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Modifications in the molecular structure of hydroxyapatite induced by titanium ions

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The purpose of this study was to contribute to the understanding of the effect of titanium ions released from a metallic substrate on the molecular structure of hydroxyapatite. The effects of pH, time of incubation and concentration of titanium ions were investigated. The solids were analysed by X-ray diffraction, Fourier transform infrared spectroscopy, Fourier transform Raman spectroscopy, energy dispersive X-ray analysis and thermogravimetric analysis. The results clearly indicate the presence of a titanium phosphate, $Ti(HPO_4)_2 \cdot nH_2O$ ($n = 1$–3), which probably has a double layered structure. The formation of this compound is dependent on the titanium concentration and its crystallinity increases with the time of incubation.

1. Introduction

The excellent corrosion resistance of titanium and its alloys to physiological chloride solutions is well documented [1, 2]. Nevertheless, metallic implants inserted in the body eventually undergo some degradation through a variety of mechanisms, including corrosion. The results of both animal and human studies have shown that local, remote and systemic ion distribution into the tissues may occur. In fact, high concentrations of Ti have been found in tissues adjacent to Ti implants [3, 4], in some cases reaching values of several thousand parts per million [4].

Plasma sprayed hydroxyapatite (HAp) coatings on Ti and Ti alloy implants have been used in an attempt to obtain reliable implant-to-bone fixation. The use of the hydroxyapatite coating aims to improve osseointegration of the orthopaedic implants, the coating being expected to act as a barrier to elemental transfers from the underlying substrate to the surrounding tissues. It has been suggested that the bioactivity of the HAp coating is also due in part to the reduction of the possible adverse effects of metal dissolution products by shielding the underlying metal substrate [5–7]. However, studies developed by Ducheyne *et al.* [8] showed that the HAp coating on metallic implants of Ti-6Al-4V alloy had a major effect in enhancing interfacial bonding but at the same time released greater concentrations of aluminium [9].

This study aims to contribute to the understanding of the effects that Ti ions released from the metallic substrate may have on the degradation of HAp coatings and on the HAp as a bone mineral constituent.

The capacity of HAp to incorporate metal ions in its lattice has been associated with the presence of osseous pathologies. For instance, to justify the appearance of a pollution-related disease that was found in Japan 30 years ago, Aoki [10] presented the hypothesis that cadmium ions are assimilated in the HAp of bone tissues. According to the author, the fact that cadmium ions are very quickly exchanged with the calcium ions of HAp could cause infection as well as necrosis of bone tissue and prevent bone growth. Also, the presence of aluminium has been associated with several bone pathologies, including osteomalacia [11–13].

2. Materials and methods

Commercial HAp supplied by CAM Implants, with granulometry smaller than 32 μm and a specific surface area of 69.8 m^2, was used. All solutions were prepared with de-ionized water and the chemical reagents were of pro. analysis (p.a.) grade.

As it was impossible to obtain commercially produced titanium phosphate to be used as reference material, an attempt to precipitate the compound was made. A solution of 0.02 M of $Na_2HPO_4 \cdot 12H_2O$ and 0.01 M of $TiCl_4$ was prepared with de-ionized water, and 50 ml of this solution was kept at 37 °C for different incubation times. After centrifugation, the solids obtained were washed with de-ionized water. These solids are designated here as the 'mixture'.

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2.1. Dissolution studies

Dissolution studies of HAp were carried out using a saline physiological solution of 0.9% NaCl to which the metal cation was added in the form of a concentrated salt solution (Titrisol Merck Standard – $TiCl_4$ in 18% HCl). A solid-to-liquid ratio of 500 mg of HAp to 50 ml of solution was used. The samples were tested in polyethylene flasks, maintained at a temperature of 37 °C (± 0.2 °C) in a warm air cabinet equipped with an orbital shaker. An agitation speed of 250 r.p.m. was used throughout the experiments. The influence of the incubation time and the Ti concentration on the extent of dissolution were studied. Samples with Ti concentrations ranging from 1 to 2000 p.p.m. were prepared. The time of incubation ranged from 5 min to 230 days. After incubation, the solid and liquid phases were separated by centrifuging at 4000 r.p.m., and the supernatant liquid was analysed by atomic absorption spectroscopy for Ca and Ti ions. The total P in the liquid phase was determined by ultraviolet spectroscopy using the molybdenum blue method.

2.2. Solids analysis

The solid samples were thoroughly washed with deionized water to eliminate Cl and Na ions, and dried in a stove at 60 °C for 24 h. The solids were then analysed using X-ray diffraction techniques, Fourier transform infrared (FT-IR) spectroscopy, Fourier transform Raman (FT-Raman) spectroscopy, energy dispersive X-ray analysis and thermogravimetric analysis. X-ray diffraction was performed on a Philips PW 1710 diffractometer. When higher sensitivity was required, the Debye-Scherer X-ray diffraction technique was used. FT-Raman spectra were obtained on a Bruker FRA 106 FT Raman spectrometer in conjunction with a Bruker IFS 88 FT-IR optical bench, using a near infrared Nd^{3+} YAG as the excitation source. All samples were run at a spectral resolution of 6 cm^{-1}. The operating laser power was 180 mW and the number of scans varied according to the sample characteristics. Infrared spectra were obtained on a Bruker IFS 88 FT-IR optical bench, and all the samples were prepared as KBr discs. The thermogravimetric analysis was performed with Metler TA 4000 equipment, with a thermobalance TG 50 linked to a computer TC 11 with a precision of 2 °C and sensitivity of 1 µg. The energy dispersive spectroscopy (EDS) analysis was performed with a scanning electron microscope JEOL JSM-35C in conjunction with an energy dispersive spectrometer Tracor-TN 2000.

3. Results

3.1. Dissolution tests

Fig. 1 represents the variation in Ca and P concentrations in the liquid phase as well as the Ca/P concentration ratio as a function of the Ti concentration, for an incubation time of 10 days. The Ca and P concentrations increased up to a value of approximately 500 p.p.m. of Ti. For higher concentrations of Ti in solution, the Ca concentration stabilized while the P concentration decreased. The values obtained for Ca and P correspond to a non-stoichiometric dissolution of HAp. After incubation, Ti was below the detection limit of the instrumentation (1 p.p.m.). Therefore, it may be concluded that practically all the Ti was incorporated in the solid phase. Results obtained for powders incubated for different periods of time in 0.9% solutions containing 500 p.p.m. Ti showed that stationary concentrations of Ca and P are reached after the first minutes of incubation.

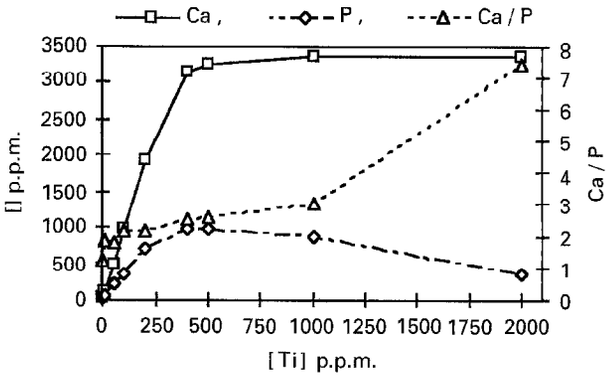

Figure 1 Influence of Ti concentration on the dissolution of HAp.

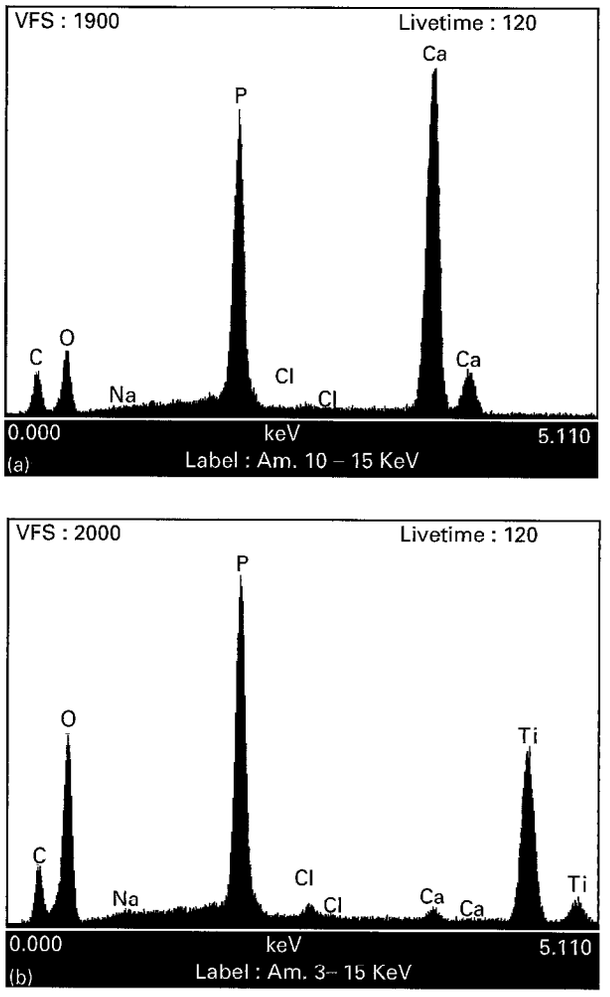

Figure 2 Spectra from EDS of powders obtained after 10 days' incubation of HAp in: (a) 0.9% NaCl solution; (b) 0.9% NaCl solution with a concentration of 500 p.p.m. of Ti cation.

3.2. Energy dispersive analysis

Fig. 2 shows the energy dispersive spectra of HAp and of a powder obtained after a 10 day incubation period

of HAp in a 0.9% NaCl solution with a Ti concentration of 500 p.p.m. Comparing both spectra, one concludes that the appearance of the Ti peak is accompanied by a significant depression of the Ca peak. The EDS analyses for the various Ti concentrations used indicated that as the concentration of Ti in the solution was increased, a decrease in the Ca signal and an increase in the Ti signal were observed, while the P signal kept constant, suggesting substitution of Ca by Ti. Since the pH of a solution with a Ti concentration of 500 p.p.m. is sufficient to dissolve HAp, the presence of either a titanium phosphate or some form of apatite with a high degree of substitution of Ti for Ca is possible. For the time being, we will refer to this solid as a 'Ti compound'. Thus, for the concentrations mentioned, the possibility exists that some type of dissolution-precipitation reaction occurred, leading to the formation of a Ti compound. The EDS analysis of the solids has also shown that formation of the Ti compound occurs very quickly. Similar spectra were obtained for times of incubation of 5 min and 10 days.

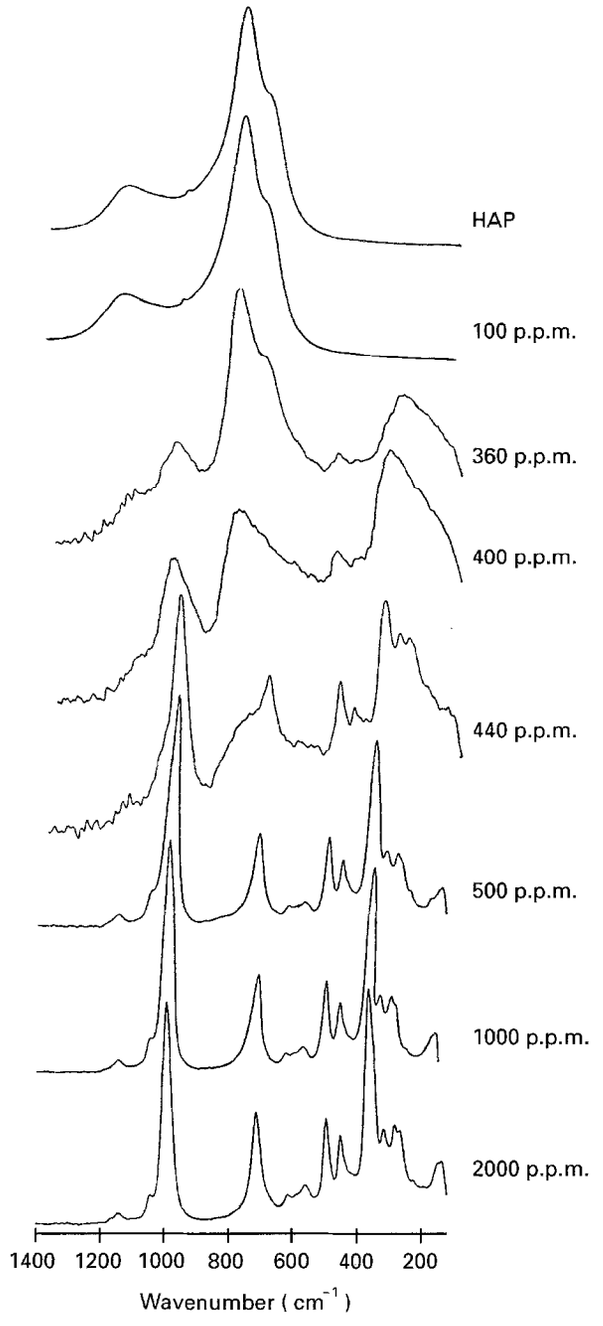

Figure 3 Overlay of Raman spectra of HAp and of powders obtained after 10 days' incubation of HAp in 0.9% NaCl solution with different concentrations of Ti cation.

3.3. FT-Raman and FT-IR spectroscopies

In general, all the FT-Raman spectra showed high signal-to-noise ratios and no fluorescence. Fig. 3 shows the FT-Raman spectra of HAp powders incubated for 10 days in solutions with different Ti concentrations. For comparison, the corresponding FT-Raman spectrum of HAp is also shown. HAp is an extremely good Raman scatterer and, consequently, the spectra produced are intense. The spectrum of pure HAp has been shown to be dominated by two bands, at 773 cm^{-1} (possibly associated with Ca-OH vibration [14]) and at 1144 cm^{-1}, with a smaller band at 959 cm^{-1} associated with the phosphate functionality of HAp. A similar spectrum was obtained by Tudor *et al.* [15] for a commercial HAp with a different origin.

Fig. 3 shows that Ti has a drastic effect on the HAp structure. For a concentration of 100 p.p.m. there are no differences between the spectrum obtained and the one that corresponds to HAp, but for higher Ti concentrations new bands arise. For a Ti concentration of 400 p.p.m. the FT-Raman spectrum is dominated by four broad bands at 1003, 774, 479 and 278 cm^{-1}. These undergo considerable changes in the concentration interval of 400–500 p.p.m. of Ti, as it may be seen in Fig. 3. The spectrum for 500 p.p.m. is relatively more complex, with the appearance of sharper bands at 987, 708, 484, 439, 348, 300 and 265 cm^{-1}. Some of these Raman vibrational bands, namely those at 987 cm^{-1} (the most intense) and 439 cm^{-1}, can be attributed to phosphate interactions [16–18]. This indicates that the Ti compound may be a newly formed titanium phosphate compound. The FT-Raman spectra of the samples with Ti concentrations of 1000 and 2000 p.p.m. exhibit bands similar to that with 500 p.p.m. but slightly sharper. The change in sharpness also suggests that the crystallinity of the compound may have increased.

Fig. 4 shows the FT-Raman spectra of powders obtained after incubation of HAp in a 0.9% NaCl solution with a Ti concentration of 500 p.p.m. for different periods of time. Again, dramatic changes are observed. After an incubation period of 5 min, the spectrum is dominated by four broad bands which show considerable alteration after 10 days of incubation. This latter spectrum is relatively more complex, showing more and also better defined bands. Therefore, it is clear that formation of the Ti compound is time dependent. The evolution of the spectra with incubation time and Ti concentration appear to be similar. No differences are observed when the spectrum of a sample with an initial concentration of 500 p.p.m. of Ti incubated for 10 days is overlapped with the spectrum of the synthesized 'mixture' (see materials and methods), thus suggesting that similar compounds are present in both cases. Results

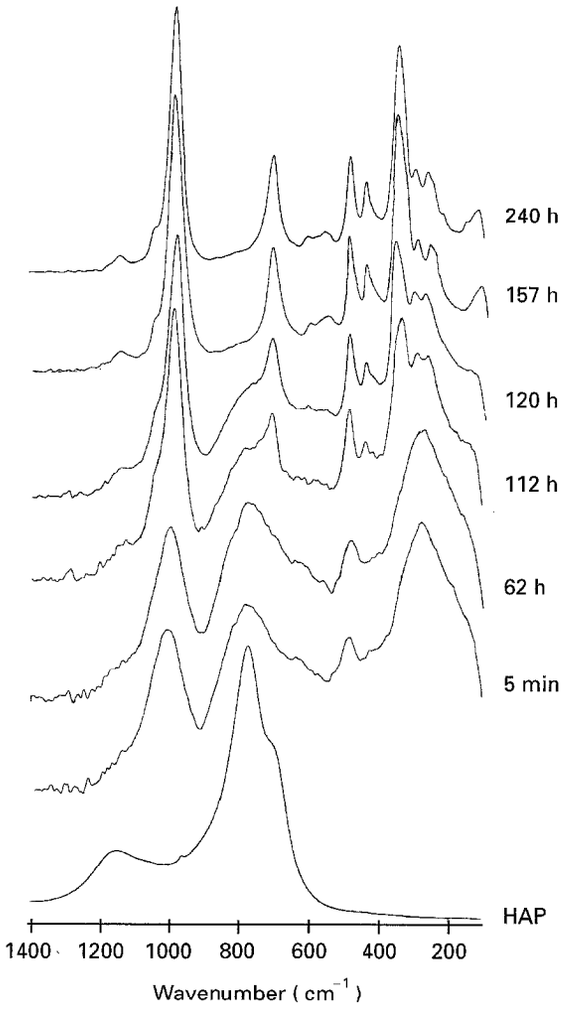

Figure 4 Overlay of Raman spectra of HAp and of powders obtained after different times of incubation of HAp in 0.9% NaCl solution with a Ti concentration of 500 p.p.m.

obtained by FT-IR spectroscopy (not shown) complemented the results of FT-Raman spectroscopy. The FT-IR spectra of the Ti compound exhibit a band at 1632 cm^{-1}, which is a characteristic vibrational mode of water, suggesting the presence of a hydrated compound.

3.4. X-ray diffraction

X-ray diffraction of powders collected after a 230 day testing period revealed that the addition of Ti to HAp in concentrations close to 400 p.p.m. leads to the destruction of the structure of the HAp (Fig. 5). In the less concentrated samples (Ti concentration $<$ 400 p.p.m.), some of the peaks characteristic of the HAp powder diffraction pattern are still present. Identical data were obtained for a time of incubation of 10 days. As the Ti concentration is increased, a new, poorly crystalline, phase is formed. The formation of the new phase is dependent on Ti concentration and its crystallinity increases with the time of incubation. The X-ray diffraction data (diffractograms and radiograms) indicate that this phase consists of a double layered compound ($Ti(HPO_4)_2 \cdot nH_2O$). This hypothesis is based on the findings of Christensen *et al.* [19], who reported the formation of two layers of Ti phosphates, also prepared by a liquid phase method. Elsewhere [20] we describe the structure of this double layered compound in more detail. The spectrum of the Ti compound is quite similar to that of the 'mixture', suggesting that they are similar in structure.

3.5. Thermogravimetric analysis

The thermal behaviour during heating cycles (25–1000 °C) of the powders obtained after incubation of HAp in solutions with different concentrations of Ti (500, 1000 and 2000 p.p.m.) was very similar, suggesting that the samples should be approximately identical in composition. The mass balance of the weight loss during heating enables us to conclude that the Ti compound present in the solids is hydrated, with the number of water molecules ranging from one to three [1–3].

4. Discussion

The results obtained in this study suggest that the interaction of Ti with HAp depends on the concentration of the metallic ion present in solution. The solid obtained after incubation of HAp in solutions with a Ti concentration equal to or smaller than 240 p.p.m. presents an X-ray diffraction spectrum similar to the one obtained for pure HAp over the time scale studied here. For a concentration of 320 p.p.m. of Ti, there are still some of the peaks of HAp in the X-ray diffraction spectrum, but they are much less intense. Also, the same peaks are seen in the FT-Raman spectra of solids obtained after incubation of HAp in solutions with a concentration of Ti equal to or smaller than 320 p.p.m., as in the spectrum produced by pure HAp. These results indicate that in the range of concentrations mentioned ($\leqq$ 320 p.p.m.) the formation of another compound, detectable by Raman spectroscopy or X-ray diffraction, did not occur. However, chemical analysis of the supernatant liquid obtained after incubation revealed that Ti was not present in solution, indicating that the metal ion had been incorporated in the solid or adsorbed on its surface, as supported by the EDS results. For the Ti concentrations mentioned above, the influence of the metal ion on the concentration of Ca in solution is not clearly different from the effect of pH (the pH in solution is lowered due to the fact that the metal ion is added in the form of an acid solution). In contrast, the P concentration in solution was lower than would be expected if only the pH effect is considered. The results obtained suggest that substitution of Ca by the metallic element in the HAp lattice occurs according to the reaction:

$$Ca_{10}(PO_4)_6(OH)_2 + \tfrac{n}{2}Ti^{4+} \rightarrow (Ca_{10-n}Ti_{n/2})(PO_4)_6 (OH)_2 + nCa^{2+} + n/2\ \square$$

where $\square$ represents a vacancy. It is well known that Ca can be replaced by different cations in the HAp structure [21, 22]. However, to the best of our knowledge, the present substitution has not been reported before. In terms of the size of the cations, the substitution seems possible, as Ti^{4+} ($r = 0.068$ nm) has an ionic radius much smaller than Ca^{2+} ($r = 0.099$ nm).

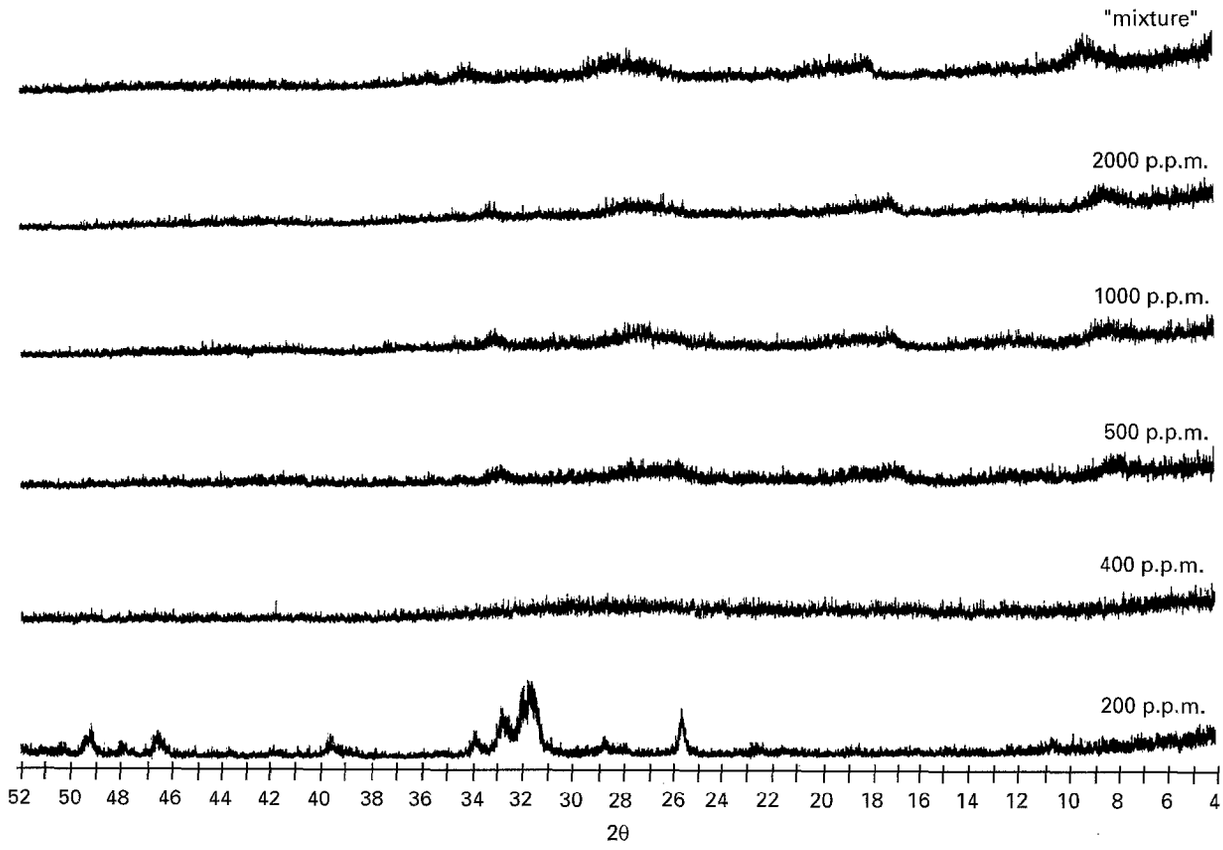

Figure 5 Overlay of X-ray diffraction spectra of powders obtained after 230 days incubation of HAp in 0.9% NaCl with different concentrations of Ti cation and of the 'mixture' powder obtained after the same time of incubation.

This substitution would lead to cationic vacancies due to the differences in electrical charge between the two cations.

For higher concentrations of Ti, a dissolution-precipitation process seems to occur, leading to formation of a new compound. The data obtained suggest that it may be a hydrated hydrogen titanium phosphate of the type $Ti(HPO_4)_2 \cdot nH_2O$. This compound may also form at low Ti concentrations, but its presence is undetectable with the techniques used.

This study has also shown that the FT-Raman analysis is sensitive to changes in the chemical composition and molecular structure of HAp powders. The Ti phosphate samples were poorly crystalline and, therefore, limited information on their nature could be obtained using conventional X-ray diffraction techniques. FT-Raman spectroscopy was very useful for the characterization of these materials, as it allows the study of amorphous compounds.

5. Conclusions

Ti ions interact with HAp and, depending on the concentration, will either enter the HAp lattice or form a Ti phosphate, $Ti(HPO_4)_2 \cdot nH_2O$ ($n = 1$–3). These observations are of relevance when Ti cations are released from orthopaedic implants, since the type of compound formed is bound to interfere with the normal process of bone formation. The results obtained in this work also suggest that HAp, when used as a coating material, will prevent the release of Ti metal ions from a metallic substrate to the surrounding tissues. However, the fate of the Ti-containing compounds present within a HAp coating when the latter degrades, particularly during the process of phagocytosis, should be investigated.

Acknowledgements

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