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Investigation on the biomimetic influence of biopolymers on calcium phosphate precipitation—Part 1: Alginate

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ABSTRACT

The understanding of how macromocules act in precipitation of inorganic phases is the key knowledge that is needed to establish the foundation to mimic nature and produce materials with high mechanical modulus besides outstanding optical and thermal properties. This study investigated how addition of small amounts of alginate (7–70 ppm), that presents many carboxylic groups, affects phase distribution and morphology of calcium phosphates, obtained through precipitation and further submitted to calcination and sintering. The results lead to the conclusion that alginate action is dynamic, where alginate molecules act as templates to nucleation, and most of the biopolymer remains in solution even when all calcium phosphate has precipitated. However, despite the effect on phase composition being mainly related to the system's kinetics, alginate does present thermodynamic interaction with the precipitates. It is probable that it acts by reducing the free energy of nucleation, as in heterogeneous nucleation processes.

1. Introduction

Nature promotes, from a precisely controlled process, the precipitation of inorganic phases within biomacromolecule matrices [1]. The action of these biopolymers often provides inorganic and hybrid structures with remarkably different properties in size distribution, orientation of crystals [2] and self-oriented domains that could not be obtained by common routes of obtention used in laboratory.

The understanding of how macromocules act in precipitation of inorganic phases is the key knowledge that is needed to establish the foundation to mimic nature and produce materials with high mechanical modulus besides outstanding optical and thermal properties. These routes, called as biomimetic processes [3–8], would be a very easy way to produce materials exploring ionotropic, molecular recognition and nucleation-and-growth methods in mild conditions: temperature and saline concentrations of living organisms.

The interest in understanding how chemical functions would influence inorganic phases has inspired many scientists to explore co-precipitation [9] and heterogeneous phase depositions [10–12]. So far, some understanding of nucleation energy role and the differences between homogeneous and heterogeneous action of biopolymers is attained [13].

Calcium phosphates are widely used in medicine and dentistry due to its similarity to bone inorganic phase [14,15], which represents approximately 65% of hard tissues, in weight. This way, calcium phosphate ceramics are frequently bioresorbable and osteoconductive [16], and so, ideal to filling bone defects or coating metallic implants, thus allowing a better post-surgical recover. Not surprisingly, they consist in a largely studied group of inorganic compound in terms of obtention and processing.

On the other hand, as organic matrices, the most common chemical functionalities that are found in nature are carboxyl and amino terminals. Usually in the form of macromolecules such as polypeptides and polysaccharides. In our case, polysaccharides were used, as they are much more stable and controllable in "in vitro" studies than polypeptides are.

Alginate is a naturally occurring biopolymer, more specifically a carbohydrate, extracted from brown algae. Its monomers are D-mannuronic and L-guluronic acid, present in varied proportions. It is suitable for use as a biomaterial, since it presents low toxicity and easy interaction with peptides, due to its free carboxylic acids [17].

The aim of this study is investigate how addition of small amounts of alginate, that presents many carboxylic groups, affects phase distribution and morphology of calcium phosphates, obtained through precipitation and further submitted to calcination and sintering (just as a step of characterization and a way to evidence phase differences in the pristine amorphous form). In this way, a better understanding of the role of some biopolymer chemical functionalities on calcium phosphates interaction is aimed, besides contributing to obtain more precisely engineered bioceramics and composites.

2. Materials and methods

Calcium nitrate ($CaNO_3$ $4H_2O$) and ammonium phosphate ($(NH_4)_2HPO_4$), provided from Merck were used as calcium and

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Table 1
Produced samples.

Sample	[Chitosan] ppm	Final pH
Acid control	0	5.4
Alkaline control	0	8.4
Acid alginate 1	7.2	5.4
Alkaline alginate 1	7.2	8.4
Acid alginate 2	72	5.4
Alkaline alginate 2	72	8.4

phosphate sources. A starting solution of sodium alginate (1 g L^{-1}), from Synth was prepared and subsequently diluted to the desired concentration, which was calculated taking into account the final medium volume. Deionized water was used in all solutions. Concentration of calcium and phosphate solutions was such that a Ca/P ratio of 1.67 was obtained in all experiments.

The reaction was carried out in a polyethylene Becker containing initially 50 mL of pure water (control sample) or alginate solution, in continuous agitation, as reaction medium. Two different concentrations of alginate were used, 7 and 70 ppm. Calcium and phosphate solutions were added drop-wise, by using two channels of a peristaltic pump. The addition rate of calcium ($Ca(NO_3)_2$ $4H_2O$) was twice the rate of phosphate ($(NH_4)_2HPO_4$), in order to maximize the interaction alginate–calcium. The addition of solutions started simultaneously, but calcium solution ended before phosphorus'. After mixing by using a magnetic stirrer, the medium was kept without agitation, in reaction for 1 h and the pH was then measured. The final medium volume was of 570 mL. Thus, six different samples were produced, as shown in Table 1.

After reaction, each experimental run was divided in two portions. The first one, denominated acidic sample, was filtered, washed with 1 L of deionized water and dried at 100 °C during 12 h. Five drops of concentrated NH_4OH were added to the second, which was called alkaline sample (until reaching pH of *ca.* 8,4 ± 0,1). The idea was to understand how the biopolymer (alginate) would influence the reaction media in these two different conditions. The reaction was carried out for an extra hour and then it was also filtered, washed and dried at 100 °C for the same time. pH measurements were performed prior and during NH_4OH addition.

All experiments were developed under similar conditions, seeking to minimize any variability or scattering in the results due to undesirable parameter variations. This way, time of reaction, drying and rate of reagents addition were kept in all experiments.

Analysis of powders' phase composition done by using X-Ray diffraction (XRD) using CuKα1 radiation. Phase fractions in XRD diagrams can be best analyzed by Rietveld refinement, which gives the calculated amount of present phases, however, in our case, just the qualitative identification of phases was enough for our discussion.

Scanning electron microscopy (SEM) analysis allowed assessment of changes in powders' morphology, and was performed in a Jeol Scanning Electron Microscope.

FTIR (Fourier-transformed infra-red spectrophotometry) analyses were performed in transmittance mode, using a mixture with KBr, in a wavenumber range of 4000–400 cm^{-1}. A Nicolet 800 equipment was used attached to a MTech PAS cell.

3. Results and discussion

Alginate is a biopolymer composed of two monomers: L-guluronic and D-mannuronic acids. However, their degree of dissociation is not identical. The former has a pKa of 3,65, while the later is 3,32 [18]. Since L-guluronic acid is the main responsible for the ionotropic effect [19], stereochemical differences affects alginate behavior in solution.

The results of XRD analysis are shown in (Figs. 1 and 2). The acidic control sample contains mainly brushite, also named dicalcium phosphate dihydrate (DCPD, $CaHPO_4$ $2H_2O$). Alkaline control sample also shows small peaks around $2\theta = 26.4°$ and 30.4° corresponding to monetite, or anhydrous dicalcium phosphate (DCPA, $CaHPO_4$).

The samples containing alginate also presented monetite. Although samples produced in acidic medium only show small peaks at $2\theta = 26.4°$ and 30.4°, those produced in alkaline medium have intense reflections at the characteristics angles of monetite, suggesting that it became the predominant phase.

FTIR analyses – shown in (Figs. 3 and 4) – of the control samples are characteristic of brushite. Acidic samples produced with alginate are very similar, but both alkaline alginate 1 and 2 show strong absorbance around 1400 cm^{-1}, characteristic of carboxyl groups. These bands are also found in acidic alginate 1 and 2, but are far less intense. According to Lawrie [20], alginate usually presents the peaks listed in Table 2. There are signals assignable to carboxyl groups in 1600–1700 cm^{-1}

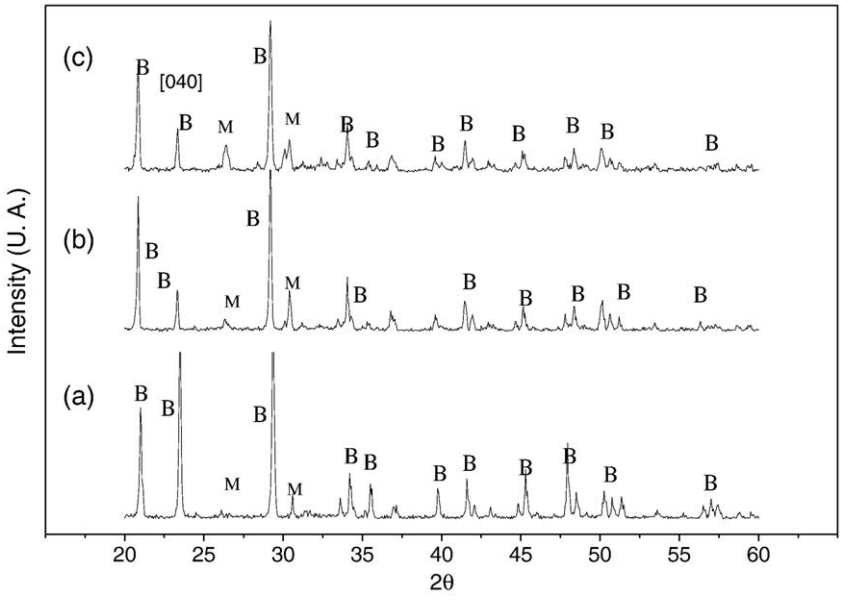

Fig. 1. XRD of samples produced in acid medium: a, blank acidic, b, acidic alginate 1, c, acidic alginate 2. B represents brushite and M monetite.