

CALCIUM CARBONATE PRECIPITATION: A REVIEW OF THE CARBONATE CRYSTALLIZATION PROCESS AND APPLICATIONS IN BIOINSPIRED COMPOSITES

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Abstract. Recently, bioinspired materials have received particular interest for their unique properties and applications as well as their non-toxicity, which make them a target for intensive research. The principal interest of this review lies on the study of calcium carbonate and its polymorphs and how they interact with different substrates, in general, and with biopolymers, in particular. This review hinges on the synthesis of calcium carbonate particles (aragonite, calcite and vaterite phases) using methodologies such as: gaseous diffusion and reactants mixing. The synthesis of the particles involves some variables which play a decisive role in the formation of calcium carbonate particles. Some of those variables include pH, temperature, concentration of solutions, concentration of additives, type of additives (organic or inorganic) and the substrate surface roughness. All of them are fundamental in the precipitation of the crystals, motivating scientists to focus more on the study of the additives on the precipitation of the crystals. Some widely used additives such as poly(acrylic acid), polyacrylamide and poly(vinyl alcohol) are discussed. Furthermore, since CaCO_3 precipitation can occur on biopolymers or other organic substrates, these biopolymers have been used to create organic-inorganic compounds exhibiting unique properties also discussed in this review. Emphasis on abundant biopolymers such as chitin, chitosan and cellulose is provided in this review and aims at bettering the understanding of potential applications of those organic-inorganic composites. Processes involving calcium carbonate precipitation are also explained in detail as well as how the previous variables play a pivotal role in the resulting compound.

1. INTRODUCTION

Bioinspired and biomimetic materials are used to fabricate products that have a nature-based structure or are fabricated by mimicking processes or phenomena found in natural materials. These materials possess the advantage of being naturally designed as well as adapted or functionalized. Such functionalization provides them with the capacity of being more resistant and elastic compared to their kin in the original or natural state. The characteristics of biomimetic materials are vast. Their

biocompatibility with the environment, non-toxicity, and biodegradability are among the most important characteristics [1,2]. Additionally, they can be produced by low energy consumption means and can render prolonged useful lifetime to the final product. As a result, the evolution of biomimetic materials has expanded the range of applications of these materials. In recent decades, in particular, scientists have focused on the study of biomimetic materials in order to manufacture new composites made of biopolymers and calcium carbonate.

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1.1. Biopolymers

Biopolymers have attained great potential in the market due to their vast applications in fields such as “bio-ceramic, bio-sensing, biomedical engineering, bio-nanotechnology and biologically assembly” [3]. As aforementioned, recent and increasing interest in these materials is due to their unique characteristics such as: biocompatibility, eco-friendly qualities and nontoxicity [1,2]. The most common biopolymers are chitin, chitosan and cellulose. These biopolymers present many applications in the medical, food, water treatment, industrial and agricultural fields; they also possess good biocompatibility with human body tissues and fluids [4].

After cellulose, chitin is considered the most abundant natural polymer. Chitin consists of groups of β -(1,4)N-acetyl glucosamine which are repeated in the structure of the polymer with multiple hydrogen bonding [5]. The glucosamine position in the structure can change to α (antiparallel), β (parallel) and γ (combined), which indicates the differences in packing and polarities of the chains. Of those structures, α -chitin is the most stable form and can be dissolved in stronger swelling agents such as aliphatic diamines [1,6]. Additionally, α -chitin has a highly crystalline structure with intra-inter hydrogen bonding, which limits the access of the solvent into the network [5]. Contrary to α -chitin, a more open structure is obtained for β -chitin that makes it susceptible to swelling; polar molecules (water or alcohol) can penetrate the structure of β -chitin and destroy the hydrogen bonds of the structure [5]. Consequently, N-dimethylacetamide/LiCl is the most common system to dissolve α -chitin for its crystalline structure [6].

Moreover, chitosan is a cationic polysaccharide with a molecular structure made of hydroxyl and amino groups and the structure consisted of D-glucosamine and N-acetyl-D-glucosamine [5,7]. This biopolymer is a derivate of chitin and is produced via the alkaline N-deacetylation of chitin, which consists of removing the acetate group and replacing it with an amide group in alkaline solutions [2,6]. The degree of deacetylation of chitin must be at least about 50% to be considered chitosan and to render it soluble in aqueous acidic media [6]. The mechanism of solubility occurs by protonation of the primary $-\text{NH}_2$ group on the C-2 position of the D-glucosamine repeating unit, by which the polysaccharide is converted to a polyelectrolyte in acidic media [8]. The properties of chitosan depend on pH, molecular weight, distribution of the acetyl groups

in the structure and the degree of de-acetylation [8]. As mentioned above, at low pH, the amide groups are protonated, increasing the solubility of chitosan in dilute aqueous solutions; conversely, at high pH, the amide groups are deprotonated, decreasing the solubility of the polymer [6]. Also, advanced functional materials derived from chitosan can be obtained by its chemical modification using the different functional groups already present in the molecule. Procedures such as N-alkylation, N-acylation, N-carboxyalkylation, and polymer grafting have been used for the chemical modification of chitosan [9,10].

As aforementioned, cellulose is the most abundant biopolymer in nature, obtained from plant tissue after purification [6,11]. This linear syndiotactic polymer consists of β -1,4-glycosidic bonds linked with D-glucopyranose units. In the crystalline structure, every monomer is rotated 180° with respect to its neighbors. Additionally, each glucose monomer consists of three hydroxyl groups (OH) in the C-2, C-3, and C-6 position. The rotational conformation of the hydroxyl group on the C-6 position can alter the hydrogen bonding pattern and the crystallinity structure affecting the dissolution of the polymer [11]. As in other polymers, some factors affecting the dissolution of cellulose are the length of the polymer chains and the degree of polymerization (DP), i.e. the number of glucose units present in the chain. As the glucose units increase, the number of hydroxyl groups present in the structure also increases. These hydroxyl groups form complex pattern of hydrogen bonds causing the need for solvents with high hydrogen bonding capacity for the cellulose dissolution. The most common solvents used include: dimethylacetamide and lithium chloride (DMAc/LiCl), dimethylsulfoxide and tetrabutylammonium (DMSO/TBAF) and N-methylmorpholine-N-oxide (NMMO). In addition, cellulose can be dissolved in aqueous alkali media such as sodium hydroxide ($\text{NaOH}/\text{H}_2\text{O}$), sodium hydroxide/urea and sodium hydroxide/poly(ethylene glycol) (NaOH/PEG). Furthermore, acidic media useful to dissolve it are trifluoroacetic acid, dichloroacetic acid, formic acid, and sulfuric acid.

As mentioned before, these biopolymers can effectively interact with non-organic elements to create new composites. One relevant example is the formulation of a calcium carbonate/biopolymer composites. These have unique properties inherited from joining a plastic organic compound with a hard ceramic one. As calcium carbonate has multiple polymorphs, creating new composites will also depend on what type of CaCO_3 conformation is used. To

Table 1. Properties of the anhydrous crystalline phases, data from [12,14].

Phase	Crystallographic Unit Cell	Specific Gravity	Toughness
calcite	hexagonal (rhombohedral)	2.71 g/cm ³	brittle
vaterite	hexagonal	2.65 g/cm ³	brittle
aragonite	orthorhombic	2.93 g/cm ³	brittle

further understand these composites behavior and how to effectively synthesize and functionalize them, a deeper look into calcium carbonate morphologies is mandatory.

1.2. Polymorphism of calcium carbonate

In the last decades, investigators have been intensively working on the development of ceramic materials capable of being utilized in the preparation of “paint, textiles, plastics, adhesives, tires, ceramics and industrial paper” [12]. Calcium carbonate, nowadays a ceramic material of high scientific interest, is normally found in the shells of arthropods and mollusks [13]. This carbonate is a polymorphic material that has three anhydrous crystalline phases, i.e. calcite, aragonite and vaterite, in order of increasing solubility and decreasing thermodynamic stability, and an amorphous phase. The difference among the crystalline phases is due to the carbonate ions distribution respect to the calcium cations inside the unit cell [12]. The carbonate ion is the principal unit of the carbonate minerals, possessing a rigid structure that contains one carbon atom surrounded by three oxygen atoms rearranged in an equilateral triangle. The linkage between carbonate groups and cations does not affect the 120° CO bond angle [14].

The calcite polymorph crystallizes in the hexagonal system, is stable at room temperature and is the least soluble phase of the polymorphs [12], [14]. In the calcite structure, calcium atoms are in a face-centered rhombohedral unit cell. Aragonite, on the other hand, crystallizes in the orthorhombic crystal system and is stable at high temperatures and high pressures [14]. Finally, vaterite crystallizes in the hexagonal crystal system and is the least stable polymorph [12]. Vaterite is the most soluble for its loose package, as shown in Table 1.

All three phases can be employed in the fabrication of organic-inorganic compounds. For instance, interesting applications are related to the fabrication of a polymer layer followed by a calcium carbonate layer. These compounds have unique

applications and deserve a closer look, which is provided in a later section.

1.3. Some applications of calcium carbonate

Calcium carbonate particles are appealing due to their applications in different fields such as: environmental engineering, chemical engineering, bioengineering among others. CaCO₃ also displays useful applications in the water purification field. For example, Kyong-Soo Hong et al. developed a starfish-shaped CaCO₃ to remove heavy metal ions such as Cu⁺², Pb⁺², Cd⁺², Zn⁺² and Cr⁺⁶ [15]. This starfish CaCO₃ filter bore higher efficiency in removing heavy metals than other conventional filters such as activated carbon, crab shell, sawdust and other CaCO₃ particles. In another work, Ganiyu Latinwo et al. used calcium carbonate particles to improve the mechanical properties of polyurethane foam [16]. They wanted to develop economic foams with high compression resistance. Furthermore, calcium carbonate has been studied for drug delivery carriers [17]. Yaran Zhang et al. studied the biocompatibility of calcium carbonate particles on Hela cells. The researchers found the minimal and safe dosage of calcium carbonate particles for maximizing therapeutic activity without negatively affecting the bio-system. A drug delivery device of calcium carbonate suspensions and calcium alginate hydrogel was proposed by Brent Lantin and his collaborators [18]. They worked on a medically useful hydrogel with calcium carbonate particles, which can be disintegrated under the effect of high frequency ultrasonic waves.

Based on all these applications, it is evident that calcium carbonate particles are practicable to develop novel materials with potential use in many fields. However, the precipitation process of the crystal is a complex process since it depends on factors affecting the nucleation process and subsequent crystal growth, which are often difficult to control. Additionally, CaCO₃ precipitation also depends of its interaction with the substrate surface used for the fabrication of a composite. All those factors can

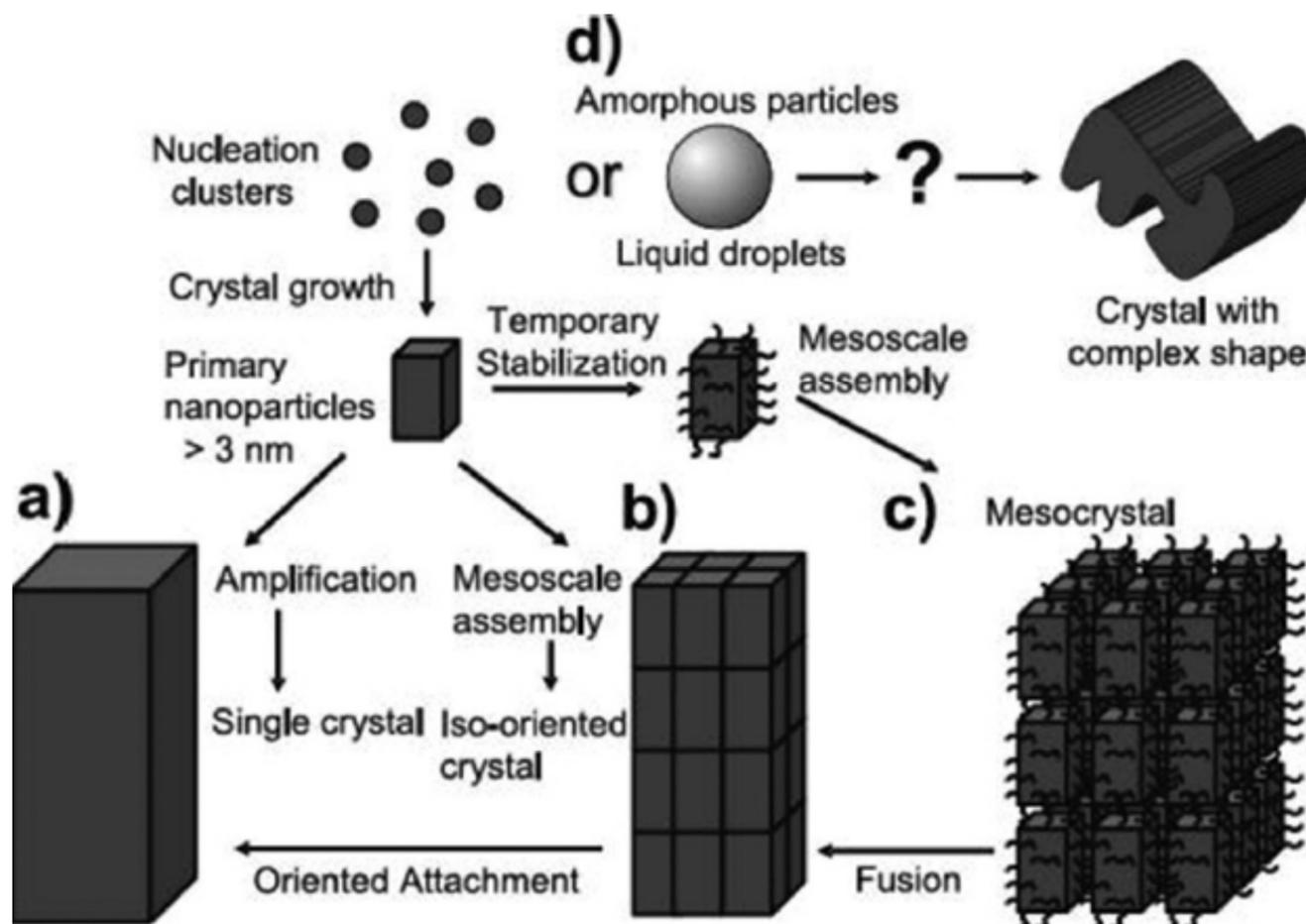


Fig. 1. Nucleation and growth of calcium carbonate. Reprinted with permission from N.A.J.M. Sommerdijk and G. De With // *Chemical Reviews* 108 (2008) 4499. © 2008 American Chemical Society.

affect the final characteristics of the composites as well as its applications.

2. CALCIUM CARBONATE FORMATION

Because the nucleation and growth of crystals have different pathways, the final structures of the crystals depend on the process selected for the crystallization. Such crystals can exist as a single crystal, iso-oriented crystal, mesocrystal or amorphous phase [19]. For these reasons, it is essential to understand the nucleation phenomena and the subsequent growth of this ceramic.

2.1. Nucleation and growth process

It is commonly accepted that this carbonate formation begins with a reaction between two most common reactants: calcium chloride (CaCl_2) and ammonium carbonate ($(\text{NH}_4)_2\text{CO}_3$). Upon the reaction, calcium cations bond with carbonate ions forming calcium carbonate embryos in solution or on a catalytic polymer surface. The embryos grow to form a critical nucleus which, if the local conditions are favorable, grow to form a primary nanoparticle. These nanoparticles can be formed into a single crystal with the addition of other ions or can result in an iso-oriented (or equiaxed) phase with the addition of atoms. When additives are present in the reac-

tion, a mesoscaled crystal forms during the process. Common additives are: polyacrylic acid or polyvinyl alcohol. These additives create a negative surface charge on the initial crystal that attracts other particles to the surface. This process continues until equilibrium is reached. Additionally, the assembly of embryos, i.e. not bearing critical nucleation size, can lead to the formation of an amorphous phase that can then form an oriented crystal favored by the additive present in the solution. The different pathways of crystallization are presented in Fig. 1. Since particles can nucleate heterogeneously on a substrate, another important factor to consider is the roughness of that substrate. In effect, surface roughness can affect the adhesion of the calcium carbonate crystals onto the surface.

2.2. Effects of the polymer substrate on the nucleation of calcium carbonate crystals

The adhesion of atoms onto a substrate is of utmost importance because the two materials (substrate and ad-atoms) come into contact and form a region in which they sustain and transfer stresses. For strong adhesive bonds, intimate molecular contact and active interactions must be present. These interactions include Van der Waals or other non-covalent interactions as a result of surface and interfacial energies.

Naturally, the morphology of the organic matrix (polymer substrate) at the nanoscale and the interaction between the organic matrix and the forming crystals does affect the heterogeneous nucleation [20]. Furthermore, as mentioned, this nucleation process is affected by surface roughness. In effect, an atom on a smooth surface has high interfacial energy due to its free bonds. For example, a cubic crystal (six sides) on a smooth surface has only two sides in contact with the surface and the other sides are in contact with the solution. This atom has four free bonds, which hamper the atom bonding to the surface. It moves from one place to another on the surface and finally returns to the solution. On the other hand, if one considers the same cubic crystal on a rough surface, the atom has more than two sides in contact with the surface. It can adhere to the surface better than in the previous case because it has less free bonds that reduce the interfacial energy. However, materials do not always behave as explained above. Consider the work by Tabor who studied the effects of surface roughness and ductility of materials on the adhesion of solids [21]. In this case, adhesion decreased as the surface roughness increased although this was attributed to the elastic modulus of the phases in contact. Another study by Chaudhury and Whitesides focused on the surface energies of poly(dimethylsiloxane) [PDMS] in air and in mixtures of water and methanol [22]. In their work they found that the interfacial interactions decreased as the methanol content in the water/methanol mixture increased. The results showed that the interfacial interactions lowered in solutions of water and methanol as the methanol content increased. However, a small interaction persisted, even in pure methanol. In both the Tabor and Chaudhury and Whitesides' experiments, the probes used were hemispherical elastomeric lenses.

Therefore, the number of free bonds can affect the interfacial energy of the calcium carbonate crystals. The energy of the crystals increases as the number of free bonds increases, affecting the adhesion of the crystal onto the substrate surface. Also, if the number of free bonds decreases so will the interfacial energy. This promotes a better adhesion of the crystals on the substrate.

Thus far, we have discussed the nucleation and growth processes for the calcium carbonate crystals as well as the influence of substrate roughness on the nucleation of the crystals. One must now consider the methodology implemented for the precipitation of the crystals. As mentioned earlier, calcium carbonate can precipitate in three known crys-

talline phases: aragonite, vaterite and calcite. Precipitation of these phases directly depends on the technique employed, i.e. by reaction and by gaseous diffusion. These two techniques will be discussed in order to predict the favored phase in each one of the techniques.

3. PREPARATION METHODS FOR CALCIUM CARBONATE FORMATION

Calcium carbonate can be produced through various methods. However, the size and morphology of the crystals can be affected by the technique used in the experimental procedure.

For instance, Hang Wang and his collaborators utilized a reactor with the two reactant precursors for synthesizing calcium carbonate crystals: calcium chloride and sodium carbonate [23]. The reactor consisted of three cells separated by baffles and polymeric membranes bearing a pore size of 1 mm and a width of 2 cm. While one corner of the reactor had calcium chloride solution and the other one, a sodium carbonate solution, at the center of the reactor there was only water. The reactor was placed in a water bath at a constant temperature. Upon increasing the temperature to the desired value, the baffles were removed allowing diffusion in the liquid phase from the edges of the reactor to the center. The precipitation of the calcium carbonate crystals occurred at the center of the reactor. To yield effective results with this experiment, control of the reactants' diffusion was necessary. This technique favored the precipitation of calcium carbonate as calcite, vaterite and aragonite [23].

In addition, the same researchers managed to precipitate calcium carbonate using another technique, which mixed the calcium chloride and sodium carbonate solutions. In contrast with the first technique discussed, the diffusion of the reactants was not controlled. As a result, the favored phases were (in order of appearance) vaterite, calcite and aragonite [23].

Herley Casanova et al. used a different method to precipitate calcium carbonate with a high pressure jet homogenizer to mix the solutions [24]. In this technique, pipe A contained a sodium carbonate solution and pipe B had a calcium chloride solution. Sodium caseinate (or casein) was added to the sodium carbonate solution and the addition of an acetic acid solution controlled the pH. The equipment was submerged in a thermostatic bath until this reached the desired temperature. Immediately the reactants started to flow through the conducts

until they met at the mixing point. Finally, the product obtained from the reaction between both flows fell into a glass flask [24]. The equipment combined two factors: mixing and fragmentation of the agglomerates of the calcium carbonate nanoparticles. The flow of the solutions was turbulent and improved the solution mixing. The high pressure led to a better dispersion and less agglomerates. The technique was successful in forming calcium carbonate nanoparticles, specifically, vaterite and amorphous agglomerates [24].

Xurong Xu et al. achieved precipitation (heterogeneous nucleation) of calcium carbonate on a substrate by going from gaseous diffusion to liquid diffusion [25]. They prepared a solution containing calcium chloride and poly(acrylic acid) and a flask with solid ammonium carbonate. After they placed both flasks in a desiccator, the ammonium carbonate started to decompose releasing carbon dioxide and ammonia vapors. The vapors were contained in the desiccator and made contact with the calcium chloride solution, causing the precipitation of calcium carbonate crystals. In this method, the polyacrylic acid provided a negative charge to the substrate surface. Subsequently, the calcium ions adhered to the substrate surface followed by the carbonate ions and thus the precipitation of calcium carbonate occurred.

While the interaction between ions that constituted the reactants was the key factor in this technique, the diffusion and reaction time represented relevant variables. As expected, higher calcium carbonate precipitation was achieved at longer diffusion times whereas the amorphous phase was obtained at lower diffusion times. As a result, only vaterite and calcite formed after 16 hours of CO₂ diffusion [25].

Rajeev Agnihotri and coworkers used another method to precipitate calcium carbonate via a calcium hydroxide and pure CO₂ gas reaction [26]. The researchers utilized a Pyrex™ reactor containing a calcium hydroxide solution whereas the gas passed through the bottom of the reactor. They added anionic surfactants to the calcium hydroxide solution to modify the surface of the particles. The setup had a pH meter and a thermocouple to monitor these variables. After the precipitation, the aqueous suspension was filtered and dried at room temperature. This procedure resulted in different particle sizes as a consequence of the variations in the calcium hydroxide and anionic surfactant concentrations [26].

These techniques were efficient for calcium carbonate precipitation and favored a specific calcium

carbonate phase. Yet, in most of the techniques depending on gaseous diffusion, the predominant phase was aragonite. On the other hand, vaterite and calcite phases were obtained via reaction. It is important to note that there are other factors that affect the final phase obtained; this is not solely due to the implemented technique. These other factors affecting nucleation and growth processes of the crystals include solution pH, temperature and the presence of additives. The next section discusses these important factors for calcium carbonate precipitation.

4. VARIABLES AFFECTING CALCIUM CARBONATE HETEROGENEOUS NUCLEATION OR PRECIPITATION

As aforementioned, numerous variables intervene in the precipitation of the calcium carbonate crystals. This requires a further review in order to draw a map of the optimal conditions required for specific types of precipitation. Variables that affect the precipitation process are listed below to better understand its effects on calcium carbonate precipitation.

4.1. PH and temperature effects on the polymer surface

We already mentioned that calcium carbonate crystallization is affected by the hydrogen potential produced during the reaction. The pH impacts the degree of dissociation of the carboxylic groups in the poly(acrylic acid) structure and the amine groups present on the chitosan film. In general, the surface charge density of the chitosan and poly(acrylic acid) (PAA) changes at different pH values. PAA exists as a polyanionic chain in the solution at values of pH above 6.3. At this pH level, the chitosan surface is close to be a neutral polymer; a great number of -COO⁻ are bonded on the chitosan surface. At pH values below 4.5, PAA exists as a polycation (-COOH) and the chitosan surface consists of NH₃⁺ groups. As the pH decreases, the PAA becomes more protonated and less charged and therefore decreases the precipitation of calcium carbonate. Additionally, the precipitation of the crystals is affected at pH values above 11.0. At such pH values, the surface charge density decreases and most of the hydrogen bonds are destroyed [27]. In general, a minimum number of carbonate groups bind to calcium cations to form the particles. As the number of -COO⁻ groups increases, so does the precipitation of calcium carbonate crystals on the surface.

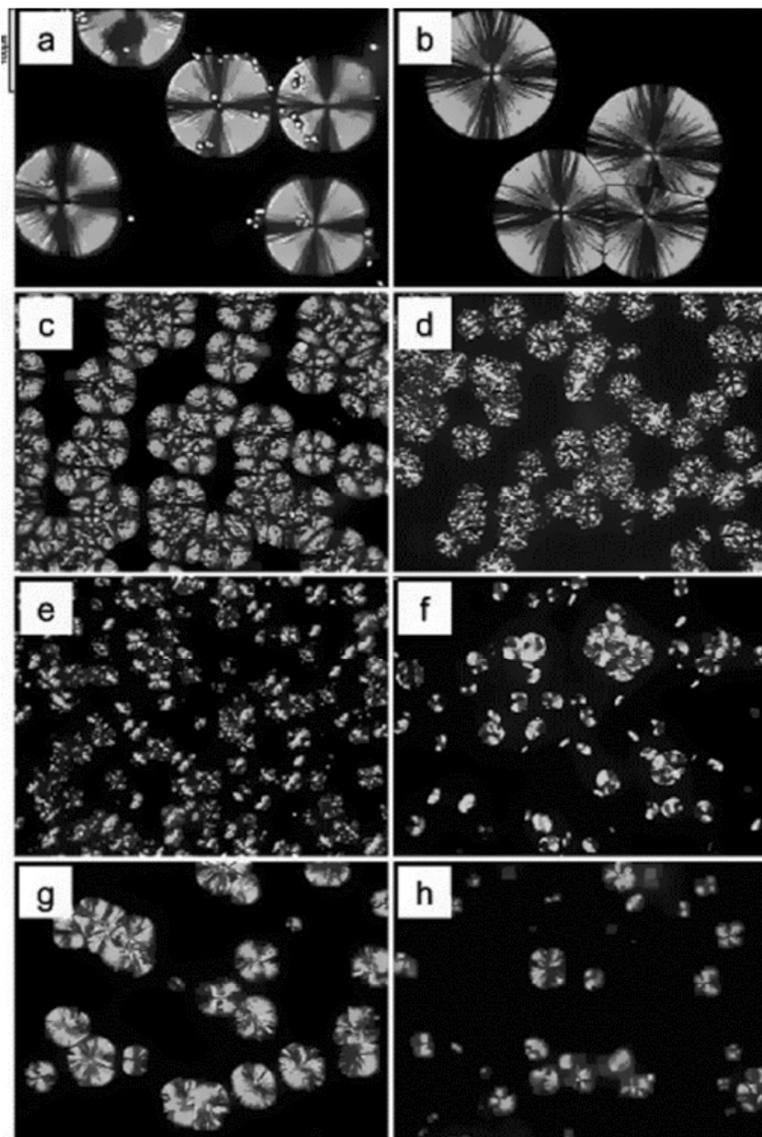


Fig. 2. Optical micrographs of calcium carbonate crystal on chitosan polymer surface, considering 0.004 wt.% PAA and different pH values; (a) pH 8.5, (b) pH 9.0, (c) pH 9.5, (d) pH 10.0, (e) pH 10.5, (f) pH 11.0, (g) pH 11.5, and (h) pH 12.0. Reprinted with permission from S.R. Payne, M. Heppenstall-Butler and M.F. Butler // *Crystal Growth & Design* 7 (2007) 1262. © 2007 American Chemical Society.

Simon Payne and a coworker studied the pH effects of the calcium carbonate precipitation on chitosan films, which were prepared by dissolving the polymer in an acetic acid aqueous solution [28]. Then, the solution was placed in petri dishes and dried. The acetic acid was neutralized, and the film was washed with distilled water and dried. Then the film was soaked in a PAA aqueous solution to modify the surface charge, and the pH value was set at 10.5. The film was immersed into a solution of 0.025 M CaCl_2 , 0.050 M NaHCO_3 and PAA; this solution was mechanically stirred and the pH constantly monitored and adjusted using sodium hydroxide. The experiment performed at room temperature was conducted at different polymer and PAA concentrations, and pH values.

Payne et al. studied how the PAA concentration modified the polymer surface [28]. They analyzed the surface of the polymer with infrared spectroscopy. The infrared spectrum shows that for neutral

and basic pH values, the 0.004 wt.% PAA concentration produced the most intense band for $-\text{COO}^-$ and NH_3^+ , the functional groups present on the chitosan surface. Furthermore, for a 0.001 wt.% PAA concentration intense bands were produced. The experiment was performed for neutral and 10.5 pH values, because this affects the presence of COO^- groups. Above 10.5 pH value, the solution becomes saturated with $-\text{COO}^-$ groups, affecting the calcium carbonate precipitation.

Additionally, the effect of PAA concentration was analyzed by optical microscopy [28]. It was observed that calcium carbonate precipitation decreased at lower poly(acrylic acid) concentrations but higher PAA levels favored the precipitation. The increase in PAA facilitated the surface supersaturation with calcium, which caused the nucleation and growth of calcium carbonate crystals. At low PAA levels, the amount of PAA adsorbed on the chitosan surface was low. This cut down the amount of calcium cations on the chitosan surface and lowered the amount of calcium carbonate precipitation on the surface. In general, the calcium carbonate nucleation resulted from a balance between the number of carboxylate groups available at the film surface and the number of ionized carboxylate groups. According to the optical microscopy analysis, the optimum PAA concentration was 0.004 wt.% in which a maximum nucleation density was obtained. Based on infrared spectral analysis and optical microscopy, a poly(acrylic acid) concentration of 0.004% was set to analyze the effects produced by a neutral or basic solution.

In addition, Payne et al. studied the pH effects on the calcium carbonate precipitation. The experimental results consisted of large and well defined spherulites obtained at pH ranging from 8.5 to 9.0 [28]. Their morphology was found to be affected as the pH increased above 9.0, as shown in Fig. 2. As the pH rose, the solution became more basic and favored the carbonate precipitation. At $\text{pH} > 10.5$, the conversion rate of hydrogen carbonate ions into carbonate ions increased. The calcium cations in solution have been sequestered, which reduced the calcium carbonate precipitation.

In another work, Jiaguo Yu et al. also analyzed the behavior of calcium carbonate precipitation at different pH levels [29]. Nevertheless, Yu and collaborators did not use a polymeric matrix as Payne et al. Firstly, stock solutions of calcium chloride (0.5 M) and sodium carbonate (0.5 M) were prepared for the calcium carbonate precipitation. Then, sodium carbonate solution was injected into an aqueous PAA solution (1.0 g/L). The pH was set to 10.0

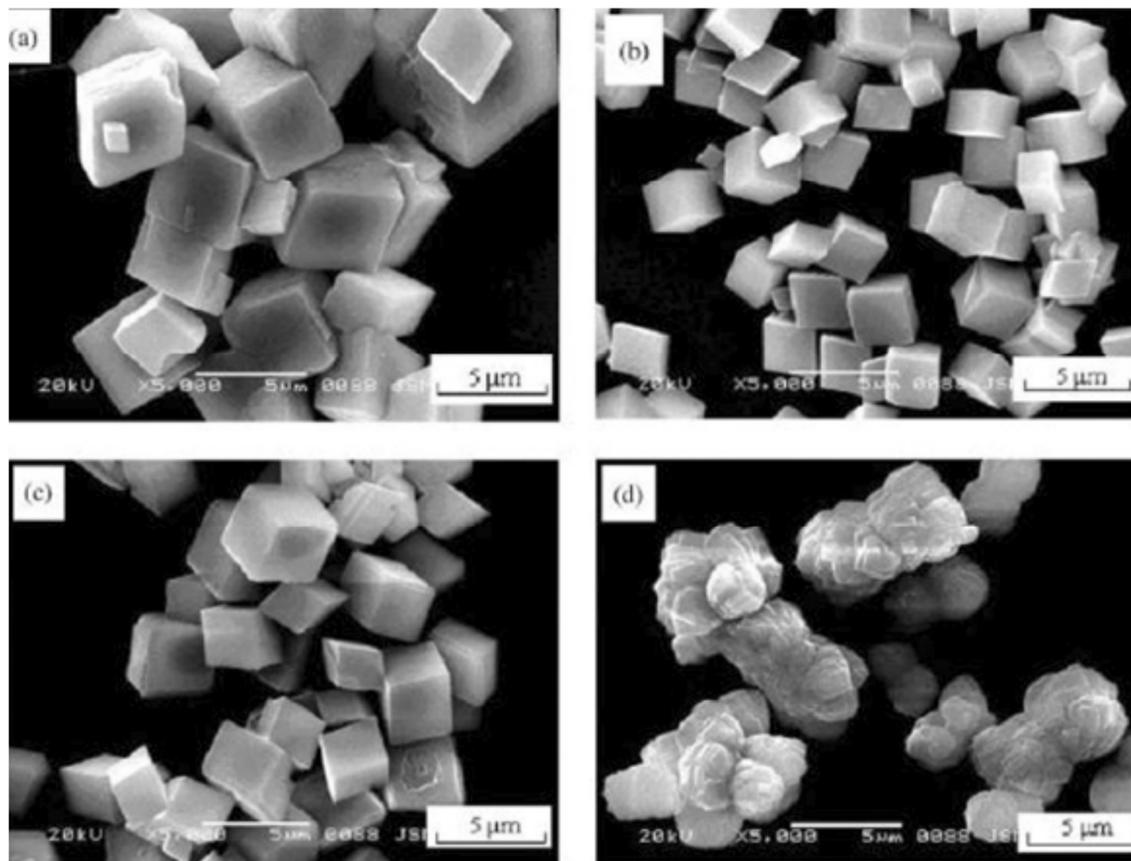


Fig. 3. SEM micrographs of CaCO_3 crystals at $80\text{ }^\circ\text{C}$ after 24 hours, considering 8.0 mM CaCO_3 , 1.0 g/L PAA , and different pH values; (a) 9, (b) 10, (c) 11, and (d) 12. Reprinted with permission from J. Yu, M. Lei, B. Cheng and X. Zhao // *Journal of Solid State Chemistry* 177 (2004) 681. © 2004 Elsevier.

using HCl or NaOH solutions. Then, calcium chloride was mixed with the previous solution under vigorous mechanical stirring. The authors analyzed different pH values, PAA concentrations, calcium carbonate levels and temperatures to study their effects on the precipitation of the crystals. This is an extensive and important publication but for the sake of brevity, in the present review we shall focus on the analysis of the pH effects on the calcium carbonate precipitation.

The experimental results at $25\text{ }^\circ\text{C}$ revealed that uniform calcium carbonate particles were obtained by lowering the pH from 10 to 9. High pH values led to a formation of round and irregular particles. At a pH of 12, a reduction in particle size occurred while a maximum particles size was obtained at a pH of 9. Higher pH values promoted the complete protonation of carboxylic groups and a polyanionic chain formed. Furthermore, higher pH values raised the solution supersaturation and the nucleation rate, which naturally lessened the particle size. Moreover, the cubic morphology of the particles was not affected at pH values from 9 to 11 at $80\text{ }^\circ\text{C}$, as shown in Fig. 3. At $80\text{ }^\circ\text{C}$ and a pH of 12, smaller irregular particles were obtained.

Summarizing, the pH of the solution does play an important role in the calcium carbonate precipitation. In some cases, the pH is used to modify the structure of the substrate surface. As mentioned earlier, the substrate is utilized to create a com-

pound based on an organic-inorganic layer. This modification is of great importance since calcium carbonate precipitation increases proportionally with the formation of $-\text{COO}^-$ groups. Additionally, the additives affected the precipitation of the crystals. Different types of additives exist, with magnesium being one of the most used. As a consequence, two important factors for nucleation and growth are magnesium concentration and reaction time.

4.2. Magnesium effects on calcium carbonate crystallization

In biological systems, magnesium stabilizes amorphous calcium carbonate phases, which form depending on the magnesium/calcium ratio and the incubation time of calcium carbonate crystals, i.e. calcite, vaterite and aragonite. Low magnesium/calcium ratios favor calcite precipitation whereas high magnesium/calcium ratios favor aragonite precipitation [30].

Loste and her coworkers studied these Mg effects on calcium carbonate precipitation by analyzing the influence of the magnesium/calcium ratio on the carbonate precipitation [30]. Their experiments consisted of combining sodium hydrogen carbonate solutions with calcium chloride and magnesium chloride solutions. The calcium chloride concentration was set constant during the experiment according to Table 2.

Table 2. Composition of precipitation experiments, see [30].

Mg:Ca	0:1	1:1	2:1	3:1	4:1	10:1
CaCl ₂ (M)	0.06	0.06	0.06	0.06	0.06	0.06
MgCl ₂ (M)	0	0.06	0.12	0.18	0.24	0.60

The researchers analyzed the precipitations of calcium carbonate at different times and discovered that amorphous calcium carbonate (ACC) precipitated in all their experiments [30]. As mentioned in a prior section, this amorphous phase is the most soluble and least stable. The stability of the amorphous phase was discovered to depend on the Mg/Ca ratio in the solution whereas ACC was favored at higher Mg concentrations in the solution. Furthermore, higher concentrations of magnesium delayed the transformation of the amorphous phase and, as a consequence, retarded the transformation onto other phases.

In the experiments, the precipitation of calcium carbonate began with the amorphous phase, which then transformed to other crystalline phases such as calcite, vaterite, aragonite, calcium carbonate monohydrate (CaCO₃·H₂O), nesquehonite (MgCO₃·3H₂O) and magnesium calcite [Table 3]. In the first minutes, the amorphous phase was favored in all the experiments. After the precipitation of the amorphous phase, calcite particles were obtained

in all the experiments. However, vaterite phase was favored only in the first two experiments (ratios of 0:1 and 1:1) after 2 minutes. The experiments with Mg/Ca ratios of 2:1, 3:1, 4:1 and 10:1 facilitated the precipitation of calcite, aragonite and calcium carbonate monohydrate. Additionally, nesquehonite came up in the experiments with Mg/Ca ratios of 4:1 and 10:1. In the experiment with 10:1, magnesium calcite was favored.

Furthermore, the same authors analyzed the precipitates obtained at the top and bottom of the petri dishes and found that calcium carbonate particles precipitated in the bulk solution and then settled on the bottom of the petri dish [30]. The particles at the top of the petri dishes were in contact with the air/solution interface. Conversely, at the bottom of the petri dishes, the particles were in contact with the solution for a longer time. According to the results, the morphology of the crystals changed as the magnesium concentration increased in the solution whereas significant changes in the calcium carbonate precipitation occurred in all the experiments above 2:1. Calcium carbonate at the bottom of the petri dishes consisted of small calcite particles. However, a thin film of calcite particles was obtained at the air/solution interface in which the oxygen ions have interacted with the solution. This interaction contributed to the precipitation of calcium carbonate particles until a continuous layer of particles formed.

Table 3. X-ray diffraction (XRD) results obtained for each experiment at different times, data from [30].

Mg:Ca ratios	Time	Calcium carbonate phase
0:1 and 1:1	0 min	amorphous
	2-60 min	calcite and vaterite
2:1	0-10 min	amorphous
	30-60 min	amorphous and calcite(traces)
	24 hours	calcite, aragonite(traces) and calcium carbonate monohydrate(traces)
3:1	0 min	amorphous
	2 min	amorphous and calcite(traces)
	14 hours	calcite and aragonite(traces)
	48-72 hours	calcium carbonate monohydrate
4:1	0 min	amorphous
	10-60 min	calcite
	24-72 hours	aragonite
	72 hours-6 days	aragonite(traces), calcite and calcium carbonate monohydrate
	6 days -	nesquehonite
10:1	14 hours	amorphous
	24 hours	amorphous and calcite(traces)
	14 days	nesquehonite, calcium carbonate monohydrate and magnesium calcite

The addition of magnesium and reaction time considerably affected calcium carbonate formation. Magnesium altered the saturation of the solution, which means that Mg raised the presence of ions in the solution. The increasing saturation affected the already precipitated phases in which the amorphous phase was the first one to form followed by its crystallization into other phases as the reaction time increased.

There are other additives that are also used for precipitating calcium carbonate, the most common ones being poly(acrylic) acid, polyacrylamide and poly(vinyl alcohol). In general, these additives bear upon the substrate surface and interact with the calcium ions present in the solution. As a consequence, it is of great importance to further analyze the interactions occurring among the additives, the substrate, and the calcium and carbonate ions.

4.3. Effects of additives on a polymer surface

The synthesis of inorganic-organic layers by biomineralization methods has become of mounting interest to scientists and researchers. In this case, the ceramic nucleation process can be affected by several factors such as the degree of saturation of a solution and the surface charge on the polymer surface, issues already addressed in the present review. Additives can be used to produce a negative charge on the polymer surfaces, e.g. PAA, poly(vinyl alcohol), poly(ethylene glycol), poly(acrylamide), polypeptides and glycols-polyols.

The negative charge on the surface of the polymer change the initial polymer structure [28]. Of our particular interest is chitosan, which is, as previously mentioned, a cationic polymer with amine groups (NH_2) and poly(acrylic acid) a polyanion with carboxylic groups (R-COOH), which interacts electrostatically with the amine groups forming a polyelectrolyte complex. Then, calcium cations are attracted to the negative charge and the carbonate ions bond to the calcium cations. After the reaction, precursor calcium carbonate molecules are created on the biopolymer structure. The polyelectrolytes provide cohesion and adhesion between the inorganic and organic layers. When dealing with polyelectrolytes, variables like pH, composition, conformation, charge density, and solvents become relevant [31]. At this point, we need to underscore that the polymeric additives facilitate calcium carbonate nucleation and promote the formation of a specific phase.

4.3.1. Poly(acrylic acid)

As aforementioned, PAA is an anionic polyelectrolyte with its negative charge resulting from carboxylate (COO^-) functional groups. According to the literature, PAA is one of the most widely used additives and functionalizes the surface of the substrate forming a polyelectrolyte complex that assists in the deposition of calcium ions.

He et al. studied the PAA effects during calcium carbonate precipitation on chitosan films [32]. Their experiment consisted of two types of films: chitosan and acetylated chitosan films. The chitosan films were obtained by dissolving the polymer in an acetic acid aqueous solution. This solution was dried and annealed to remove both water and solvent producing films with 8% of degree of acetylation. The acetylated process was performed to raise the acetylation degree to 80%. The resulting acetylated films were obtained using the chitosan films without annealing. After soaking the chitosan films with methanol, they were immersed in a solution of acetic anhydride and methanol. Finally, the films were washed with methanol and dried. Afterwards, the calcium carbonate precipitation on chitosan films took place in a closed box containing two vessels. The first one held a chitosan film or acetylated chitosan film with calcium chloride solution. The second one contained ammonium carbonate in solid state. Calcium carbonate precipitation occurred via gaseous diffusion [32].

The PAA concentration was studied by the Fourier-transform infrared (FTIR) spectroscopy analysis. The results showed that the intensity of the bands diminished as the PAA concentration in the solution intensified. According to the FTIR analysis, the absorption band at 875 and 745 cm^{-1} corresponded to a vaterite phase and the one at 857 cm^{-1} , to an aragonite phase [32].

Calcium carbonate phases were affected by the PAA concentration in the solution and the degree of acetylation (DA). Vaterite was favored at low poly(acrylic acid) concentration and 8% DA. However, aragonite formed at low PAA concentration and 80% DA. At PAA concentrations higher than 1×10^{-2} g/L, vaterite appeared with 8% or 80% of DA. It is apparent, therefore, that the degree of acetylation played an important role in the calcium carbonate precipitation at low concentration of the additive [32].

At low PAA concentrations two phenomena occurred. PAA exists as a polyelectrolyte composite with calcium ions, i.e. PAA-Ca^{+2} . The supersaturation resulted as a consequence of the electrostatic forces between the ester group $-\text{COO}^-$ and the am-

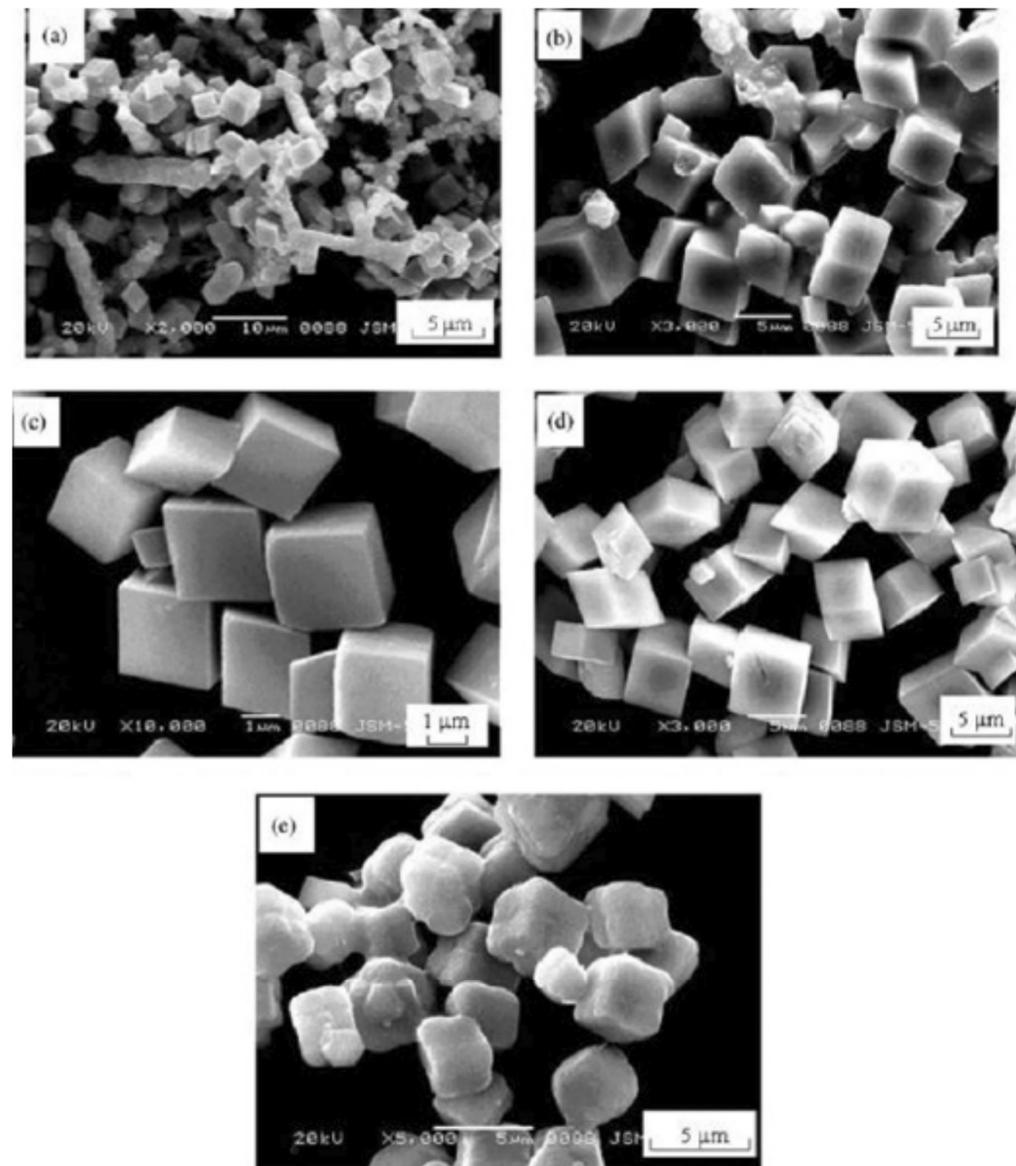


Fig. 4. SEM micrographs of CaCO₃ crystals at 80 °C, considering 8.0 mM CaCO₃, pH:10 and different PAA concentrations; (a) 0.2, (b) 0.5, (c) 1.0, (d) 2.0. and (e) 5.0 g/L. Reprinted with permission from J. Yu, M. Lei, B. Cheng and X. Zhao // *Journal of Solid State Chemistry* 177 (2004) 681. © 2004 Elsevier.

monia group NH₃⁺. Also, the interaction between the hydrogen bonds and the -COO⁻, -OH and -NH₂ groups increase the solution supersaturation around the polymer matrix. On another part, PAA-Ca²⁺ can remain as a mobile polyelectrolyte in the solution and the CO₂ molecules are absorbed increasing the solution supersaturation. These phenomena further raised the supersaturation with CaCO₃ [32]. In general, the aragonite phase came forth at low PAA concentrations because it grows on chitosan surfaces rich in OH groups. On the other hand, vaterite formed at high concentrations because it grew on surfaces comprised of NH₂ and OH groups [32].

In another research, Jianguo Yu et al. examined the effects of PAA on the precipitation and nucleation of calcium carbonate crystals [29]. Initially, stock solutions of calcium chloride (0.5M) and sodium carbonate (0.5M) were prepared. The solution of sodium carbonate was injected into the aqueous PAA solution (1.0 g/L) with the pH set to 10.0, using HCl or NaOH solutions. Under vigorous mechanical stirring, then calcium chloride was mixed with the previous solution. Some of the variables considered in this research were PAA concentration, cal-

cium carbonate concentration, temperature, and pH. We will discuss the analysis of the effects of the polyacrylic acid concentration at pH = 10.0 [29].

A scanning electron microscopy analysis revealed that the calcium carbonate morphology had been affected by the additive concentration at 25 °C as presented in Fig. 4 [29]. Lower PAA levels showed calcium carbonate crystals with rhombohedral and rectangular shapes. The authors attributed these morphologies to the insufficient amount of PAA ions to bind the calcium cations. On the contrary, at higher concentrations of the additive (1.0 to 2.0 g/L), the calcium carbonate crystals changed their cubic morphology to large irregular spheres. These shapes resulted from a strong interaction between the carboxylic groups of the PAA and the calcium carbonate crystals that had precipitated previously. When the additive concentration reached 5.0 g/L, twinned calcium carbonate particles formed. On another segment of this same publication, the authors revealed that at 80 °C the morphologies of the calcium carbonate crystals changed, as shown in Fig. 4 [29]. Lower PAA concentrations allowed obtaining branched irregular particles, rhombohedral

and cubic shapes. As the additive concentration rose, the branched irregular morphology of the particles changed to cubic. Higher PAA levels allowed forming branched particles. When the additive concentration reached 1.0 g/L calcite particles (cubic-shaped) resulted. As the additive concentration reached 5.0 g/L, the cubic particles became rounder and had more aggregates of small cubic particles. High temperature also affected the calcium carbonate precipitation favoring the formation of monodispersed cubic particles [29].

Another critical study on the PAA effects on calcium carbonate precipitation is due to Ouhenia et al. [33]. Their experimental procedure consisted of preparing three solutions: calcium chloride (CaCl_2), potassium carbonate (K_2CO_3), and PAA. First, the calcium chloride and poly(acrylic acid) solutions were mixed at different temperatures. Afterwards, the ensuing solution was mixed with potassium carbonate. Finally, the researchers collected the precipitates by filtration through a cellulose membrane and dried them [33]. Once again the results corroborated that the temperature did affect the calcium carbonate particles precipitation. The study analyzed the volume fraction of the polymorphs (calcite, vaterite and aragonite) obtained with and without the presence of poly(acrylic acid). Furthermore, temperature effects were considered. For calcite particles, the presence of the additive reduced the volume fraction of the crystals formed at 25 °C in the solution compared to those without PAA. Also, higher temperatures lowered the volume fraction of calcite particles for both cases. Contrarily, the presence of the additive raised the volume fraction of vaterite particles compared to those without PAA. At 50 °C the volume fraction of vaterite particles was higher than calcite and aragonite; however, it decreased above 80 °C for both cases. Additionally, the presence of the additive increased the volume fraction of aragonite crystals compared to those without PAA. At temperatures above 50 °C aragonite particles formed. Even more, higher volume fraction of aragonite particles were obtained at 80°C than calcite and vaterite particles [33].

At this point one can safely conclude that poly(acrylic acid) has been one of the most studied additives by researchers and scientists. In general, the presented studies considered distinct variables such as additive concentration, acidity of the solution and temperature. Additionally, the above studies employed different techniques such as gaseous diffusion and reaction. By controlling these variables, precipitation of the three calcium carbonate crystalline phases was achieved although all of them

could not be obtained in the same experiment. Just as these researchers decided to study the effects of poly(acrylic acid), other investigators focused on studying another important additive: polyacrylamide.

4.3.2. Polyacrylamide

Polyacrylamide (PAM) is another anionic polyelectrolyte commonly used to modify or adjust the morphology of calcium carbonate. For instance, Wang et al. used the carbonation in combination with PAM, as an organic substrate and the only modifier [34]. In their experimental procedure they prepared a polyacrylamide gel and mixed it with calcium hydroxide particles ($\text{Ca}[\text{OH}]_2$) at different temperatures. Then, they bubbled CO_2 and N_2 into the said solution while controlling the pH. The resulting calcium carbonate precipitates were rinsed with distilled water and dried [34]. Wang et al. identified the morphology of the crystals by the Fourier-transform infrared (FTIR) spectroscopy analysis. Cubic calcite precipitated without polyacrylamide, but needle-shaped aragonite and calcite crystals were obtained in the presence of the additive. The FTIR spectra showed the characteristic peak of calcite crystals at 875 cm^{-1} when no polyacrylamide was added while 1082 cm^{-1} for aragonite crystals in presence of polyacrylamide.

Additionally, differential thermogravimetric analysis demonstrated the decomposition of calcium carbonate and polyacrylamide depending on an organic/inorganic interfacial interaction [34]. At low organic concentrations, aragonite amount raised. Nevertheless, the aragonite fraction started to decrease at organic concentrations higher than $0.75 \times 10^{-7}\text{ mol/l}$. The authors attributed this phenomenon to two possible mechanisms: 1) Based on the Ostwald step rule, they claimed that the least stable polymorph formed first and then transformed into the more stable phase; and 2) the polyacrylamide substrate inhibited the formation of aragonite, probably due to the ($-\text{NH}_2$) groups adsorbed on the crystal, which blocked further calcium carbonate growth [34]. Furthermore, the crystal size of calcite became smaller for larger concentrations of the additive. Based on these findings, one can conclude that the polyacrylamide could have delayed the nucleation of calcite particles and stabilized the aragonite phase [34].

Wang et al. determined the fraction of aragonite crystals at different temperatures. Aragonite crystals were not obtained at temperatures below 60 °C while a higher aragonite fraction came along at 80

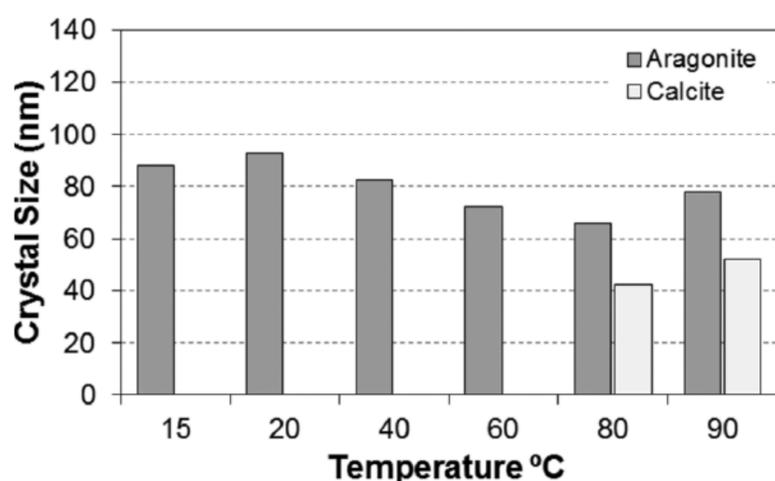


Fig. 5. Influence of temperature on crystal sizes. Reprinted with permission from C. Wang, J. Zhao, X. Zhao, H. Bala and Z. Wang // *Powder Technology* **163** (2006) 134. © 2006 Elsevier.

°C; yet, the amount of aragonite particles decreased above that temperature. Once again it was corroborated that temperature affects the crystal size of both phases, i.e. aragonite and calcite, as presented in Fig. 5. Larger calcite particles formed at 20 °C but the size decreased within the 20 °C to 80 °C range and increased above 80 °C. When the sizes of calcite and aragonite particles were compared, the researchers found that calcite particles were larger than aragonite ones. A maximum particle size of aragonite was obtained at a temperature of 90 °C. In general, the crystal size of aragonite and calcite particles increases above 80 °C. Wang et al. stated that the NH_2 groups of the additive could adsorb onto the crystal surface, which increased at higher temperatures. The nucleation of calcite crystals could be affected by the supersaturation of carbonate ions on the crystal surface causing as the particle size to decrease as the supersaturation rose.

Table 4. Calcium carbonate precipitates (0.10 M) using polyacrylamide from methanol/water solutions at a temperature of 23-24 °C after 9 days, data from [35].

Methanol/Water	PAAm (%)			
	0	0.50	1.0	5.0
0:10	C	C	C	C
1:9	-	C, (V)	-	-
2:8	-	C, (V)	-	-
3:7	-	C, (V)	-	-
4:6	C	V	-	-

A = aragonite, C = calcite, V = vaterite; symbol in parentheses indicates that the phase is a minor amount.

By mixing two solutions, Won Kim et al. investigated the effects of some nonionic polymers additives on the calcium carbonate precipitation [35]. The first solution was calcium chloride while the second one consisted of ammonium carbonate and polyacrylamide (PAAM). The calcium carbonate concentration was different, but equal amounts of the main reactants were mixed. The calcium carbonate precipitate was separated from the solution by centrifugation, and washed with distilled water and acetone to eliminate the residues [35].

The researchers were able to analyze the effects of the PAAM amount and the methanol/water ratio on the calcium carbonate precipitation [Table 4]. Calcite particles were obtained for all polyacrylamide levels in a water solution and in a 4:6 methanol/water solution at 0% of PAAM. Nonetheless, the addition of methanol caused the precipitation of vaterite and calcite at 0.50% of PAAM. Vaterite particles were obtained at a methanol/water ratio of 4:6. Some characteristics of PAAM include its ability to form hydrogen bonds and its poor adsorption rate because water dissolved the organic additive. As result, calcite particles are precipitated in a water solution. However, the addition of methanol into the water solution retarded the vaterite transformation into calcite [35].

The studies presented considered the polyacrylamide concentration effects, the methanol/water ratios, and the temperature effect. By following the same line as with the study of PAA, these researchers managed to precipitate different calcium carbonate phases. However, there was always a specific phase that was favored in the experiments. Apart from the studies made on PAA and PAAM, there is another important additive worth mentioning that is also used in the CaCO_3 precipitation: the poly(vinyl alcohol).

4.3.3. Poly(vinyl alcohol)

This polyalcohol is obtained from vinyl acetate by a polymerization process and further base-induced hydrolysis. It is well known that poly(vinyl alcohol) is an anionic polyelectrolyte with a negative charge arising from the $-\text{OH}$ groups. It is an anionic, water soluble additive that enhances the calcium carbonate nucleation. Nucleation of calcium carbonate crystals depended on the contact between the crystals and the polymer. In general, the surface of the polymer underwent a treatment with an additive which then were adsorbed by the polymer surface. Fig. 6 shows how the additive produced some charge into the polymer allowing the growth of the calcium carbonate crystals [36].

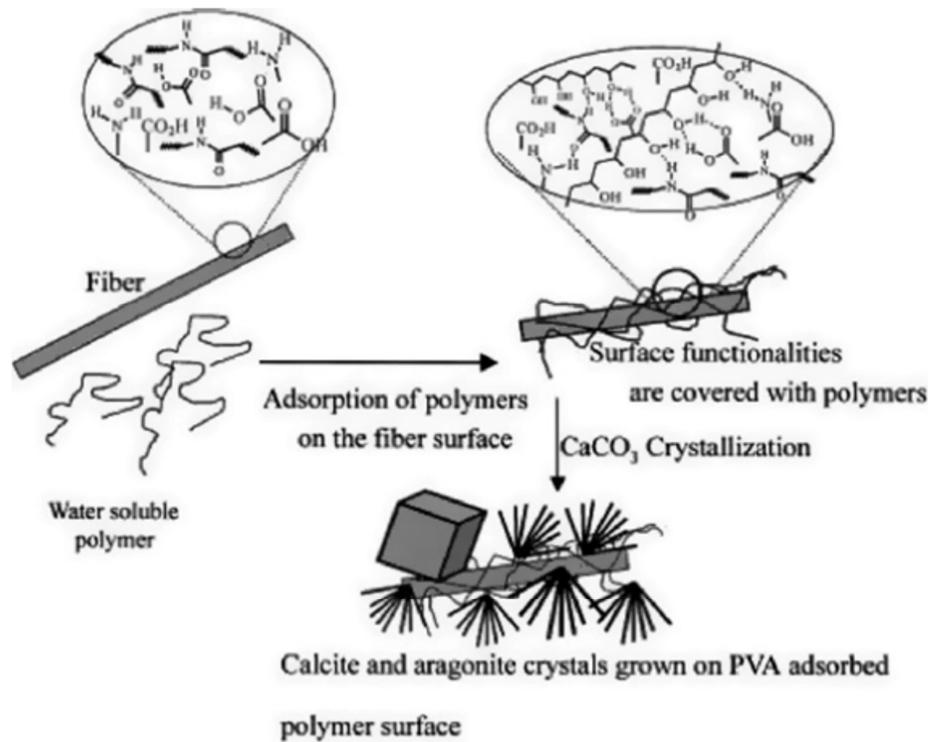


Fig. 6. Mechanisms of nucleation and growth of calcium carbonate crystals. Reprinted with permission from R. Lakshminarayanan, S. Valiyaveetil, and G.L. Loy // *Crystal Growth & Design* 3 (2003) 953. © 2003 American Chemical Society.

Lakshminarayanan et al. further explored the effects of poly(vinyl alcohol) on the growth and nucleation of calcium carbonate crystals. Crystal growth was studied in two types of polymers: Nylon 66® and Kevlar 29®. Also, this study considered the pretreatment performed with a hydrochloric acid solution and an alkaline (NaOH) solution on both types of polymers. Then, the polymer fibers were submerged into the additive solution, which was adsorbed by the fiber surface. These treated fibers were then submerged into a calcium bicarbonate solution where calcium carbonate crystals (vaterite, calcite and aragonite) began to grow adhered to the polymer surface by passing CO_2 to the solution [36].

The results showed that the fibers pretreatment with acid and alkaline media influenced the nature of the calcium carbonate phases formed on the fibers surface. The presence of 2000 ppm of polyacrylamide in the polymer favored the growth of ara-

gonite while calcite crystals formed in the absence of the additive [36].

For untreated fibers, i.e. not exposed to the acid and alkaline media, facilitated the formation of vaterite and calcite for a lower concentration of the additive (200 ppm). When this concentration was raised to 2000 ppm, vaterite formed on both untreated fibers. Also, the results revealed that the pretreatment favored the aragonite phase on both polymers. For Kevlar 29 treated with an acid medium, the aragonite and vaterite phases formed for a low additive concentration. However, a higher concentration promoted aragonite formation. In Nylon 66 treated with an acid medium, the favored phases were aragonite and calcite using a low concentration of the additive. Higher concentration of PVA produced aragonite [Table 5]. While the pretreatment increased the number of carboxylate and amino groups on the fiber surfaces, these polar groups interacted with the

Table 5. Calcium carbonate precipitation, before and after be treated the fibers with poly (vinyl alcohol) additive (PVA), data from [36].

Description	Calcium Carbonate Polymorphs	
	200 ppm	2000 ppm
Untreated Kevlar 29	vaterite and calcite	vaterite
Untreated Nylon 66	vaterite and calcite	vaterite
Acid-treated Kevlar 29	aragonite and vaterite	aragonite
Alkali-treated Kevlar 29	aragonite	aragonite
Acid-treated Nylon 66	aragonite and calcite	aragonite
Alkali-treated Nylon 66	aragonite	aragonite

PVA molecules through the hydrogen bonds. As result, the PVA molecules accumulated on the fiber surface facilitating the nucleation of aragonite particles. On another hand, the vaterite phase formed on untreated fibers.

In summary, in this study the fibers were treated with an acid or alkaline solution prior to the precipitation of the calcium carbonate crystals, with the additive concentration taken into account too. In the same line as the studies presented, they managed to precipitate the three phases even though not every phase formed under the same conditions. It is worth it to notice how the nucleation and growth processes of the crystals have been affected by a handful of variables like the ones presented in this review. The precipitation of these crystals is complex but very interesting since researchers can carry out different studies that permit to select a specific process to form one of the three calcium carbonate phases. Following the same trend set by prior investigations, we decided to study calcium carbonate precipitation in order to formulate an organic-inorganic composite. To this purpose, our research work focused on variables such as PAA concentration, the addition and concentration of magnesium chloride (MgCl_2) and the reaction time.

5. SYNTHESIS OF CHITIN/CALCIUM CARBONATE COMPOSITES

As mentioned earlier, calcium carbonate has been used in the fabrication of organic-inorganic composites. These materials, particularly those based on chitin, have gained interest from researchers due to their applications in different areas such as environmental engineering, biomedical engineering, and bionanotechnology.

5.1. Chitin film preparation

We prepared the chitin solution by dissolving 0.75 wt.% of the polymer in an N,N-Dimethylacetamide (DMAc) solvent and lithium chloride (LiCl) (5 wt.%), which enhanced the chitin solubility in the solvent system. After the polymer was dissolved, the chitin solution (15 mL) was cast into a petri-dish and coagulated with 2-propanol to promote the gelling. The resulting sol gel was used for the calcium carbonate precipitation without the drying process. Fig. 7 shows the resulting chitin surface with some roughness. Also bubbles that came up due to the coagulation process of the chitin solution are present.

Our investigation started with the fabrication of chitin matrix because we would later use it for the

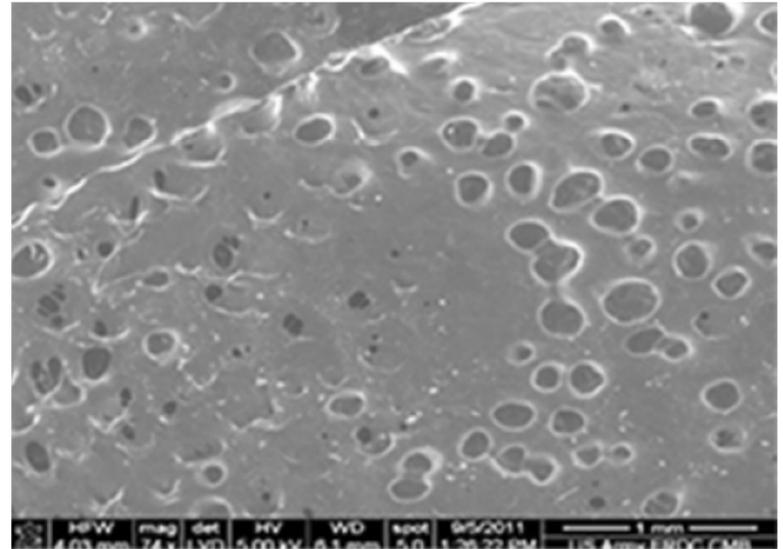


Fig. 7. SEM micrograph of a chitin film.

production of composites. After fabricating the polymer, we worked on the inorganic phase, i.e. calcium carbonate. In producing the carbonate, a few variables were considered to optimize the crystals precipitation, based on the literature review. Of particular importance was the poly(acrylic) acid concentration.

5.2. Calcium carbonate precipitation via reaction

The chitin sol gel was prepared by dissolving the polymer into an N,N-dimethylacetamide (DMAc) and lithium chloride (LiCl) (5 wt.%). After that, the chitin solution was coagulated with 2-propanol and the resulting sol gel was used for the calcium carbonate precipitation.

We soaked the chitin sol gel into a 0.4 wt.% PAA solution to alter the charge density of the polymer film and then, submerged the film into a mixture of CaCl_2 (0.25M) and $(\text{NH}_4)_2\text{CO}_3$ (0.25M) in which the calcium carbonate precipitated.

Upon the first part of this research, calcium carbonate precipitated using 0.25 M of CaCl_2 and $(\text{NH}_4)_2\text{CO}_3$ and 0.4 wt.% PAA. As shown in Fig. 8, a smaller amount of particles formed on the chitin surface but the particles had a defined morphology. The precipitation process of calcium carbonate is affected by the concentration of ammonium carbonate and calcium chloride, as shown in Figure 8. In our research, we worked with 0.25 M ammonium carbonate concentration not enough to raise the solution pH. It is well known that lower pH values ($\text{pH} < 10$) decrease the crystal precipitation. At high pH values ($\text{pH} > 11$), the nucleation rate increased and shortened the induction time which is required for the formation and subsequent transformation of the calcium carbonate particles.

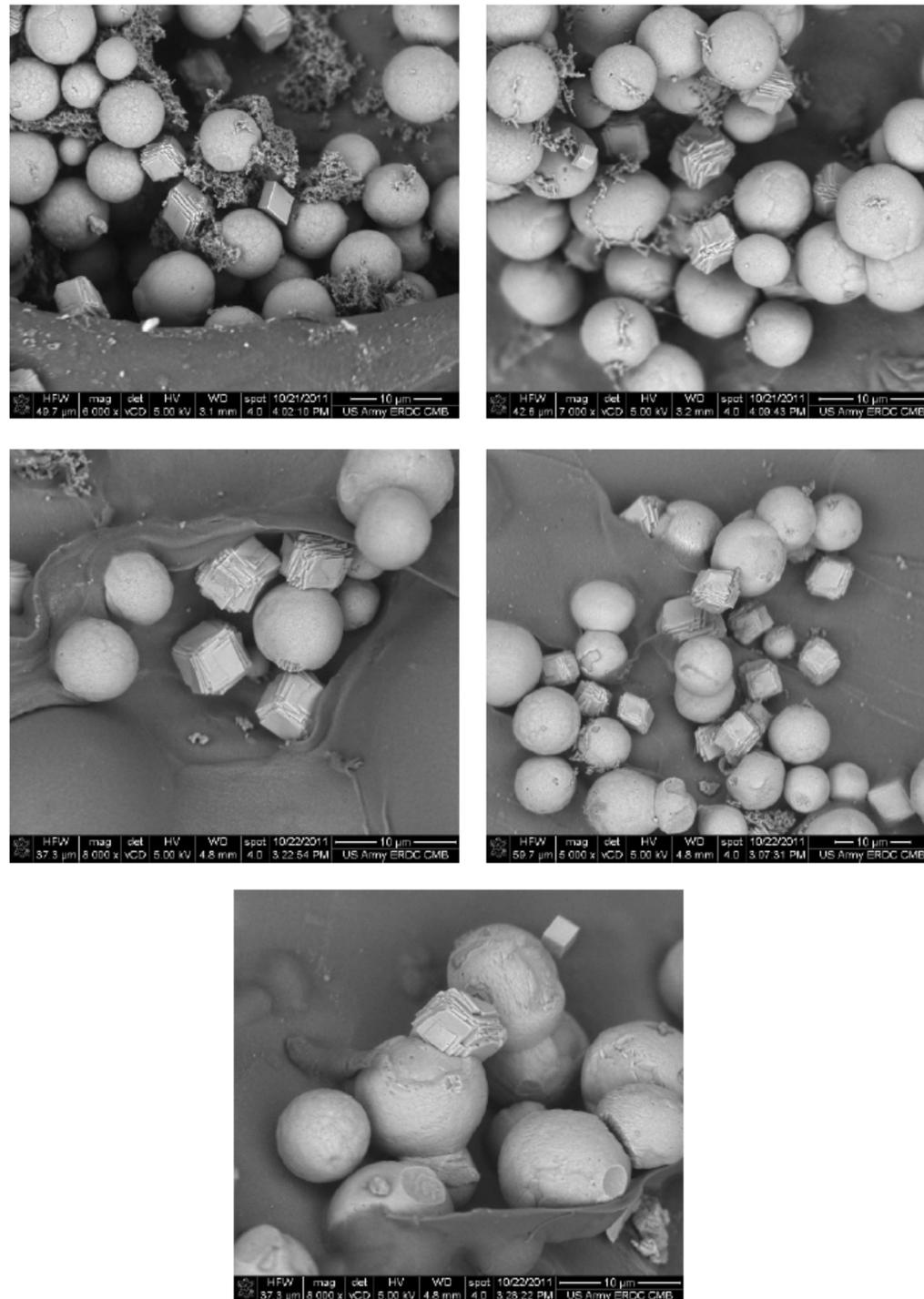


Fig. 8. Calcium carbonate precipitation on chitin surface: vaterite (spherical crystals), calcite (cubic crystals) and amorphous particles. Calcium carbonate phase precipitated at 0.25 M CaCl_2 , 0.25 M $(\text{NH}_4)_2\text{CO}_3$, and 0.4 wt.% PAA.

In addition, the PAA concentration affected the precipitation of CaCO_3 crystals, as presented in Fig. 8. PAA concentrations above 0.4 wt.% inhibited the crystal growth. Initially, 0.4 wt.% PAA was used, which resulted in a low precipitation. As mentioned before, the authors found that the amount of calcium carbonate particles decrease on the chitosan surface at lower PAA concentrations. Additionally, they indicated that high PAA concentrations (above 0.004 wt.%) simply increased the amount of PAA in the solution, affecting its surface adsorption.

At this point we could conclude that the PAA concentration and the ammonium carbonate affected the precipitation process of calcium carbonate. Since we worked with a 0.25 M ammonium carbonate concentration, i.e. not enough to raise the solution pH, we started using a 0.50 M concentration of $(\text{NH}_4)_2\text{CO}_3$ instead. Similarly, a 0.4 wt.% concen-

tration of PAA inhibited the crystal growth. Therefore, we decided to realize an additional study of the effects PAA on calcium carbonate precipitations for our experimental conditions using 0.001 wt.% and 0.004 wt.% PAA.

5.3. Effects of poly(acrylic acid) on calcium carbonate precipitation

From the previous sections, one can infer that additives have a great deal of importance in the precipitation of calcium carbonate crystals. In this section, we discuss the effects of PAA concentration in the CaCO_3 precipitation to select better conditions for the precipitation of the crystals.

With that purpose in mind, we selected two PAA levels: 0.001 wt.% and 0.004 wt.%. For the composite fabrication, the chitin sol gel was immersed

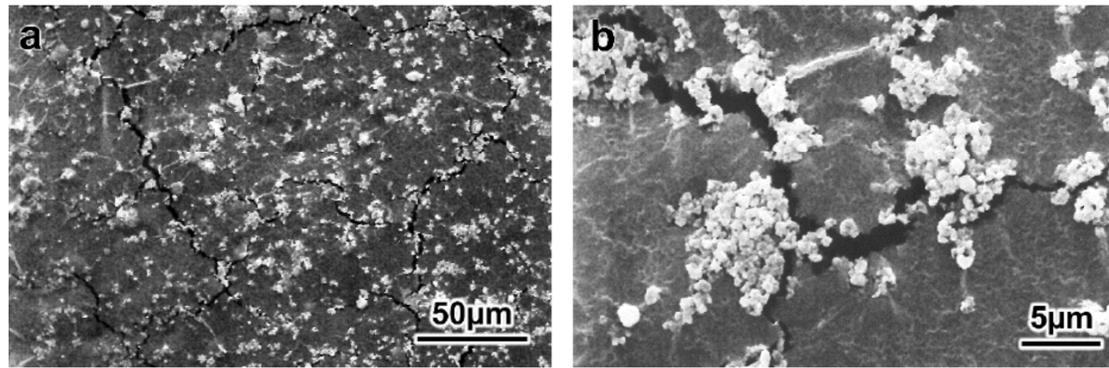


Fig. 9. Calcium carbonate precipitation under (a) 0.25M calcium chloride, (b) 0.50M ammonium carbonate and 0.001 wt.% poly(acrylic acid).

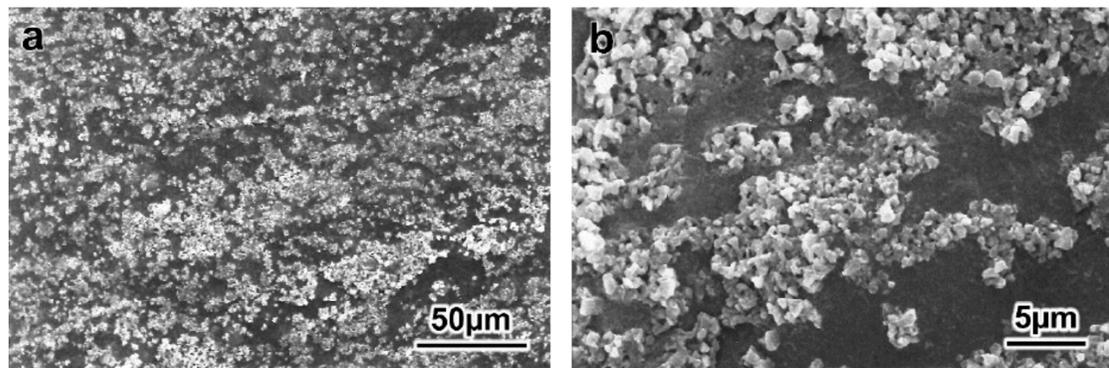


Fig. 10. Calcium carbonate precipitation under 0.25M calcium chloride, 0.50M ammonium carbonate and 0.004 wt.% poly(acrylic acid).

in an aqueous solution of PAA (0.001 wt.% or 0.004 wt.%, as stated) followed by the addition of CaCl_2 (0.25 M) dissolved in ethanol. An ammonium carbonate solution (0.50 M) was added to a calcium chloride and poly(acrylic acid) solution. The sol gel was immersed into the solution for 24 hours and let it dry at room temperature. Figs. 9 and 10 present the PAA effect on the nucleation and growth processes of the calcium carbonate crystals. As the additive concentration increased, the precipitation of the calcium carbonate crystals on the chitin surface increased. These CaCO_3 particles were later found to be amorphous.

In the precipitation process, PAA interacted with the calcium ions to form polyelectrolytes (PAA-Ca^{+2}), which then reacted with the polymer and became adhered onto its surface. Next, CO_3^{-2} ions reacted with the polyelectrolytes, forming the crystals. Additionally, some amount of polyelectrolytes remained in the solution without interacting with the polymer, thus, inhibiting the nucleation and growth of the crystals. In summary, the precipitated carbonate, which are initially amorphous then transformed into calcite, vaterite or aragonite as the reaction time passed. This time interval is related to how much time the polymer is in intimate contact with the solution containing the reactants used for the carbonate precipitation.

After studying the PAA effects in the formation of CaCO_3 , we turned our attention to the effects of another well-known additive: MgCl_2 (under gaseous

diffusion conditions). Magnesium chloride promotes the crystal precipitation, as mentioned before.

5.4. Calcium carbonate precipitation via vapor diffusion

The chitin sol gel was prepared by dissolving the polymer in a solvent of DMAc and LiCl (5 wt.%). After the polymer dissolution, the solution was coagulated with 2-propanol. This sol gel was used for the calcium carbonate precipitation.

It was mentioned before that vapor diffusion is another way of precipitating calcium carbonate crystals as aragonite phase. This precipitation is affected by the concentration of MgCl_2 and the diffusion time. In our case, the crystallization was achieved by NH_3 and carbon dioxide diffusion from the decomposition of ammonium carbonate, i.e. $(\text{NH}_4)_2\text{CO}_3$. The gaseous diffusion experiment was conducted in a closed box where the chitin sol gel was immersed in a solution of calcium chloride (0.25 M), PAA (0.004 wt.%) and magnesium chloride. Solid ammonium carbonate was placed in an open vial inside the closed box. Our results demonstrated that magnesium chloride indeed promoted CaCO_3 precipitation via gaseous diffusion. The aragonite phase precipitated at low MgCl_2 concentrations while, on the other hand, high MgCl_2 concentrations favored amorphous CaCO_3 formation. We deem important to underscore the role of the diffusion time on the carbonate precipitation. High diffusion time promoted the assem-

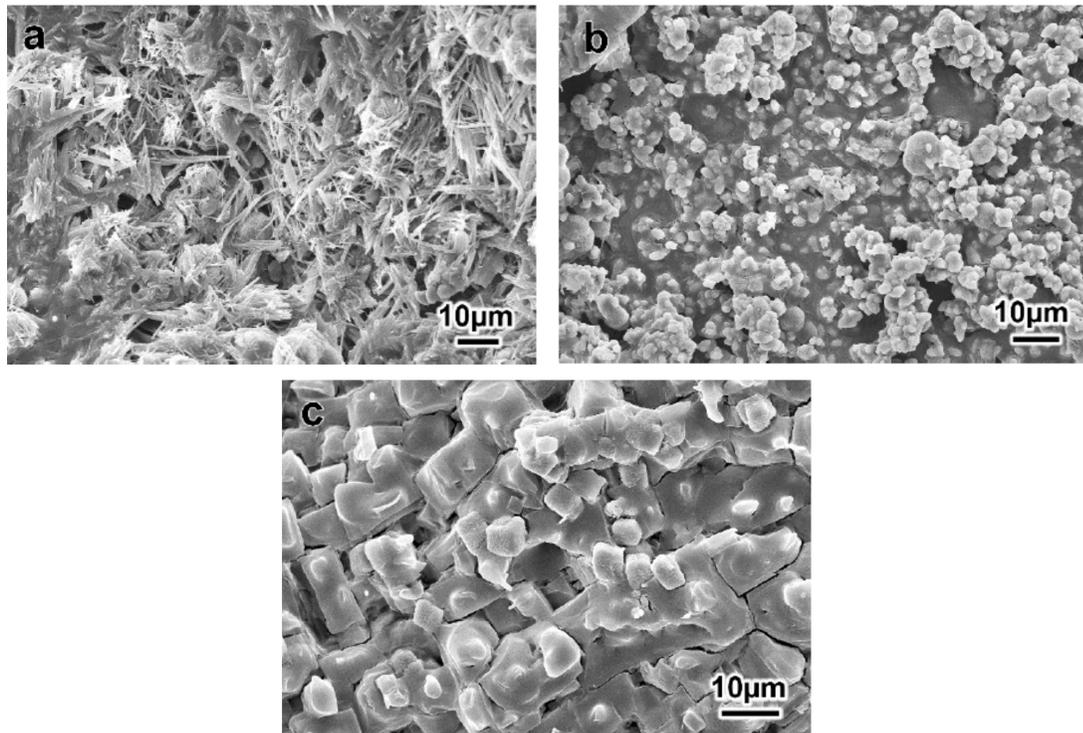


Fig. 11. Precipitation of calcium carbonate crystals: (a) 0.10 M MgCl_2 for 2 days, (b) 0.50 M MgCl_2 for 2 days, (c) 0.10 M MgCl_2 for 4 days.

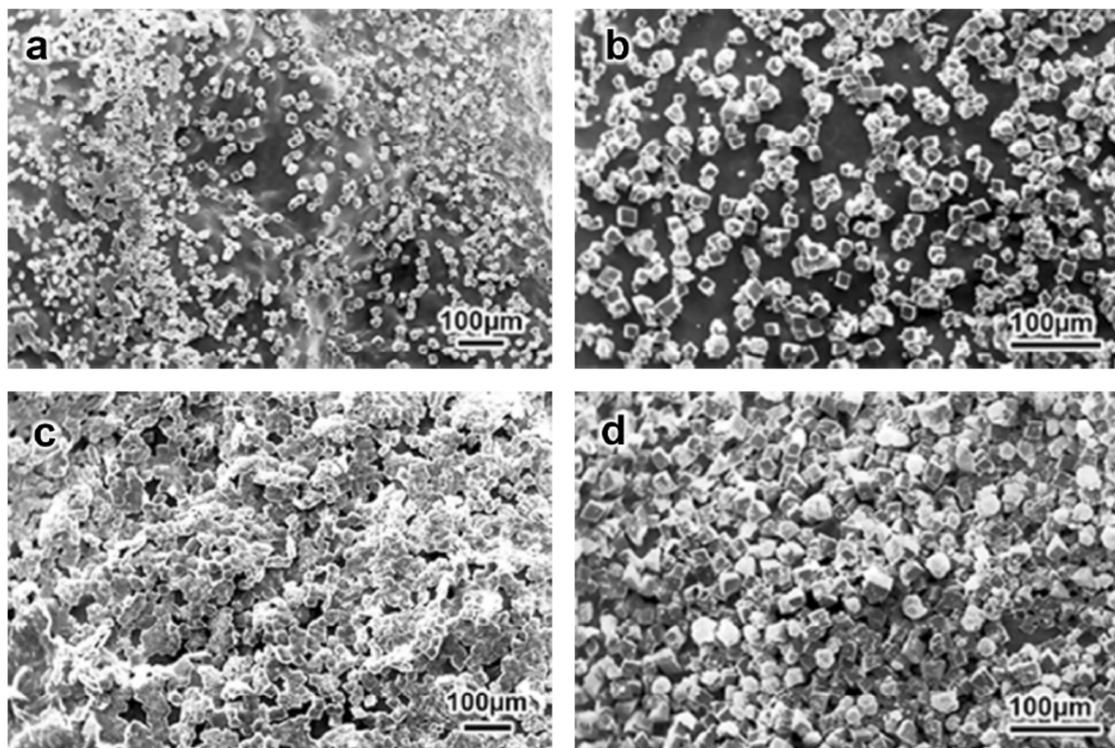


Fig. 12. Calcium carbonate precipitation via vapor diffusion: (a) small desiccator for 2 days, (b) large desiccator for 2 days, (c) small desiccator for 4 days and (d) large desiccator for 4 days.

bling of the crystals until a CaCO_3 layer is formed, as shown in Fig. 11c. This phenomenon can occur as a consequence to a supersaturation degree in the solution containing CaCl_2 and MgCl_2 . When both CaCl_2 and MgCl_2 concentrations rose, an increase of calcium and magnesium cations occurred. These cations reacted with the carbonate ions to form either calcium carbonate or magnesium carbonate. Next, the incipient crystals started to interact with the polymer and appeared joined together on the surface of the polymeric matrix. Magnesium cations could also interact with the CaCO_3 crystals and incorporate themselves in the crystalline structure.

As shown above, MgCl_2 and the diffusion time affected the CaCO_3 precipitation. However, diffusion

times depend on the dimensions of the system for the gaseous diffusion process. As consequence, we centered our attention on the desiccator size.

5.5. Effects of the close box size on the calcium carbonate precipitation

For this experimental segment we use desiccators of different sizes. The area of the larger desiccator was 3534 cm^3 , while the smaller desiccator consisted of 155.5 cm^3 . The sol gel was laid into a petri dish with calcium chloride (0.25M), magnesium chloride (0.1 M) and PAA (0.001 wt.%). Each petri dish was placed in a given desiccator containing pieces of solid ammonium carbonate. Naturally, the

carbonate decomposed into ammonia and carbon dioxide, which promoted the precipitation of the crystals. Fig. 12 shows the effects of the desiccator size on the carbonate precipitation.

When comparing the calcium carbonate precipitation in a small desiccator at 2 days and 4 days, we can observe a high amount of particles at 4 days. The particles accumulated until a non-uniform layer formed and the particles present an irregular morphology. Moreover, the same behavior is observed in a large desiccator at 2 days and 4 days. The crystal present a similar cubic morphology for both times.

Once again we underscore that the precipitation of the crystals depends on the desiccator size, as shown in Fig. 12. When comparing the precipitation occurring in a small and a large desiccator after 2 days, one can appreciate a marked morphology difference. In the small desiccator, the amorphous phase is favored, while a cubic morphology is observed in the large one. For a diffusion time of 4 days, the precipitation of calcium carbonate behaved similar than for 2 days. In this case, an amorphous phase precipitated at 2 days and a cubic morphology was observed after 4 days.

We were able to verify that the precipitation of calcium carbonate crystals depended on variables such as the calcium chloride, ammonium carbonate and poly(acrylic acid) concentration, the diffusion time and the size of the system where the precipitation of the crystals under vapor diffusion takes place. Initially, we found that the precipitation of calcium carbonate crystals decreased when the same concentration of CaCl_2 and $(\text{NH}_4)_2\text{CO}_3$ was used. At the same concentration, the solution was less alkaline; this promotes less CaCO_3 precipitation.

Furthermore, the additive concentration affected the precipitation of the crystals. A higher PAA concentration increased the nucleation and growth of the crystals. However, PAA above 0.004 wt.% decreased the formation of the crystals. In this case, more polyelectrolytes (PAA-Ca^{+2}) remained in the solution without interacting with CO_3^{-2} ions, which is necessary in the precipitation of the crystals. On another hand, the precipitation of the crystals increased with the addition of MgCl_2 . The inorganic additive increased the supersaturation degree of the solution, promoting the precipitation of the amorphous phase. Supersaturation leads to the formation of more carbonate phases, as a result of more calcium and magnesium ions present in the solution. Additionally, the size of the system in which the vapor diffusion takes place affected the precipi-

tation of the crystals. The amorphous phase was favored in the small desiccator. In closing, this work consisted of an extensive study of the principal variables that affected the precipitation of the inorganic phase, which were then thoroughly discussed.

6. SUMMARY

Once again, it should be underscored that the concentration of additives, i.e. PAA, polyacrylamide and PVA, strongly affects the nucleation and precipitation of calcium carbonate crystals. Normally, two methods for the CaCO_3 precipitation are used: gaseous diffusion and reaction. Some research groups as well as our studies demonstrated how the PAA affects the formation of the carbonate particles via chemical reaction and gaseous diffusion. As presented previously, Payne et al. considered different PAA concentrations at a specific pH value (pH = 10.5) [28]. On another hand, He and coworkers used different poly (acrylic acid) levels and two percentages of deacetylation (8%DA and 80%DA) but did not evaluate the pH of the solution [32]. In our case, we used two PAA concentrations, 0.001 wt.% and 0.004 wt.%. Even though the variables studied were different, the conclusions were very similar.

Low PAA concentrations yielded two possible outcomes [28,32]. First, a polyelectrolyte (PAA-Ca^{+2}) can be formed in the solution of calcium chloride and PAA, which then interacts with COO^- and NH_3^+ groups by electrostatic forces. These interactions lead to the formation of COOH , OH and NH_2 groups on the chitosan surface, which increases the supersaturation degree of calcium carbonate crystals in the solution. These particles then adhere onto the chitosan surface. In the second outcome, PAA remains as a carboxylic anion in the form of PAA-Ca^{+2} in the solution and inhibits the precipitation of the crystals.

High concentrations of PAA render different results. At high PAA concentrations, NH_2 groups are transformed into NH_3^+ groups. The NH_3^+ groups then interact with PAA molecules adsorbed on the chitosan surface. These PAA molecules react with Ca^{+2} ions in the calcium chloride solution and PAA-Ca^{+2} polyelectrolytes are formed. After mixing ammonium carbonate into the CaCl_2 solution, CO_2 and NH_3^+ groups diffuse into the CaCl_2 solution, and the saturation degree increases, leading to the formation of amorphous particles. Eventually these amorphous particles are adsorbed on the chitosan surface and transformed into another crystalline phase.

In addition, the pH of the solution is also found to have a great impact on the precipitation of CaCO_3

crystals. Yu and Payne studied the effects of pH on CaCO_3 precipitation by evaluating different pH values at specific PAA concentration values [28,29]. They found that for $\text{pH} < 10$, that the particles have a regular morphology and larger size when compared to the particles obtained at $\text{pH} > 10$. Also, the nucleation rate is minimal at lower pH values. However, high pH values ($\text{pH} > 11$) trigger high nucleation rates of calcium carbonate particles with irregular morphology.

In systems with weak polyelectrolytes related to additives such as PAA, polyacrylamide and PVA, the pH controls the surface charge on the polymer. The interaction between a weak polyelectrolyte and a weak acidic polymer surface produces a pH difference in the solution that subsequently affects the surface charge density on the polymer. At higher pH values, the protonation degree decreases and the surface charge density increases affecting the nucleation rate.

The addition of magnesium (Mg^{+2}) also have a pronounced effect on the calcium carbonate precipitation. Eva Loste and our research group varied the magnesium concentration in the solution where the precipitation takes place [30]. Both studies found that magnesium (Mg^{+2}) ions promoted the formation of crystals such as magnesium carbonate and calcium carbonate and inhibit the nucleation of calcium carbonate on a calcite phase, which is the most stable phase compared to vaterite and aragonite. Magnesium adheres strongly to the carbonate ions which then inhibit the growth and nucleation processes of CaCO_3 in the aqueous solution.

Some researchers also use acid or basic solution as a pretreatment to modify the structure of polymer surfaces such as He et al. study with hydrochloric acid (HCl) and sodium hydroxide (NaOH) [32]. The researchers found that the fibers pretreatment with acid and alkaline media affected the calcium carbonate phases precipitated on the fibers surface. In general, modifying the structure of a polymer's surface is achieved by manipulating many variables like the ones summarized in this review. The impact of these variables have on the precipitation process will vary differently from one to another but nevertheless have a decisive impact on the system's outcome.

Calcium carbonate is a widely studied compound with many applications in the field of materials' science. Its precipitation, as described earlier, depends on numerous variables such as temperature, pH, additive and reactive concentrations. These variables are studied in order to observe their effects in the precipitation of the crystals. An inter-

esting study is increasing the concentration of calcium chloride above 0.5 M at a specific PAA concentration, pH value and temperature. On another hand, the effects of two additives at the same conditions could be studied. In this case, the better additive is identified. Additionally, the precipitation can be conducted in desiccators with different sizes, controlling pH value, additive concentration and temperature. In addition, the gaseous diffusion of CO_2 using decomposition of ammonium carbonate and a constant flow of gaseous CO_2 represent an interesting study. Depending on the desired end result, manipulation of these variables is of utmost importance.

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