

# Morphological control of precipitated calcium carbonates and phosphates by colloidal additives

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The precipitation of calcium carbonate and calcium phosphates from solutions with and without additives such as hydroxyethyl cellulose, colloidal silica and potato starch, has been studied by X-ray diffraction and scanning electron microscopy. A constant rate of  $10^{-6} \text{ mol s}^{-1}$  of reactant was added into the mixed solutions with and without additives. Nucleation frequency and morphology were dramatically altered by the addition of colloidal potato starch. It was found to be effective for non-specific nucleation of calcite and hydroxyapatite and specific nucleation of monetite. The nucleation frequency of calcite is increased by adding starch by about a factor of  $10^4$ . Starch also alters the shape of hydroxyapatite to fibre-like.

## 1. Introduction

Calcium carbonates and phosphates, as the most common inorganics of bioceramics, are the most important in the study of the biomineralization process. Bioceramics are inorganic–organic nanocomposites functionally optimized by organisms from molluscs to humans to the specific environment in which they live. Bioceramics constitute the hard tissue of most living organisms; they are therefore optimized principally from the view point of mechanical properties [1–5], but a few other functions are also known [6].

Detailed microstructural studies of such tissue have been proceeding for some decades [7–9] with greater details emerging as the characterization tools improve. Humans have looked for replication or substitution of such hard tissue especially in the human system for centuries. For the last century, biomaterials scientists, based on established materials technologies, have provided many substitute materials for living systems – from wood to gold to ceramic to polymers in teeth, to polymer eye lenses, to titanium hip joints. In spite of a great deal of intriguing conjecture and speculation and coining of new terms such as “biomimetic materials”, no new useful materials have been synthesized using this approach, except for one. Indeed, the only commercialized true biomimetic material – a porous hydroxyapatite material for surgical implants based literally on copying coral microstructures – was developed by White *et al.* [10] and Roy *et al.* [11] of Pennsylvania State University.

Today the critical question appears to be: is there anything other than normal chemistry involved in Nature's making of bioceramics, such as seashells and

human bones? We have shown in an earlier paper [12] that by the very simple expedient of slow precipitation of  $\text{CaCO}_3$  in a beaker by counter diffusion, with the addition of hydroxyethyl cellulose (HEC), one could form actual 2–3 cm size shell-like objects composed of highly oriented stacked layers of  $\text{CaCO}_3$  with presumably small amounts of HEC in between (Fig. 1). We showed that microstructure control was HEC-specific, and this morphology control was no doubt due to selective adsorption on the  $\text{CaCO}_3$  surfaces. This control of inorganic crystal morphology by adsorption of organics has been well known since the 1930s [13]. Thus, the role of distinctively “bio” processes in mineralization appears to be minimal. On the other hand, it becomes much more interesting to study the materials science of the control of nucleation and morphology of inorganic chemicals often made in very large tonnages by the addition of small amounts of specific organics. Indeed, understanding this chemistry will illuminate the question of whether there is anything more to biomineralization than rather straightforward chemistry.

From a physical chemistry view point, the most important fact of biomineralization is that nucleation of inorganics can be controlled by organic surfaces by their functionalized groups. Owing to this fact, as is known [14], nucleation takes place at lower supersaturation than is necessary for homogeneous nucleation. Under normal conditions, homogeneous nucleation of inorganics is not probable in organisms.

The driving force of crystallization, for transition of the dissolved matter from a supersaturated solution to the equilibrium crystalline phase, is given by the

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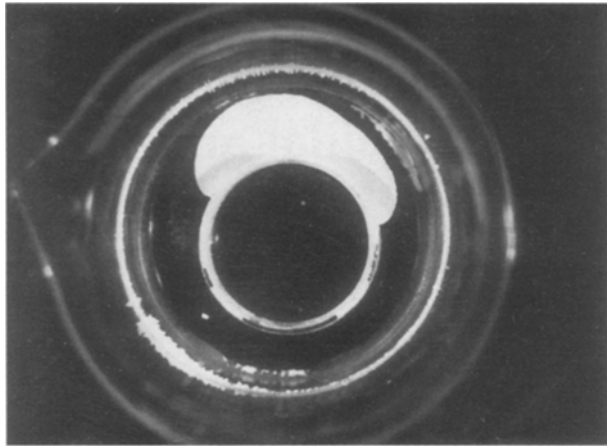


Figure 1 Shell formed in solution by reaction of  $\text{CaCl}_2$  and  $\text{Na}_2\text{CO}_3$  by counterdiffusion of ions through a liquid bridge [12].

change of standard free energy

$$\Delta G^0 = -RT \ln S \quad (1)$$

where  $S$  is supersaturation defined as

$$S = \frac{IP}{K_S^0} \quad (2)$$

where  $IP$  is the actual activity product of ions (forming crystals) in a supersaturated solution and  $K_S^0$  is their thermodynamic solubility product.

Different driving forces or degrees of supersaturation are necessary for homogeneous,  $S_1$ , heterogeneous,  $S_2$ , nucleation and for the growth of the crystals,  $S_3$ . According to the classical theory of nucleation [15, 16], the free energy for formation of a stable nucleus,  $\Delta G_1$ , by homogeneous nucleation, is given by

$$\Delta G_1 = \frac{16\pi(\sigma_{c,l})^3 v^2}{3(-RT \ln S_1)^2} \quad (3)$$

and as heterogeneous nucleation,  $\Delta G_2$  by

$$\Delta G_2 = \frac{16\pi\sigma_{c,l}^3 v^2 (1 - \cos \theta)^2 (2 + \cos \theta)}{3(-RT \ln S_2)^2} \quad (4)$$

or

$$\Delta G_2 = \Delta G_1 \frac{(1 - \cos \theta)^2 (2 + \cos \theta)}{4} \quad (5)$$

where  $S_1$  is the supersaturation for homogeneous nucleation,  $S_2$  for heterogeneous nucleation,  $\sigma_{c,l}$  is the surface energy of crystal/solution interface,  $\theta$  is the contact angle of the formed nucleus,  $c$ , on the substrate,  $S$ ,  $v$  is the solution volume for the molecule and the other symbols represent conventional constants.

As is indicated from Equations 3–5, that for low contact angles of the nucleus on the catalysing substrate, heterogeneous nucleation would be favoured. The free energy of formation of a stable nucleus, or thermodynamic barrier of nucleation is smaller for heterogeneous than for homogeneous nucleation, then from Equations 3 and 4 it follows that

$$\Delta G_1 > \Delta G_2 \quad (6a)$$

or

$$S_1 > S_2 \quad (6b)$$

For the growth of crystals or epitaxial nucleation, the barrier,  $\Delta G_3$ , is even lower, and then can be written

$$\Delta G_1 > \Delta G_2 > \Delta G_3 \quad (7a)$$

or

$$S_1 > S_2 > S_3 \quad (7b)$$

It is important to point out that the thermodynamic product of solubility,  $K_S^0$ , is generally not the same for the water (table values) – crystal system and for the same system with additives (nucleation agents); for example, it is known that the solubility of  $\text{CaCO}_3$  depends on various organic additives [17].

For the biomineralization process, it is important that it is going on at the supersaturation between  $S_2$  and  $S_3$ , at which nucleation can be controlled by organic surfaces.

For the growth of crystals, it is also important to note that the surface energy is not the same for different crystallographic faces [18]. According to the Wulff theorem, each crystallographic face must grow at the rate proportional to its surface energy [16, 18]. Minimizing the total surface energy requires vanishing or minimizing the surface area of the face with the largest surface energy. In the process of biomineralization, this principle is altered by adsorption of water-soluble polymers on some crystallographic faces [8, 9, 14]. The shape of crystals inside systems can, of course, also be influenced by space limitations [19].

Following this line of reasoning and our earlier results [12], this research therefore focused on the influence of different additives; hydroxyethyl cellulose, colloidal potato starch and colloidal silica particles (300 nm) on the precipitation of calcium carbonates and phosphates, i.e. phases, which are usually present in bioceramics.

## 2. Experimental procedure

Calcium phosphates were synthesized using  $(\text{NH}_4)_2\text{HPO}_4$  (Alfa)  $\text{NH}_4\text{H}_2\text{PO}_4$  (Fisher) and  $\text{CaCl}_2$  (Aldrich) and calcium carbonates using  $\text{Na}_2\text{CO}_3$  (Baker) and  $\text{CaCl}_2$  (Aldrich Chemicals).

As nucleation substrates, the following materials were used: colloidal silica particles ( $\approx 300$  nm), hydroxyethyl cellulose (HEC, Aldrich Chemicals) and colloidal potato starch (PS, Fisher Scientific) all in the form of 1 wt % water solutions.

Colloidal silica particles were prepared by base-catalysed hydrolysis/polycondensation reactions of silicon ethoxide according to a procedure described by Sacks and Tseng [20]. Colloidal solutions of PS were prepared by peptization of mixed (magnetic stirring) dispersion of starch at  $\approx 90^\circ\text{C}$ .

All reactions were carried out at room temperature in 100 ml volume of deionized water or in 1 wt % solutions of additives. Reactants were dissolved (0.01 mol  $\text{CaCl}_2$  in 50 ml water, likewise equivalent anionic reactants) in water. The  $\text{Na}_2\text{CO}_3$  and  $(\text{NH}_4)_2\text{HPO}_4$  reactant solutions were added dropwise

from burettes into the mixed liquid (water or solutions containing additives for nucleation). The rate of reactant additions was kept constant ( $\approx 10^{-6}$  mol s $^{-1}$  at all experiments).

Precipitation of crystals was visually evident in the increasing turbidity of solutions in all cases, using anionic reactant solutions of Na<sub>2</sub>CO<sub>3</sub> and (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>. In the case of the NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> solution, a metastable solution of CaCl<sub>2</sub> and the H<sub>2</sub>PO<sub>4</sub> [21] was obtained, which crystallized after the increase of pH by the addition of NH<sub>4</sub>OH.

The crystals obtained were washed by using multiple-forced sedimentation (centrifuge) and redispersion of crystals in deionized water. HEC and PS were removed from the system only partly. Then, the crystals were dried at  $\approx 60^\circ\text{C}$  and used for XRD detection (Scintag PAD 5 diffractometer with CuK $\alpha$  radiation and a germanium solid-state detector) and SEM observations (ElectroScan Model E-3).

### 3. Results

#### 3.1. Precipitation of calcium carbonates

The nucleation substrates show no specific nucleation influence on precipitation of calcium carbonate. In all cases (water, solution of HEC and PS) precipitated calcite of different sizes (Table I) and different shapes (Fig. 2a, b) were observed. The shape of calcite crystals precipitated in water and in HEC solutions is similar. There are brick-shaped crystals in both cases, only the size is consistently smaller in the presence of HEC. The size of calcite crystals with the addition of PS (Fig. 2b) was significantly decreased. Calcite crystals under these conditions have the shape of thin plates with a thickness about 0.1  $\mu\text{m}$ . The relative frequency ( $N/N_0$ ) of calcite nucleation (Table I), calculated from

TABLE I The mean size and relative nucleation frequency,  $N/N_0$ , of calcite crystals in dependence of used substrate

Substrate	Size ( $\mu\text{m}$ )	$N/N_0$
—	7	1
HEC	3	13
PS	0.6	$2 \times 10^4$

the size of crystals ( $N = (1/\text{volume of one particle})$ ), with respect to deionized water ( $N/N_0 = 1$ ) is slightly higher in the HEC solution, but it is significantly increased in the PS solution ( $N/N_0 = 2 \times 10^4$ ).

#### 3.2. Calcium phosphates

##### 3.2.1. Reaction of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> with CaCl<sub>2</sub>

Precipitation of calcium phosphates was visually observable after the addition of  $\approx 1.5 \times 10^{-4}$  mol calcium and phosphate ions. In this case, a specific nucleation effect of the nucleation substrates used was observed. In water solutions of SiO<sub>2</sub> and HEC, the precipitated brushite is very similar in shape, but the XRD patterns show a slight variation in peak position. The shape of the particles in all three cases is practically the same (Fig. 3a, b). There are thin, relatively large aggregated plates (10  $\mu\text{m} \times 10 \mu\text{m}$ ), in water (Fig. 3a) and HEC solution. Colloidal silica particles partly prevent the aggregation of brushite plates. The thickness of these plates appears to be less than 0.1  $\mu\text{m}$  in the presence of 0.3  $\mu\text{m}$  silica particles (Fig. 3b).

In the experimental route used, colloidal PS shows specific nucleation effects for calcium phosphates. The crystal phase and also the shape of the particles differ from the above mentioned case (H<sub>2</sub>O, SiO<sub>2</sub> and HEC). The crystal phase precipitated, changes to monetite. Two types of crystal are present (Fig. 4); mostly they are brick-like particles (size from 3  $\mu\text{m} \times 8 \mu\text{m} \times 10 \mu\text{m}$  to 4  $\mu\text{m} \times 9 \mu\text{m} \times 13 \mu\text{m}$ ) and less frequently they are rods of length 4–5  $\mu\text{m}$  and thickness  $\approx 0.5 \mu\text{m}$ . This change of phase is a rather surprising result.

##### 3.2.2. Reaction of NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> with CaCl<sub>2</sub>

Colloidal potato starch (PS) showed the largest nucleation effect in preliminary experiments; detailed studies were conducted with this additive.

Mixing of solutions of NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> and CaCl<sub>2</sub> leads, both in water and PS solutions, to the formation of metastable solutions [21] without crystal precipitation during 2 days. Increasing the pH of the solution using NH<sub>4</sub>OH caused precipitation of brushite. The pH is very nearly constant (pH = 4.0) during the

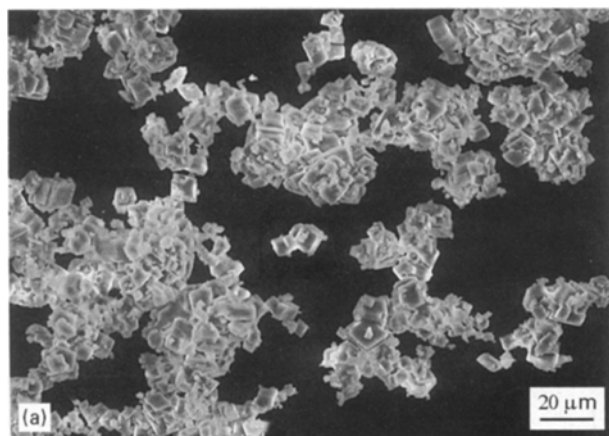


Figure 2 Scanning electron micrographs of calcite precipitated in solutions of (a) deionized water, (b) 1 wt % colloidal starch.

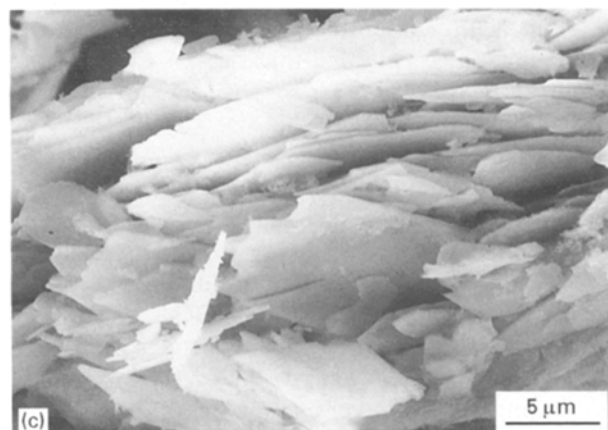
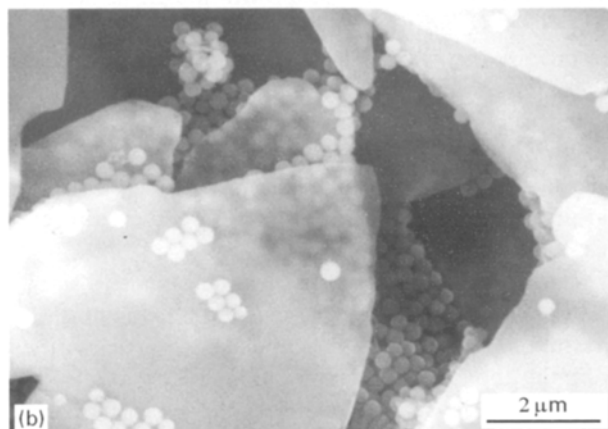
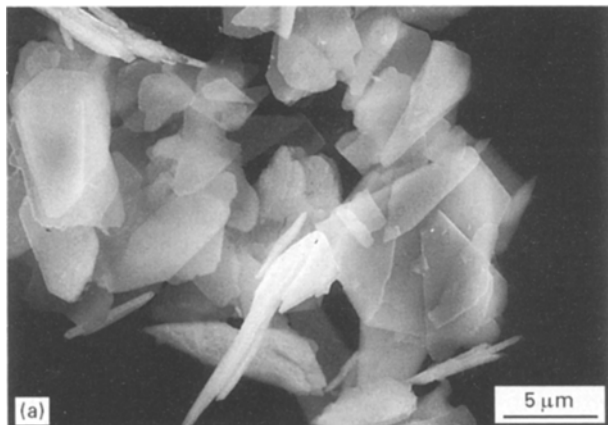


Figure 3 Scanning electron micrographs of brushite precipitated by reaction of  $(\text{NH}_4)_2\text{HPO}_4$  and  $\text{CaCl}_2$  in (a) water, (b) a 1 wt % water solution of colloidal ( $\approx 300$  nm)  $\text{SiO}_2$  particles, and (c) a 1 wt % water solution of hydroxyethyl cellulose.

addition of  $\text{NH}_4\text{OH}$  (Fig. 5) up to the stage when precipitation is finished (intensive pH change). In both cases ( $\text{H}_2\text{O}$ , PS-solution) brushite precipitated in a similar way but yielded different sized plates (Fig. 6).

In the next two experiments, both reactants ( $\text{NH}_4\text{H}_2\text{PO}_4$  and  $\text{CaCl}_2$  solutions) were continuously added and the pH of the mixed reaction solution was kept constant at a value of 5.5 or 7.4 ( $\pm 0.1$ ) by simultaneous addition of dilute solutions of  $\text{NH}_4\text{OH}$ . At pH 5.5, as well as at pH 4.0, brushite of similar shape was formed in water and also in PS solution. Using the same route of reaction at pH 7.4, hydroxyapatite (HAP) was formed in both reaction solu-

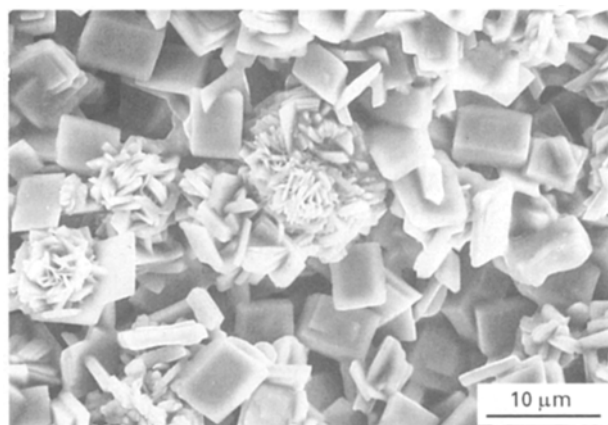


Figure 4 Scanning electron micrograph of monetite (calcium phosphate) precipitated by reaction of  $(\text{NH}_4)_2\text{HPO}_4$  and  $\text{CaCl}_2$  in a 1 wt % water solution of colloidal potato starch.

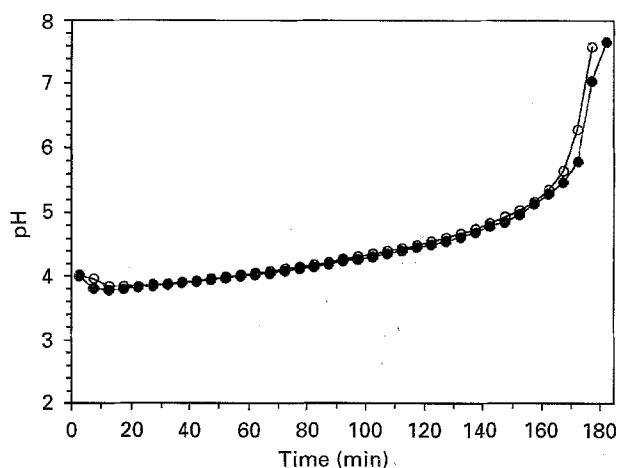


Figure 5 pH change of metastable solution of  $\text{NH}_4\text{H}_2\text{PO}_4$  and  $\text{CaCl}_2$  at 5 min intervals after addition of 0.1 ml 5 wt % solution of  $\text{NH}_4\text{OH}$  (stabilized value). (○)  $\text{H}_2\text{O}$ , (●) starch.

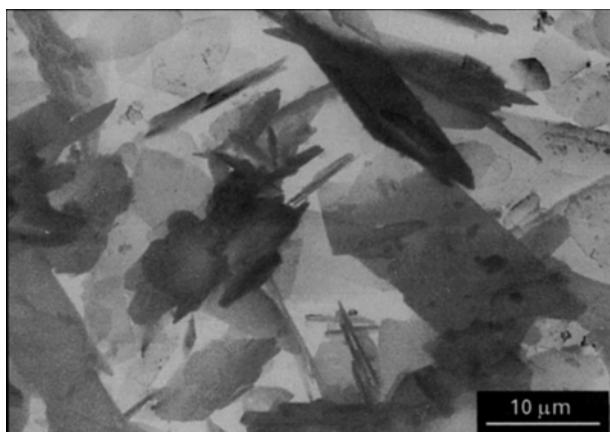


Figure 6 Scanning electron micrograph of brushite precipitated by reaction of  $\text{NH}_4\text{H}_2\text{PO}_4$  and  $\text{CaCl}_2$  at pH  $\approx 4$  in water.

tions. The nucleation frequency of HAP, as is evidenced from scanning electron micrographs (Fig. 7a, b) is very high in both cases. In water, the shape of particles is monosized (Fig. 7a), but in PS-solution, small fibres are observable (Fig. 7b).

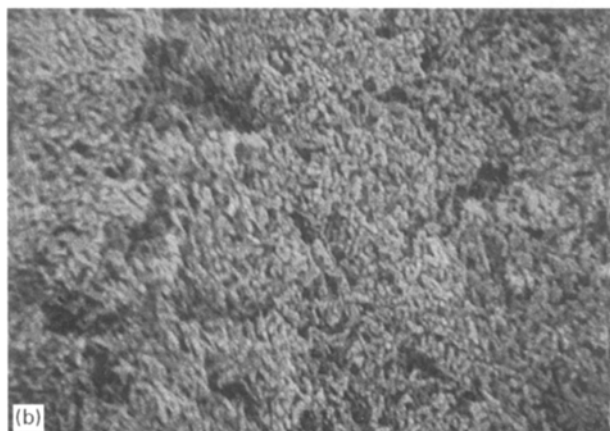
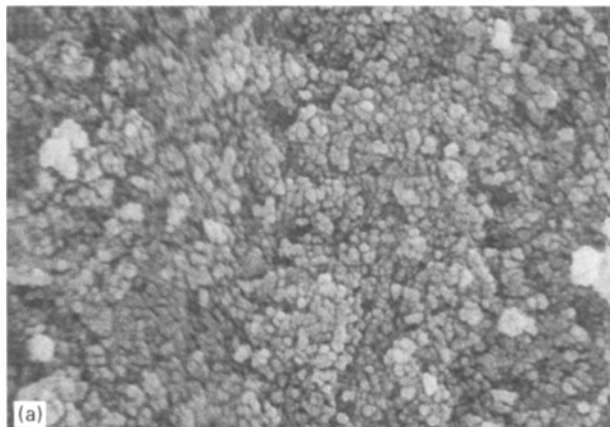


Figure 7 Scanning electron micrographs of HAP precipitated at pH 7.4 from reaction of  $\text{NH}_4\text{H}_2\text{PO}_4$  and  $\text{CaCl}_2$  in (a) water, (b) 1 wt % solution of colloidal potato starch.

#### 4. Discussion

Non-specific nucleation effects can be assumed in all colloids or polymeric species, which can adsorb ions of one or both reactants on the surface. All three particulated nucleation agents used can be regarded as such substrates. They are covered (colloidal silica) or contain OH groups (HEC, PS). The OH groups can react with or adsorb the  $\text{Ca}^{2+}$  cations [22].

Non-specific nucleation effects were observed on the nucleation of calcite, brushite and HAP by colloidal silica, HEC and PS.

The specific effect of colloidal potato starch was observed in the precipitation of monetite instead of brushite in the reaction of  $(\text{NH}_4)_2\text{HPO}_4$  with  $\text{CaCl}_2$ .

In our opinion, specificity or non-specificity of particulated nucleation agents is not only the result of the surface structure (functionalized groups) but in the first order it depends on the pH of reaction surroundings and the concentration or rate of reactant addition. In other words, for specific nucleation effects of a substrate, scanning the pH and the rate of addition of a reactant throughout the reaction route, should be done.

#### 5. Conclusion

Colloidal potato starch increased the nucleation frequency of calcite about  $10^4$  times with respect to the

same solution ( $\text{Na}_2\text{CO}_3$  and  $\text{CaCl}_2$ ) reaction route without starch.

This starch showed specific nucleation effects on monetite in comparison to the same solution  $(\text{NH}_4)_2\text{HPO}_4$  and  $\text{CaCl}_2$  reaction route in water (without additives) and in the solutions of colloidal silica particles or hydroxyethyl cellulose.

In the reaction of  $\text{NH}_4\text{H}_2\text{PO}_4$ ,  $\text{CaCl}_2$  and  $\text{NH}_4\text{OH}$  in solution (with and without additives  $\text{SiO}_2$ , hydroxyethyl cellulose) at pH  $\approx 4$  and  $\approx 5.5$ , the effects of additives on the crystallization of brushite were observed. At pH 7.4, the nucleation frequency of hydroxyapatite is very high (the size of the particles is  $\approx 10$  nm) with and without potato starch, but the shape of the particles is a little different (monosized in water and fibre like in the presence of starch).

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