Influence of Alginate on Precipitation of Calcium Phosphates

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Abstract
Calcium phosphates are widely used in medicine and dentistry. However, synthesis of these
substances through precipitation methods still presents problems regarding reproducibility. Alginate
is a polymer extracted from brown algae, whose monomers are D-mannuronic and L-guluronic
acids, and is suitable for biomedical applications. The aim of this paper is to investigate how the
poly-ionic nature of alginate affects phase composition of calcium phosphate obtained through a
precipitation method.

Introduction
Calcium phosphates are widely used in medicine as synthetic substitutes to hard tissues, due to its
similarity with bone inorganic phase. Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, is the most used
bioceramic and its combination with other more resorbable calcium phosphates create conditions
for dissolving *in vivo* while bone healing is taking place.
Several methods for obtaining calcium phosphates are available. Precipitation from aqueous
solution seems to be one of the most suitable, due to the mild reaction conditions, which allows a
relatively simple adaptation to large production schemes. However, this method presents difficulties
regarding reproducibility, apparently due to its sensitiveness to reaction parameters, such as pH and
temperature, which may lead to a final product containing several phases, some of them unexpected
and undesired.
Biopolymers affects markedly phase distribution and degree of crystallinity of materials obtained
through precipitation reactions. The biopolymer influence on mineral deposition is the key factor
that characterizes biomimetic processes and many authors have attributed this ability to the
ionotropic effect, the provision of nucleating sites and local changes in pH or reagents concentration
caused by biopolymeric chains, usually with poly-ionic nature. Thus, crystalline forms that are
normally metastable can be obtained [1] through these processes. This property determines scaffold
performance, since phase composition has direct effects in its dissolution rate and bioactivity. These
characteristics can determine the rate of bone growth and apposition to the biomaterial.
Alginate is a naturally occurring biopolymer, extracted from brown algae. Its monomers are D-
mannuronic and L-guluronic acid, presents in varying 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 [2].
In this study, the effect of alginate on calcium phosphate precipitation was investigated. Small
amounts of this biopolymer were added to the reaction media prior to calcium and phosphate
addition. This way, we expect to cast some light on the effects of alginate on phase composition and
morphology of calcium phosphates.

Materials and Methods
Calcium nitrate ($CaNO_3.4H_2O$) and ammonium phosphate (($NH_4)_2HPO_4$) were used as calcium and
phosphate sources. A starting solution of sodium alginate (1 g/L) was prepared and subsequently
diluted to the desired concentration, which was calculated taking in account the final medium

volume. Concentration of calcium and phosphate solutions was such that a Ca/P ratio of 1.67 was obtained in all experiments.

Calcium and phosphate solutions were added drop-wise in a polyethylene becker containing 50 mL of pure water or an alginate solution as reaction medium. Thus, when the reaction was carried out using the former medium a non-affected, 'blank', sample was obtained. Two different concentrations of alginate were used, 7 and 70 ppm. The final medium volume was of 570 mL. Thus, six different samples were produced, an acidic blank and an alkaline blank, acidic and alkaline alginate 1 (precipitated with 7 ppm of alginate)and acid and alkaline alginate 2 (70 ppm).

After reaction, each experimental run was divided in two portions. The first one, from now on denominated acidic sample, was filtered, washed with 1 L of deionized water and dried at 100°C during 12 hours. Five drops of concentrated NH_4OH were added to the second, which will be called alkaline sample. 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 carried out 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 was carried out through X-Ray diffraction (XRD) using $CuK\alpha_1$ radiation. Scanning electron microscopy (SEM) analysis allowed assessment of changes in powders' morphology, and was carried out in a Philips SEM model XL-30.

Results and Discussion

XRD analysis of the acidic blank sample showed that it was composed mainly of dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$, DCPD, Brushite). This was expected since the pH value during precipitation was approximately 5.4. LeGeros [3] shows the stability of brushite under these conditions. In addition, phosphate source was $(NH_4)_2HPO_4$ and no ammonium hydroxide was added. The later has a fundamental role in hydroxiapatite formation through this process, as can be seen in the following Eq. 1:

$$10CaNO_3 . 4H_2O + 6(NH_4)_2HPO_4 + 8NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20NH_4NO_3 + 6H_2O \qquad (1)$$
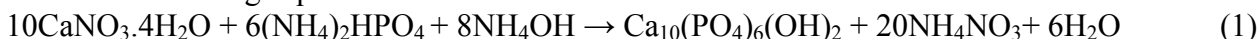
However, it is worth to point that DCPD is apparently an intermediate phase of hydroxiapatite in lower pH values [4]. Samples precipitated with alginate in acid medium presented small peaks at $2\theta \approx 26.4$ and $30.4°$. These peaks are related to anhydrous dicalcium phosphate (DCPA, Monetite, $CaHPO_4$). The result of XRD analysis is shown in Fig. 1.
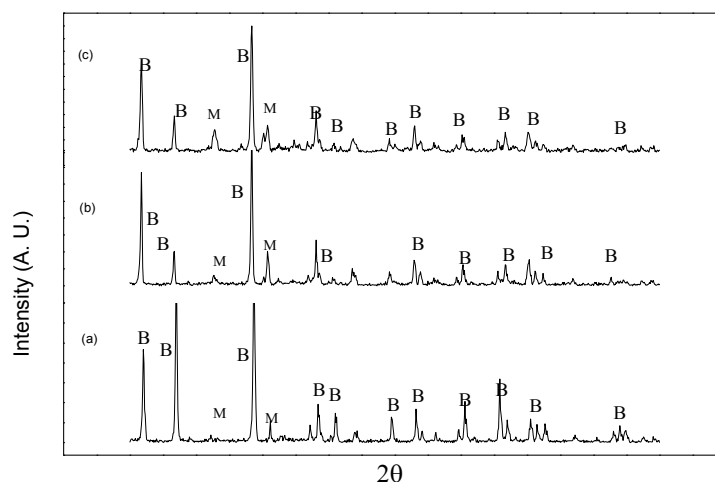
Fig.1. XRD of samples produced in acidic medium: (a) blank acidic, (b) acidic alginate 1, (c) acidic alginate 2. B represents Brushite and M Monetite.

Alkaline samples presented a very different behavior. While addition of NH_4OH resulted in a small increase of DCPA peaks in the alkaline blank, the effect on solutions containing alginate was remarkable. These peaks increased so much that their intensity is about the same of DCPD in alkaline alginate 1 and 2.XRD results can be seen in Fig. 2.

In both XRD spectra, it is possible to observe the intensity changing at 23° peak, possibly indicating a preferred orientation due to the alginate presence. This peak is associated, according to JCPDS files, with [020] plan of the brushite phase. This peak intensity also changed with the alginate concentration.
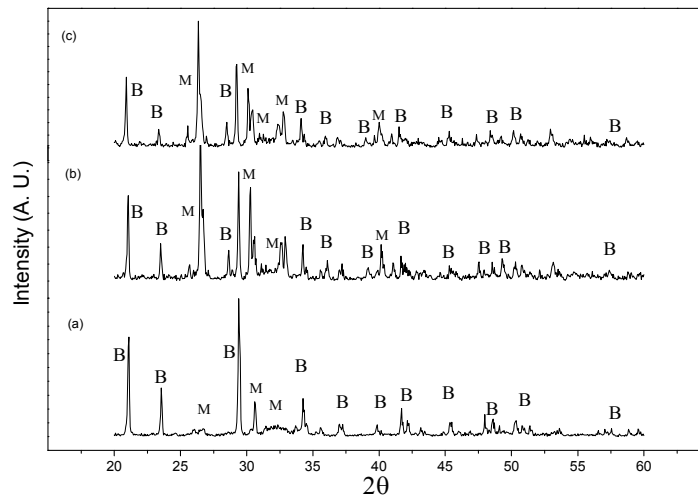
Fig. 2. XRD of samples produced in alkaline medium: (a) blank alkaline, (b) alkaline alginate 1, (c) alkaline alginate 2. B represents Brushite and M Monetite.

pH was measured before and after NH_4OH addition, and raised in all experiments from 5.40 ± 0.1 to 8.40 ± 0.1. As demonstrated by XRD results, alginate effect on DCPA formation is strongly pH dependent.

This can be explained when the chemical structure of alginate is taken in account. Alginate is a polymer of D-mannuronic and L-guluronic acids, and contains hydroxyl and carboxyl groups. At low pH values, the degree of dissociation of these groups is small. However, as pH increases, a higher degree of dissociation is reached, and the biopolymer will present a net negative charge. Consequently, electrostatic interaction among these groups and Ca^{2+} ions becomes more intense.

In DCPD, water molecules are bounded to calcium ions. However, when alginate is present at high pH values, electrostatic interaction will prevail, and water bonding will be avoided. This way, DCPDA, which is the anhydrous form of DCPD, will be formed.

SEM analysis showed some important morphological differences between samples produced in low pH medium with and without alginate. The sample acidic blank presents smaller particles than those found in acidic alginate 1. In fact, the particles were so small that even in a magnification of 5.000x most of them could not be visualized. On the other hand, particles in acidic alginate 1 are easily seen. SEM analysis of both can be seen in Fig. 3, 4 and 5.

The effect of alginate on particles' size can be explained through an analysis of crystal formation. Crystallization processes can be divided in two phases, nucleation and growth. In the acidic blank sample calcium phosphate nuclei should be formed prior any crystal growth could take place. However, alginate particles present during calcium phosphate precipitation act as nuclei, and growth is the dominant step, resulting in larger particles.
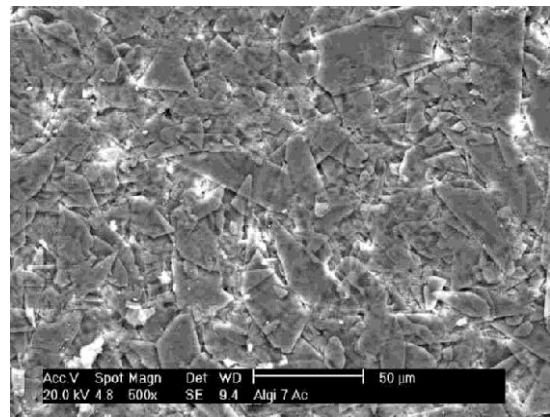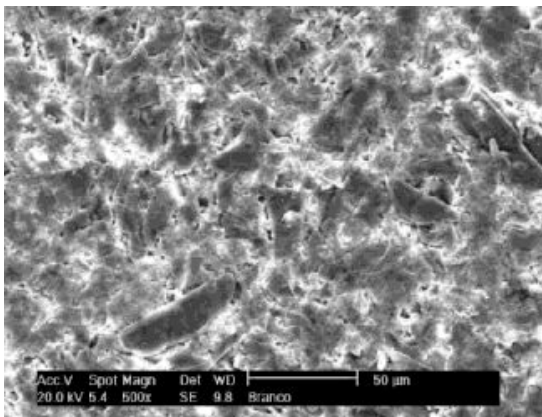
Fig.3. SEM analysis showing acidic blank (left) and acidic alginate 1. Magnification: 500X
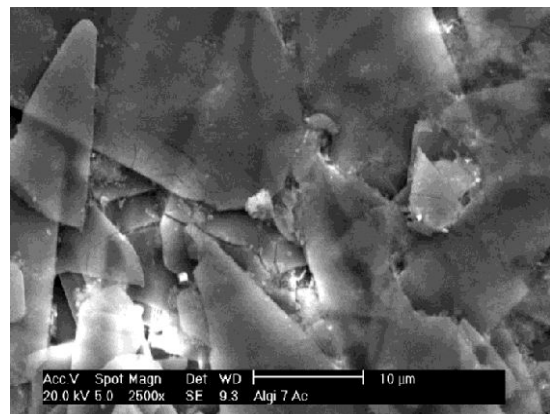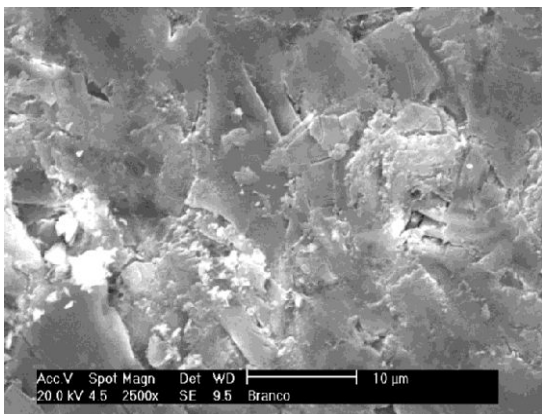
Fig. 4. SEM analysis showing acidic blank (left) and acidic alginate 1. Magnification: 2.500X
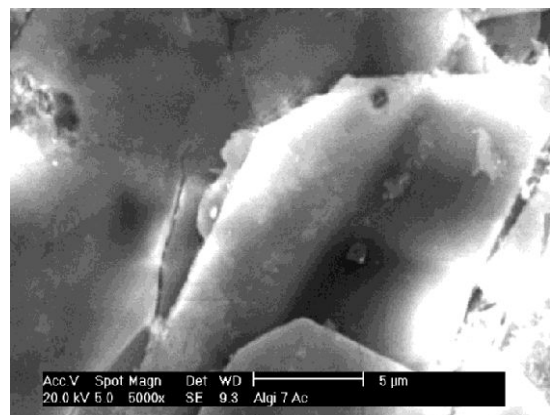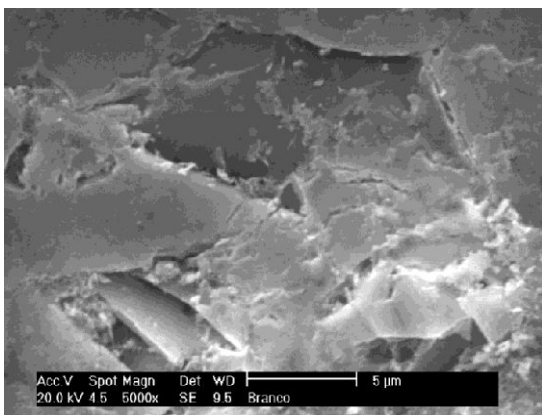
Fig. 5. SEM analysis showing acidic blank (left) and acidic alginate 1. Magnification: 5.000X

Conclusion

Alginate addition during calcium phosphate precipitation affects both phase distribution and morphology of the powders. The first effect is related to degree of dissociation of carboxyl and hydroxyl groups, which is higher at alkaline pHs. Alginate particles also act as heterogeneous nuclei, resulting in larger particles.

References
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