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Crystal growth of aragonite in the presence of phosphate

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ABSTRACT

The crystal growth of aragonite was investigated at pH 7.8, 37 °C and constant solution supersaturation from aragonite-seeded supersaturated solutions. The effect of the presence of orthophosphate ions in the supersaturated solution on the kinetics of crystallization of aragonite was investigated over the range of orthophosphate concentrations of 0.25 μM–1 mM. In the presence of orthophosphate in the range of 0.25 μM–8 μM, the crystal growth rate of aragonite decreased with increasing phosphate concentration. At orthophosphate concentration levels exceeding 2 μM, induction times were measured and were found to increase with orthophosphate concentration. At orthophosphate concentration levels > 8 μM, the crystal growth of aragonite was inhibited, suggesting the blockage of the active growth sites by the adsorption of orthophosphate ions. Adsorption was confirmed by the investigation of orthophosphate uptake on aragonite, which was: i) found to depend on the equilibrium concentration of orthophosphate in aqueous solutions saturated with respect to aragonite; ii) not influenced by the ionic strength of the electrolyte up to 0.15 M NaCl, showing that electrostatic interactions between orthophosphate and $CaCO_3$ did not play a significant role in this concentration range. Adsorption data of orthophosphate on the aragonite crystals gave satisfactory fit to the Langmuir adsorption model and was confirmed by XPS analysis.

1. Introduction

Calcium carbonate exists in six polymorphic forms: calcite (thermodynamically the most stable), aragonite, vaterite, amorphous $CaCO_3$, and two hydrated crystalline phases, calcium carbonate monohydrate and hexahydrate. Precipitation, crystallization and dissolution of calcium carbonate species have been intensively studied over the past decades because of the significance of these processes in the formation of scale or deposits, in biological mineralization and in various environmental conditions [1,2].

The presence of foreign substances in the supersaturated fluid media in which growth of calcium carbonate polymorphs takes place may interfere with the crystal growth process because of the interactions of these substances with the lattice ions of calcium carbonate at the respective active crystal growth sites [3,4]. Early investigations have shown that very low phosphate concentrations decreased the rates of calcite precipitation [5,6]. The inhibition effect was attributed to the competition between carbonate and phosphate ions to bind to the available calcium ions. Katsifaras and Spanos [7] have found that vaterite was stabilized in the presence of low phosphate concentrations and that its growth rate was reduced at the same time. This was attributed to the adsorption of the orthophosphate ions on vaterite crystals. Evidence concerning the adsorption of orthophosphate has been reported from dissolution studies of calcium carbonate polymorphs [8]. In addition, the presence of orthophosphate in solutions supersaturated with respect to the calcium carbonate, in combination with pH, has been suggested to play a key role in the stabilization of less stable polymorphs [9]. Environmental studies concerned with the interaction of carbonates and phosphates have quantified the interaction between inorganic orthophosphate and calcite and aragonite mineral surfaces [10–14]. Studies on the interaction of calcium carbonate with phosphate by Stumm and Leckie [15] suggested that the uptake of phosphorus is a complex process consisting of chemisorption and surface precipitation of calcium phosphates. Millero et al. [16] has reported large extents of uptake and desorption of phosphate on aragonite surfaces, which were suppressed in the presence of seawater. It has also been claimed from reports in literature that phosphate is chemisorbed on calcium carbonate substrates and would become less susceptible to electrostatic forces [17]. Both studies pointed out the role of the ionic strength on the orthophosphate-

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carbonate interaction.

The need of biomaterials for the replacement or healing of damaged bone tissues has been one of the driving force for the development of materials based on calcium carbonate, particularly those of marine origin that have been shown to be biocompatible and bioactive [18,19]. Calcium carbonate based cements have been proposed and shown to be effective biomaterials [20–22] and aragonite has been presented as a promising implant material [23]. The presence of inorganic orthophosphate in the body fluids in contact with calcium carbonate is believed to play an important role for the ability of $CaCO_3$ polymorphs to act efficiently as biomaterials. In particular, their *in vivo* evolution and resorption behavior will most probably be impacted by the presence of these orthophosphate ions [24].

Concerning the effect of the presence of orthophosphate on the kinetics of crystal growth of aragonite, a limited number of studies have been conducted under conditions in which the chemistry of the supersaturated solutions was maintained relatively constant, although limitations concerning the concentrations [25–27] or the establishment of steady state conditions past an initial rapid de-supersaturation of the solutions have been applied [28].

In this study, a highly reproducible seeded growth technique is employed to investigate the crystallization of aragonite crystals in presence and absence of orthophosphate ions. The investigation was done under conditions of constant supersaturation, achieved by the addition of titrant solutions in which the concentrations have been calculated appropriately, to ensure replacement of the ions transferred from the supersaturated solutions to the crystallizing solid, ensuring that the interaction of orthophosphate ions during the growth was not disturbed by factors such as the solution de-supersaturation. Moreover, the adsorption of orthophosphate on aragonite crystals was quantified by equilibrium studies and interpretation according to the Langmuir isotherm.

2. Materials and methods

2.1. Synthesis of aragonite seed crystals

Aragonite seeds were prepared by precipitation at 100 °C. Briefly, 0.1 M $CaCl_2 \cdot 2H_2O$ (Normapur) solution was added dropwise into 0.1 M Na_2CO_3 (Normapur) solution containing 1 mM $SrCO_3$ (Alfa Aesar). The formed precipitate was filtered through membrane filters, washed with 1 L of deionized water, freeze-dried and stored in a freezer to prevent any evolution of aragonite before use. The specific surface area of the synthesized aragonite seed crystals was measured in triplicate using a multiple point BET apparatus and was equal to $3.0\ m^2\ g^{-1}$.

2.2. Characterization of aragonite crystals

Aragonite seeds and grown crystals were characterized by FTIR spectroscopy (Nicolet 5700 spectrometer, ThermoElectron), X-ray diffraction (XRD, Inel CPS 120 diffractometer – with a Co anticathode, λ=1.78897 Å), and scanning electron microscopy (SEM, LEO 435 VP and FEI QUANTA FEG microscopes, samples were silver-plated before observation).

2.3. Crystal growth experiments at constant composition

All solutions were freshly prepared using analytical reagent grade chemicals without further purification, dissolved in triply distilled CO_2-free water, and were filtered (0.45 μM Millipore® filters) before use. Supersaturated solutions were prepared using $CaCl_2 \cdot 2H_2O$ (Ferrak Berlin), $NaHCO_3$ (Merck) and NaCl (Merck) salts. The supersaturated solutions (200 mL) were prepared into a double walled, water jacketed glass reactor. The reactor was kept at 37 ± 0.1 °C by circulating water from a thermostat. The reactor was tightly sealed and a light stream of nitrogen gas was passed above the solution to prevent intrusion of atmospheric CO_2. The pH of the supersaturated solutions was adjusted by the addition of standard sodium hydroxide or hydrochloric acid solutions as needed. The pH was monitored by a pH-meter (Metrohm®) and the electrode was calibrated before and after the experiments with NIST standard buffer solutions at pH 6.84 and 7.38. During the course of crystal growth, the concomitant pH drop triggered the addition of titrant solutions including calcium and carbonate concentrations that were calculated appropriately to counter-balance the ions removed from the supersaturated solution during aragonite crystallization.

During equilibration the solution pH remained constant, thus confirming the stability of the supersaturated solutions. After equilibrium was attained, an accurately weighted amount of aragonite seed crystals (40 mg) was introduced in the supersaturated solution, triggering the initiation of crystal growth of the aragonite seed crystals. For the experiments in the presence of inorganic orthophosphate ions, Na_2HPO_4 (Merck) was dissolved in the initial supersaturated solutions to obtain orthophosphate concentrations in the range of 0.25–1 mM. No orthophosphate was added into titrant solutions; therefore, there was no compensation for possible phosphate consumption during the experiments. Preliminary work showed that adsorption of phosphate on calcium carbonate was very fast and thus no pre-equilibration of the seed crystals was necessary before starting the experiment.

The formation of aragonite in the supersaturated solutions may be described by Eq. (1):

$$Ca^{2+}(aq)+x\ H_2CO_3(aq)+y\ HCO_3^{-}(aq)+u\ CO_3^{2-}(aq) \rightarrow CaCO_3\ (arag\cdot, s)+z\ H^{+}(aq) \tag{1}$$

where 2x+y=z

As may be seen from Eq. (1) protons are released during the crystallization process. A drop in the pH as small as 0.005 pH units triggered the addition of titrant solutions from two mechanically coupled syringes of a computer controlled automatic titrator. The titrants and the corresponding concentrations in the two syringes were calculated as shown in Eqs. (2) and (3):

$$Ca_T=10\ Ca_s+2\ Ca_s \tag{2}$$

$$C_T=10\ C_s+2C_s+2(C_A,\ C_B) \tag{3}$$

where Ca_T is total calcium in the titrant solution and Ca_s the respective concentration in the supersaturated solution. C_T is the concentration of the total carbonate in the titrant solution and C_s the corresponding concentration in the supersaturated solution. C_A and C_B are the concentrations of standard acid or base, respectively, added to adjust the pH of the supersaturated solutions at the desired value. The compositions of the two titrant solutions were calculated so that their addition ensured the constancy not only of the solution pH but also of the activity of all ions present in the supersaturated solutions. Eq. (2) consists of two parts: $10Ca_s$ is an arbitrarily selected concentration, based on preliminary experiments. This part corresponds to the calcium added to compensate calcium precipitating as calcium carbonate. The value used for this part, was selected on the basis of the optimum value to avoid variations of the calcium concentration in solution greater than 1%. If this factor is too high the titrant volume-time curves show extended steps and if it is too low, it is impossible for the titrants to compensate for the formation of precipitates. The second part of Eq. (2) refers to the amount of titrants needed to avoid dilution of the initial calcium concentration in the solution. Eq. (3), gives the concentration of the titrant solution used for the compensation of carbonate going to the precipitate. The first term corresponds to the concentration needed to keep carbonate constant in solution through the replacement of the precipitating carbonate and the second term is to account for the dilution of the initial supersaturated solution. The third term in Eq. (3) accounts for the acid/base concentration needed to adjust the solution pH to the desired value. Having additions from