

NUCLEATION AND SELECTIVE GROWTH OF POLYMORPHS OF CALCIUM CARBONATE ON ORGANIC-INORGANIC HYBRID FILMS

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ABSTRACT

Inorganic/organic ternary hybrid films that are constituted of gelatin (G), poly(monomethyl itaconate) (PMMI) and silica (SiO₂), are used as a solid matrix to study the crystallization of calcium carbonate (CaCO₃). These films are transparent and stable in aqueous solutions due to the formation of interpenetrated networks. In these films it is possible to modulate the concentration of the different functional groups and the flexibility of the film structure by changing the composition. These changes in composition affected the morphology of the CaCO₃ crystals and the polymorph obtained on the films. Depending on the film compositions, fried-egg-like or spherical calcite particles were observed on the films. Films of a specific composition induced the nucleation of vaterite, the less stable polymorph of calcium carbonate.

Keywords: Chitosan, Organic-Inorganic Hybrid materials, Calcium Carbonate, Biom mineralization.

INTRODUCTION

The term biom mineralization refers to the process by which live organisms produce inorganic-organic composites, in which the organic component exerts substantial control on the mineralization process. Biom mineralization is a complex process where particles of uniform size, novel crystal morphology and with specific crystallographic orientation are produced. In nature, there are infinite examples, from bones, mollusk shells and teeth to small magnetic particles produced by bacteria. The extraordinary control that nature exerts on crystal size, shape and crystallographic orientation is surprising, and the properties of the resultant materials such as high solidity, resistance to fracture and aesthetic values represent a challenge for the synthesis of similar materials. These processes are so complex at the molecular scale that even for the simplest hard tissues the details are not fully known, although significant progress has been made.¹⁻⁴

The predominant component of mollusk shells is calcium carbonate (CaCO₃), which is abundant in biominerals and is one of the most widely studied systems. In living organisms the biomacromolecules that participate in the manufacture of these biocomposites can be classified as water-soluble proteins and water-insoluble macromolecules such as chitin and collagen, which function as solid matrices. These two kinds of macromolecules have an important role in the control of the morphology and the specific kind of polymorphs of the CaCO₃ crystals.⁵ Much of the research done in this field has been aimed at understanding the influence of basic factors such as the microstructure of the growth medium, the functional groups within the matrix, and the presence of proteins in solution or adsorbed on the surface of the lattice.

Among the approximations or models applied to the study of the mineralization of CaCO₃, soluble polymers with specific functional groups⁶⁻⁸ and insoluble substrates in the presence of soluble polymers or inhibitors, which usually contain acid groups, have been used.⁹⁻¹¹ In the first case it is postulated that the polymer selectively adsorbs onto specific crystal surfaces of the mineral and in this way affect its morphology and polymorphism. On the other hand, when insoluble substrates are used in the presence of so called inhibitors, it seems necessary to have the latter in solution to favor heterogeneous crystallization and at the same time inhibit the formation of nuclei in solution.

Crystallization in gels has been studied and the gels have served to control the ion diffusion processes in the medium. Fallini et al. induced the orientated crystallization of CaCO₃ by cross-linked gelatin films with entrapped poly-L-aspartate.¹²⁻¹⁴ The structure of the polyelectrolyte-gelatin assembly was changed through mechanical deformation. No orientation of the mineral phase was observed with entrapped poly-L-glutamate at the same concentration. These results suggested that the orientated crystallization is controlled by the β -sheet structure of poly-L-aspartate. In addition, collagen bundles and the orientated polypeptide chains can contribute to the control of the polymorph of the calcium carbonate.

In this work we study the effect of ternary hybrid films constituted of gelatin (G), poly(monomethyl itaconate) (PMMI) and silica (SiO₂), on the crystallization of CaCO₃ from aqueous solutions. The inorganic component of the films was prepared by the sol-gel process of tetraethylortosilicate (TEOS) which allows an intimate association of the SiO₂ phase with the organic components. The ternary hybrids films obtained in this way were robust and sufficiently stable to study the crystallization of CaCO₃ without destroying the matrix. It was also possible to control the flexibility of the films by varying the concentration of the inorganic component. In addition to their physical properties, the composition of the hybrid films can be easily changed over a wide range, thereby modifying at will the availability of active groups that can potentially affect the crystallization process.

2. EXPERIMENTAL

Gelatin type B, 300 bloom, from Aldrich was used as a 3% solution in dilute formic acid (5%). In order to obtain the siloxane sol, a solution of tetraethylortosilicate (TEOS) from Aldrich in ethanol was hydrolyzed with an aqueous solution of formic acid diluted in ethanol in order to have a molar composition of TEOS:H₂O:formic acid:EtOH = 1:1:0.01:10. This solution was kept at 50 °C in an open container during one day.

Monomethyl itaconate (MMI) was prepared by reaction of itaconic acid with methanol under fairly acidic conditions by the method described by Baker et al. for lower monoesters.¹⁵ Poly(monomethyl itaconate) (PMMI) was obtained by radical polymerization of MMI in bulk under N₂ at 75 °C in the presence of 0.2 mol% of azobisisobutyronitrile as radical initiator. PMMI was purified by repeated dissolution in methanol and precipitation in diethyl ether. The apparent number-average and weight-average molecular weights ($\bar{M}_n = 1.1 \times 10^5$ g mol⁻¹, $\bar{M}_w = 1.6 \times 10^5$ g mol⁻¹) was determined by gel permeation chromatography (GPC) using a Waters 600 HPLC fitted with a Shodex 803 column and a differential refractive index detector. Water containing 0.1 M NaCl was used as eluent at a flow rate of 1 ml/min. Column calibration was performed by using Pullulan standard samples.

Film preparation: Siloxane sol and PMMI solution in ethanol were mixed in the appropriate ratios and stirred for 30 min. The gelatin solutions were then incorporated and the mixture was stirred for 24 hours. Films of 140-145 μ m thickness were obtained by depositing the hybrid solution in a polystyrene Petri dish. The samples were dried at ambient temperature for two weeks.

Swelling experiments: The swelling behavior was measured by immersing pre-weighed dry samples of about 200-250 mg in pH 4, pH 3 or pH 9 solutions at 37 °C. The water uptake was calculated by measuring the weight gain of the samples in a given time interval. Excess surface water was blotted out with filter paper before weighing. The weight degree of swelling (W_{sd}) was calculated using the following equation:

$$W_{sd}(\%) = \frac{W_s - W_d}{W_d} \cdot 100$$

where, W_s and W_d denote the weight of the swollen and dry samples, respectively.

Mineralization of CaCO₃: 100-150 mg of G/SiO₂/PMMI hybrid films having different composition were placed in Petri dishes with 10 mL of 200 mM CaCl₂ solution. The solution was prepared with deionized water or with pH 9 buffer solutions. Calcium carbonate crystals were grown on the films at 25 °C by slow diffusion of (NH₄)₂CO₃ vapor into the calcium chloride solution, as described by Arias et al.¹⁶ Thereafter, the films were washed thoroughly with water, dried and analyzed.

Morphological investigations were carried out with a Leica DLML optical microscope and a JEOL (JSM 5410) scanning electron microscope with an EDS (Energy Dispersive Spectroscopy) microprobe to carry out elemental analysis using the Vantage 1.4 software. X-ray diffraction (XRD) was performed using a Siemens D-5000 X-ray diffractometer with Cu-K α radiation. The polymorphs of CaCO₃ were determined on the basis of powder X-ray diffraction patterns.

RESULTS AND DISCUSSION

The G/SiO₂/PMMI ternary hybrid films were obtained by the sol-gel process of TEOS, which allows an intimate integration of the organic and inorganic components under mild synthetic conditions. In this way brittle transparent films were obtained, sufficiently stable to be handled without disintegrating in the working solution. The transparency of the material indicates that there is no phase separation at a scale larger than that of the wavelength of visible light, probably due to the formation of interpenetrated polymer networks, as reported for a similar system.¹⁷

During the contact between the films and the CaCl₂ solution there is swelling of the films, showing a hydrogel behavior. The swelling of a hydrogel is produced by the difference between the osmotic pressure of the mobile ions in the gel and in the external solution, described by Donnan's theory. A high concentration of ions increases the flow of water into the gel by osmosis, leading to increased swelling of the gel. The electrostatic interactions between the chains of macromolecules, which depend on pH, also play an important role in the swelling properties. Figure 1 shows the values of W_{sd} as a function of time at pH 9 and pH4 for hybrid films of different composition (Table 1).

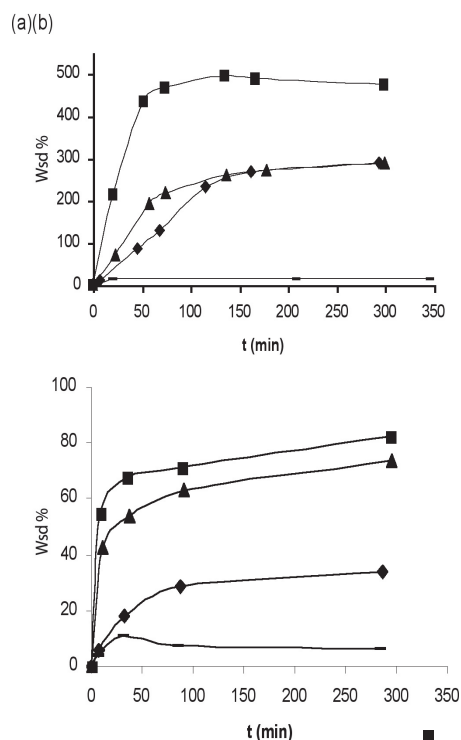


Figure 1. Water sorption isotherm for GSP1 (\blacklozenge), GSP2 (\blacksquare), GSP3 (\blacktriangle) and GS0 (\circ) at pH 9 (a) and pH 4 (b) at 37 °C.

Table 1. Change of W_{sd} (%) $_{\infty}$ with composition and pH at 37 °C.

Sample	Composition G:SiO ₂ :PMMI	pH 9	pH 4	pH 3
GS0	50:50:0	15	6	17
GSP1	45:30:25	290	35	42
GSP2	60:20:20	480	85	-
GSP3	65:30:5	290	74	120
GSP4	60:5:35	-	-	-

Table 1 shows the values of W_{sd} (%) $_{\infty}$ at equilibrium obtained for films of different compositions as function of pH. At pH 9 a swelling value of ca. 500 % is obtained for GSP2. However, GSP1 and GSP3 samples, having the same SiO₂ content but very different PMMI/Gelatin ratio, show the same W_{sd} value. The influence of the inorganic phase with respect to the flexibility of the film structure is clearly seen. Therefore, as the SiO₂ content of the hybrid film increases, the material swelling capacity decreases considerably. This was attributed to the more rigid structure of the silica phase.

Sample GSP4, containing only 5 % of SiO₂, disintegrates in the buffer solutions and can not be used in the crystallization studies. This shows that the inorganic component plays the mayor role in stabilizing the films. At acid pH, the values of W_{sd} are much smaller because it is a value that is closer to the isoelectric point of the gelatin used in this study.

Crystallization of CaCO₃ on the hybrid films. To study the crystallization of CaCO₃, a system extensively tested in the work of Arias et al. was used.¹⁶ The influence of crystallization conditions such as pH was also studied. The Ca²⁺ precursor ions are incorporated into the film either as counterion of PMMI and/or complexed by the functional groups of the gelatin.

Figure 2 shows images of the hybrid films obtained using an optical microscope under polarized light. These experiments were performed without buffer solution. It can be observed that under these conditions no crystallization occurred on the binary film constituted by gelatin and silica (GS0). Only rhombohedral calcite crystals were observed in the solution. On the other hand, the ternary hybrid films have a noticeable influence on the crystallization of CaCO₃, structures different from the typical rhombohedral calcite crystals are observed on the surface of these films after carefully washing them with distilled water. The ternary film GSP1 (45:30:25) seems to stabilize the formation of spherulitic structures on the films. On the other hand, when the percentage of silica is decreased in film GSP2 (60:20:20), the crystallization of oval crystals occur.

In the binary film G/SiO₂ the functional groups that could affect the nucleation and growth of CaCO₃ crystals must be associated with the silica silanol groups and are not longer available to interact with the crystals under formation. Furthermore, the swelling value obtained for this film is very low which also indicates that the hydrogel structure flexibility has a significant influence on the nucleation and growth of the calcium carbonate.

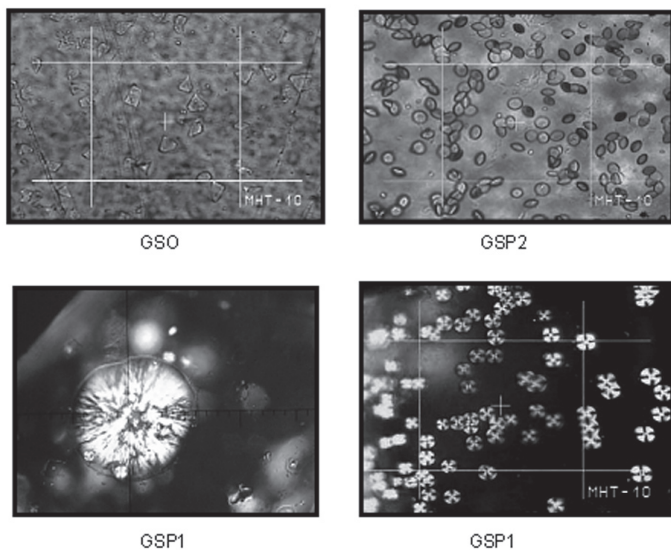


Figure 2. Optical microscope images of the CaCO₃ formed in the hybrid films GSO, GSP1 and GSP2 under polarized light.

Films GSP1 and GSP2 were analyzed by X-ray diffraction and the diffractogram obtained for GSP2 is shown in Figure 3.

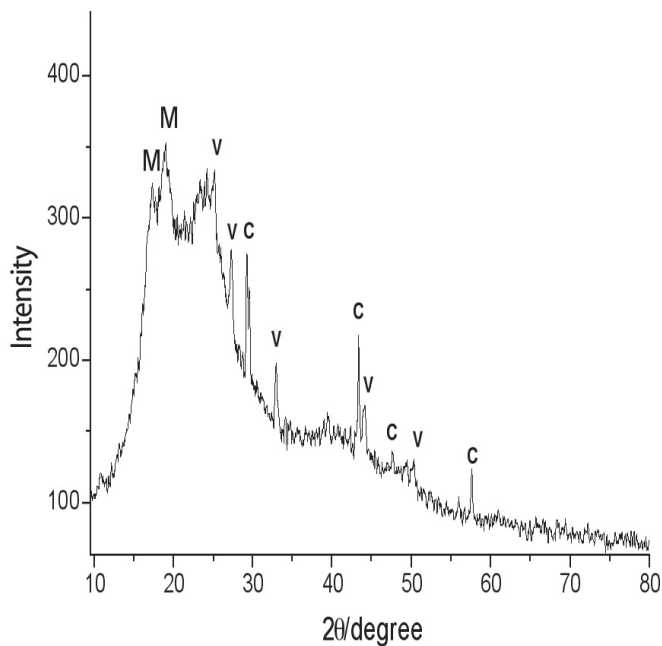


Figure 3. X-ray diffraction pattern of CaCO₃ formed on the hybrid film GSP2. CaCl₂ 200 mM without buffer solution. (c, calcite; v, vaterite; M, matrix).

In the diffractogram of GSP2 the reflections correspond to the diffraction patterns of vaterite and calcite, while in film GSP1 only formation of calcite is identified. The reflections are overlapped with the wide signal characteristic of the amorphous nature of the film, however the reflections at 2θ = 17 and 19 probably correspond to a rearrangement of the matrix induced by the crystallization conditions.

Figure 4 shows the micrographs obtained for these films. Film GSP2 is practically covered with 5 and 14 μm oval particles arranged in different areas of the film. This morphology is typical of vaterite, the metastable polymorph of calcium carbonate.

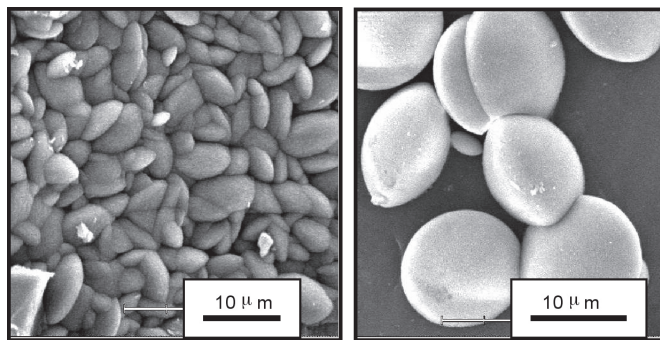


Figure 4. SEM images of CaCO₃ crystals formed on GSP2 film without using buffer solution.

The results of the X-ray diffraction as well as observation under polarized light indicate that these particles are made of calcite and vaterite crystallites. The regular shape of these particles suggests that they may be mixed particles in which the polycrystalline core of a polymorph (calcite) is surrounded by an outer polycrystalline layer of the other polymorph (vaterite), as has been seen in previous cases in which polycrystalline calcite cores are surrounded by a polycrystalline layer of aragonite.¹⁸

Figure 5 shows the images obtained by scanning electron microscopy for GSP1 film (Figure 4 left). Both surfaces of the film show the formation of circular structures of approximately 200 μm. The results obtained by EDS in two different zones (X and Y) are also shown, as well as the image obtained by using back scattering or retrodispersed electrons (Figure 4 right). In this image, the range of grays identifies the already assigned elements so that those closest to C, i.e. the lighter ones, are shown darker, while the heavier elements appear lighter. The set of analyses carried out corroborated that these structures are thin calcite films having a circular geometry in the shape of fried eggs.

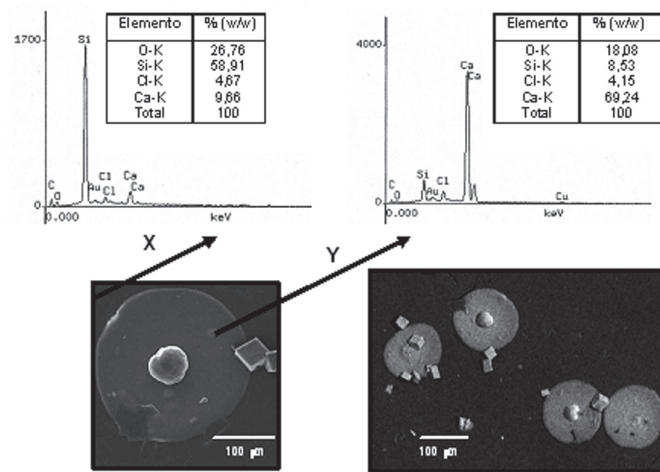


Figure 5. Micrographs obtained for GSP1 without using buffer solution. Images obtained by SEM (left) and by back scattering (right). The insets show the results obtained by EDS.

Even though the biomineralization and crystallization processes associated with CaCO₃ have been studied extensively, there are relatively few reports regarding the preparation of CaCO₃ films.^{10,11,19-21} Recently, Cho et al.²² reported the formation of CaCO₃ films on silicon wafers, and they showed evidence regarding the multistep crystallization of these structures, which involves the formation of ACC films and their subsequent transformation into crystalline structures.

The identification of the metastable vaterite phase on the surface of the GSP2 film with a slightly higher percentage of gelatin and less SiO₂, shows the role of the biopolymer functional groups in controlling nucleation and CaCO₃ crystal growth. In this film not only the change in the morphology occurs, but

also a metastable phase become stabilized. The diffractogram obtained after seven months keeps showing the presence of the metastable phase vaterite without apparent changes.

One of the characteristics of gelatin is its high glycine, proline and hydroxyproline content. Structurally, the gelatin macromolecules contain repetitive sequences of X-Y-glycine triplets, where X and Y are generally proline and hydroxyproline.²³ A typical fragment is -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro. The acid groups from glutamic acid and aspartic acid, which are ionized at the pH of the experiment (8.5) at which crystallization occurs ($pK_a = 4$ and 4.5) may give rise to a higher local concentration of Ca^{2+} ions. However, it is difficult to discard the possibility that the acid groups from PMMI could also act as nucleation centers. In addition, the matrix hybrid structure could also be acting as a template and have an important role in the observed results.

It is possible to vary the concentration of the different functional groups in these hybrid films by simply changing the composition and also by controlling the flexibility of the structure by changing the silica content. Both factors modify the microenvironment of the nucleation and growth sites. This allowed controlling not only the morphology but also the type of polymorph of the calcium carbonate that was formed on the films.

The crystallization studies were also carried out using a $CaCl_2$ solution prepared with pH 9 buffer. Figure 6 shows the diffractograms of the $CaCO_3$ crystals formed on films GSP2 and GSP1. In this case the diffraction patterns indicate only the presence of calcite over the hybrid films. The morphology of the crystals can be observed in Figure 7.

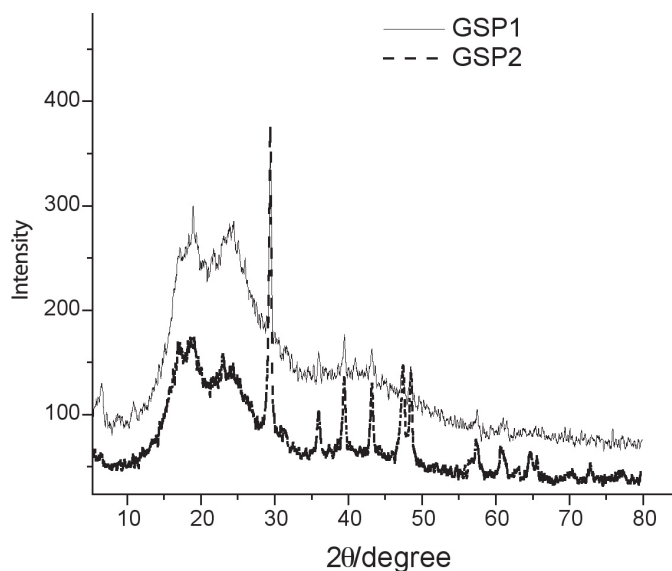


Figure 6. Diffractogram of the crystals formed on films GSP2 and GSP1 using 200 mM $CaCl_2$ with pH 9 buffer solution.

Under these conditions, both films show crystallization of calcite with spherical morphology and particles of approximately 10-12 μm . The film with the highest gelatin percentage (GSP2) is practically covered with these particles which have a smooth surface, while on GSP1 a sporadic nucleation is seen, and the surface of the particles is rough. However, although in both cases there is a change in the morphology, no formation of vaterite is seen when using pH 9 buffer solutions. This shows the sensitivity of the experimental conditions used on the results obtained.

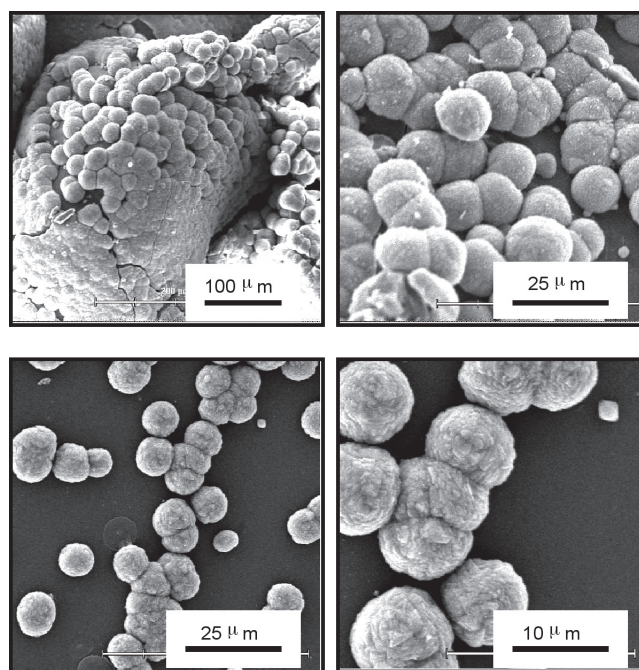


Figure 7. Micrographs of $CaCO_3$ formed on GSP2 film (top) and GSP1 film (bottom) using pH 9 buffer solution.

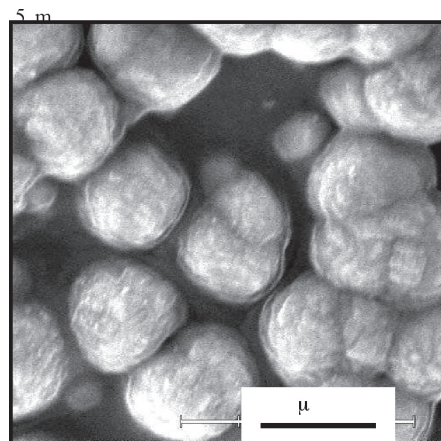


Figure 8. Micrograph obtained for GSP2.

In the case of GSP2 film, it was possible to observe that these calcite spheres may start their formation in the inner layers of the film (Figure 8). Hybrid films with different percentages of gelatin may show up to 500% swelling at pH 9. This ensures the formation of spaces within the film, where the formation of nuclei may begin, and these nuclei may continue growing at the expense of the diffusion of more ions.

CONCLUSIONS

The effect of hybrid ternary films on calcium carbonate crystallization was studied in the present work. The results show that the hybrid films can be used as matrices to control the polymorph that is formed on the films. The importance of the physico-chemical characteristics of the matrix in controlling the morphology and polymorphism of the crystals formed is evidenced through this study. In these hybrid films it is possible to favor the crystallization of a metastable polymorph of the calcium carbonate and inhibit its transformation into the more stable phase, or modify the morphology of the mineral by changing the composition of the films and/or the crystallization conditions.

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