of implant surfaces during the early phase of osseointegration. *Biomaterials.* 2007;28(16):2677–86.
37. Autefage H, et al. Adsorption and release of BMP-2 on nanocrystalline apatite-coated and uncoated hydroxyapatite/ β -tricalcium phosphate porous ceramics. *J Biomed Mater Res B Appl Biomater.* 2009;91B(2):706–15.
38. Lebugle A, et al. Study of implantable calcium phosphate systems for the slow release of methotrexate. *Biomaterials.* 2002;23(16):3517–22.
39. Misra DN. Interaction of some alkali metal citrates with hydroxyapatite: ion-exchange adsorption and role of charge balance. *Colloids Surf A.* 1998;141(2):173–9.
40. Iafisco M, et al. Adsorption and conformational change of myoglobin on biomimetic hydroxyapatite nanocrystals functionalized with alendronate. *Langmuir.* 2008;24(9):4924–30.