

The Effect of Chitosan on Hydroxyapatite Precipitation

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Abstract

The process of coprecipitation of biocomposite hydroxyapatite/chitosan from aqueous solution at low temperature in alkali environment was examined. We have shown that initially we have the formation of amorphous octocalcium phosphates ($\text{Ca}_8(\text{HPO}_4)(\text{PO}_4)_5, n\text{H}_2\text{O}$: OCP) and the transferring from OCP to amorphous calcium phosphate ($\text{Ca}_9(\text{PO}_4)_3, n\text{H}_2\text{O}$: TCP), and then from TCP to calcium-deficient hydroxyapatite ($\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_{2-x}$: ACP) and hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$: HAP). The transformation of ACP to HAP was inhibited in the presence of chitosan. The result suggests that there is an affinity binding between ACP and chitosan and subsequently blocking the active growth site of ACP.

Keywords : Hydroxyapatite, Chitosan, Coprecipitation, Conversion, Inhibition

1. Introduction

A composite biomaterial of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$: HAP) and chitosan (naturel amino-polysaccharide) has been proved to possess good osteoconductivity and biodegradability for orthopedic use. Different preparation methods of HAP/chitosan biocomposite have been reported, such as mixing of a HAP powder in chitosan solution and coating of HAP particles onto a chitosan sheet. However, the composites obtained by these means were inhomogeneous and often caused inflammation when implanted. A composite with a homogeneous structure has been prepared by a coprecipitation method. But in most of the previous studies, the mechanism and kinetics of HAP/chitosan precipitation in aqueous solution are still misunderstood. The aim of this study is to investigate the formation process of HAP in the presence of chitosan. The synthesis of the biocomposite was carried out by reaction between H_3PO_4 /chitosan and $\text{Ca}(\text{NO}_3)_2, 4\text{H}_2\text{O}$ solution at 25°C in alkali environment.

2. Experiment and Result

The powder of purified chitosan was solubilized in a bi-distilled water solution containing 1% of acetic acid. After agitation, the solution is filtered then added to the orthophosphoric acid solution.

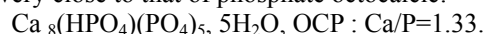
The orthophosphoric chitosan/acide report/ratio was adjusted with the final composition of the biocomposite HAP/Chitosan (100/0 and 80/20 percentages in mass).

The acid solution orthophosphorique/chitosan is added then to the calcium nitrate solution (0.5M) with 25°C and under magnetic agitation. The atomic report/ratio Ca/P was

adjusted to 1.667 (report/ratio stoichiometric of the hydroxyapatite). The pH of the mixture was maintained to 10 per addition of the ammonium hydroxide. Once the addition of the acid mixture orthophosphorique/chitosane is completed, maturation continues for the times determined in a closed balloon. The result obtained after maturation is filtered then stored with the freezer with -5° .

The values of Ca/P ratio at different reaction times were carried out by spectrophotometer with atomic emission (ICP-ACP). The curve in fig.1 highlights the evolution of the atomic reports/ratios Ca/P during the formation of the HAP in both the presence and absence of the chitosane.

The first formed phases have atomic reports/ratios Ca/P smaller compared to those quoted in the literature [1]. In temperatures equal to 0, the atomic reports/ratios Ca/P are very close to that of phosphate octocalcic:



The composition of amorphous octocalcium phosphate ($\text{Ca}_8(\text{HPO}_4)(\text{PO}_4)_5, n\text{H}_2\text{O}$: OCP) formed at the beginning was confirmed by spectrum FTIR analysis (Fig.2a) and by diffraction of x-rays at the small angles (Fig.3).

The variation of the atomic reports/ratios is very fast for the first formed precipitates. The analyses by spectroscopie IRTF and diffraction of x-rays (Fig.4) enabled us to highlight the transferring from OCP to tricalcium phosphates ($\text{Ca}_9(\text{PO}_4)_3, n\text{H}_2\text{O}$: TCP). The atomic reports/ratios increase gradually, thus representing the formation of a lacunar apatite into calcium ($\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_{2-x}$: ACP) and the evolution towards stoichiometric hydroxyapatite.

The curves of the Fig.1 take the same form. However one can note a slower evolution of the atomic reports/ratios Ca/P in the case of the precipitation of the hydroxyapatite in the presence of the chitosan. It appears that the chitosan plays the role of an inhibitor in the formation of apatite. The

phenomenon seems more accentuated at time equal to 360 minutes when the atomic report/ratio Ca/P is equal to 1.590 in the case of the composite, whereas it is 1.661 in the case of the pure HAP. The analyses by spectroscopy IRTF (Fig.2) highlight the presence of ions HPO_4^{2-} with a more important concentration in the case of the biocomposite (Ca/P=1.590) (Fig.2c) in comparison with pure apatite (Ca/P=1.661) (Fig.2b).

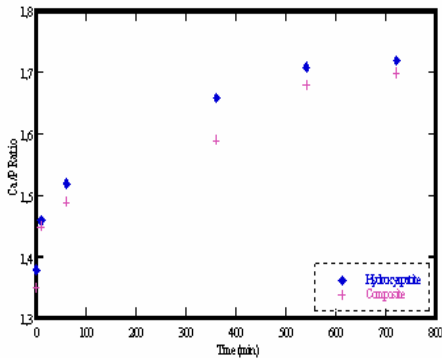


Fig. 1. The variations of Ca/P ratio with reaction time

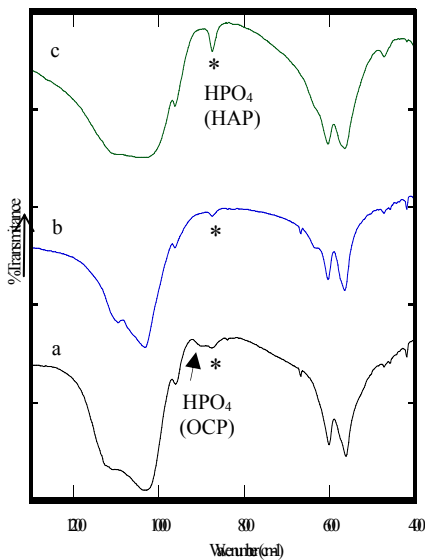


Fig. 2. Infrared spectra in the preparation of hydroxyapatite (a: Ca/P= 1.380, b: Ca/P=1.661) and biocomposite (c: Ca/P= 1.590)

In an alkaline environment, the amino form of the chitosan ($-\text{NH}_2$) confers to it excellent properties, chelating towards the calcium ions, in charge of the evolution of the system. The chitosan also has the possibility of being adsorbed on the apatite crystals by means of interaction of the hydrogen bonds type. These two phenomena seem to lead to an inhibition of the crystalline growth of apatite. The chitosan does not block all the active sites and the system evolves, in presence as in absence of the chitosan, to a stoichiometric apatite.

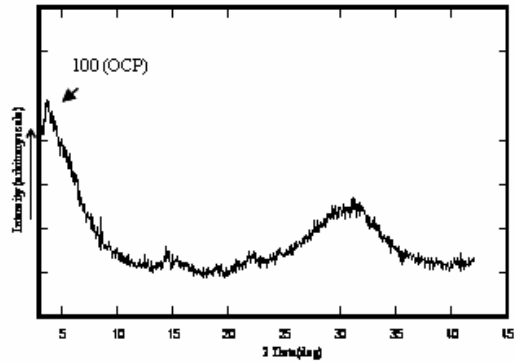


Fig. 3. X-Ray diffraction patterns of a synthesized powder hydroxyapatite (Ca/P=1.380)

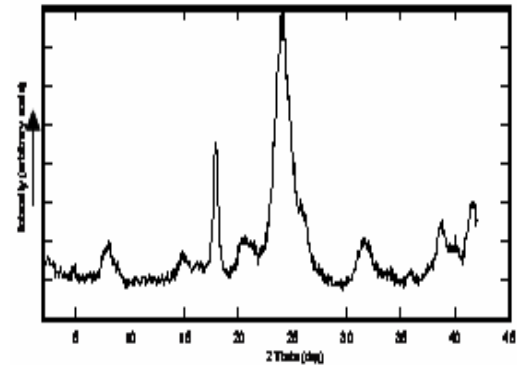


Fig. 4. X-Ray diffraction patterns of a synthesized powder hydroxyapatite (Ca/P=1.520)

3. Summary

We have shown that the process of formation of the hydroxyapatite includes the formation of amorphous octocalcium phosphates and the transferring from OCP to amorphous tricalcium phosphates and then from TCP to calcium-deficient hydroxyapatite (ACP) and HAP. The transformation of ACP to HAP was inhibited in the presence of chitosan. The result suggests that there is an affinity binding between ACP and chitosan and subsequently blocking certain of the active growth site of ACP.

4. References

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