J Mater Sci: Mater Med (2006) 17: 403–406
DOI 10.1007/s10856-006-8466-9

Calcium carbonate crystallization in the presence of taurine

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Received: 21 March 2005 / Accepted: 18 August 2005

Abstract The kinetics of calcite ($CaCO_3$) crystallization on calcite seed crystals in the presence of taurine was investigated by the constant composition method. The presence of taurine ($4 \times 10^{-5} - 4 \times 10^{-4}$M) in the supersaturated solutions lead to calcite crystals with a characteristic discontinuous planes of growth and poor habit, as compared to the hombohedral morphology of the seed crystals. The acceleration effect of taurine on the crystal growth rate was 17–96%. The apparent order of the crystal growth was found to be 2.0±0.2 typical for a surface diffusion-controlled spiral growth processes.

1. Introduction

Calcium carbonate is the most abundant mineral in nature followed by calcium phosphate, silica and other minerals [1]. It has been reported as gallstones [2], pancreatic stones in both human and cattle [3, 4] animal phyla, algae, in mollusc shells [5, 6] and in human atherosclerotic aorta [7, 8]. The biological systems use organic molecules (mostly proteins, polysaccharides and aminoacids) that function variously as nucleators, cooperatice modifiers, inhibitors and as matrices or molds for minerals. The exert exact control over the processes of biomineralization, which results in unique inorganic-organic hybrids (e.g. seashells, bone, teeth, diatome and many others) with their structure and morphology regulated to optimally fit their functions as materials [9–16].

Taurine is a conditionally-essential amino acid which is not utilized in protein synthesis, but ruther is found free or in simple peptides. Taurine has been shown to be essential in certain aspects of mammalian developments and "*in vitro*" studies in various species have demonstrated that low levels of taurine are associated with various pathological lesions, including cardiomyopathy, retinal degeneration and growth retardation, especially if deficiency occurs during development [17, 18]. There is no dietary requirement for taurine, since the body can make it out of vitamin B6 and the amino acids methionine and crysteine. People with diabetes have lower-than-average blood levels of taurine [19]. Taurine along with the amino acids methionine and cysteine are the major components in food supplements for athletes. It is present in high concentrations in algae, codfish, oysters, clams and in the animal kingdom, including insects and arthropods, but is generally absent or present in traces in the bacterial and plant kingdom. In many animals, including mammals, it is one of the most abundant of the low-molecular-weight organic constituents [20]. Taurine was first isolated from ox bile in 1827 where it is found in high concentrations. Along with methionine, cystine and cysteine, it is a sulfur amico acid (H_2N-CH_2-CH_2-SO_3H), does not polarize light and consequently dones not have an L- or D-configuration [21].

The aim of the present work is to investigate the effect of taurine on the growth of calcium carbonate crystals by the constant composition technique [22, 23] and to attempt to answer the following questions:

Does taurine affect the nature, the rate or the particle size of the calcium carbonate forming phases? The methodology of the constant solution composition was applied for studying the crystallization process of calcium, carbonate, in seeded

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growth experiments, because of the advantages the method presents in accurately assessing the rates of crystallization and the nature of the precipitating crystalline polymorphs [6, 8, 11, 13, 16].

2. Experimental procedure

The experiments were performed in a thermostated double-wall Pyrex vessel at 25±0.1°C. The total volume of the working solution was 0.200 dm^3 and the stable supersaturated solutions employed were prepared by mixing equal volumes of calcium nitrate and sodium bicarbonate solutions made from standardized stock solutions as described in detail elsewhere [6, 8, 11, 13, 15, 16, 22, 23]. The pH of the solution was measured by a glass/saturated calomel electrode (Metrohm), standardized before and after each experiment with NBS buffer solutions [24]. The pH of the working solution was adjusted to 8.50 by the addition of standard potassium hydroxide (Merck, Titrisol) and the pH remained constant at least for 10h, indicating the lack of any precipitation in the solution. Prior to the pH adjustment, quantities of 5, 15, 30, 50 mg of taurine was added to the working solutions in order to obtain concentrations of 4×10^{-5}, 1.2×10^{-4}, 2.4×10^{-4} and 4.0×10^{-4} M of the additive in the working solution. Taurine was purchased from Sigma (mp 300 °C) and used without further purification. The calcium carbonate precipitating process was initiated by the inoculating of 100 mg of synthetic calcite crystals, prepared according to the method described by Reddy and Nancollas [25] and characterized by power X-ray diffraction (Phillips PW 1830/1840 using Cu K_α filter), FT-IR spectroscopy (Perkin Elmer 18PS FT-IR using kBr pellets) scanning electron microscopy (Jeol GSM 5200 and LEO supra 35VP), and thermogravimetric analysis (Du Pont 910). The specific surface area of the seed crystals, as determined by a multiple-point BET method, was found to be 3.2 m^2g^{-1}. Upon the introduction of the calcite crystals into the supersaturated solutions, the precipitation started immediately, accompanied by a drop in the pH solution, A pH drop as small as 0.005 pH units was sufficient to trigger the addition of titrant solution from two mechanically coupled burettes of an appropriately modified pH stat (Methrohm Dosigraph with 614 Impulsomate). The two burettes contained calcium nitrate and sodium carbonate titrants having the stoichiometry of the precipitating salt (calcium carbonate = 1:1) [6, 8, 11, 13, 15, 16, 22, 23]. By inclusion in the titrants of the appropriate calcium nitrate and sodium bicarbonate concentrations, as well as the appropriate potassium nitrate concentration for the ionic strength, the initial conditions could be maintained constant throughout the precipitation process. Calcium nitrate, potassium nitrate, sodium bicarbonate and sodium carbonate were purchased from Merck (pro analysis).

The crystal growth rates, R, were easily and accurately obtained from the recorder traces of the titrants (corresponding to the moles of calcite precipitating) as a function of time [22, 23]. The constancy of the solution compositions was checked by sampling regularly, filtering the samples through membrane filters (0.22 μm Gelman), and analyzing the filtrates for calcium by atomic absorption spectroscopy (Varian 1200). At the end of each experiment, the solids were collected by filtration and examined by scanning electron microscopy (SEM), FT-IR spectroscopy, powder X-ray diffraction and thermogravimetric analysis (TGA).

3. Results and discussion

The calcium carbonate system, under our experimental conditions, was considered closed [23]. Ionic activities of all species in solution were computed by taking into account the appropriate equilibria, mass balance conditions, and solution electroneutrality [16, 22, 23]:

$$\Delta G_x = -(R_g T/2) \ln(IP/K^o_{s,x}) = -(R_g T/2) \ln \Omega_x \qquad (1)$$

where R_g is the gas constant, T is the absolute temperature, IP is the activity product of calcium carbonate in the working solution, and $K^o_{s,x}$ is the equilibrium constant of the polymorph x. In supersaturated carbonate solutions, a number of polymorphs may be formed, including calcium carbonate monohydrate, vaterite, aragonite and calcite. For vaterite, $K^o_{s,v} = 1.222 \times 10^{-8}$ [26]; for aragonite, $K^o_{s,\alpha} = 4.613 \times 10^{-9}$ [26]; for calcite, $K^o_{s,c} = 3.311 \times 10^{-9}$ [26]; and for calcium carbonate monohydrate $K^o_{s,m} = 1.411 \times 10^{-7}$ [27]. In the experiments presented herein, calcite was exclusively formed both in the presence and in the absence of taurine as concluded by examination of the powder X-ray diffraction spectra, FT-IR spectra and thermogravimetric analysis [28, 29]. SEM micrographs are shown in Fig. 1. Unreacted calcite seed crystals are well-formed, sharp-edged rhombs (Fig.1(a)). Figure 1, (b) and (c) shows that in the presence of taurine the normal growth of calcite is disturbed, crystal growth planes are not uniform, growth surfaces and growth edges appear broken and discontinuous.

The experimental conditions for the seeded growth experiments are pH 8.50, 25°C, taurine concentration = 2.4×10^{-4} M, total calcium (Ca_t) = total carbonate (C_t), and $\Delta G_{calcite} = -3.08, -2.09, -2.72, -2.51, -2.27$ kJ . mol^{-1} for $Ca_t = 3, 2.75, 2.5, 2.25, 2$ mM respectively. As may be seen from Fig. 2, taurine had an acceleration effect on the crystal growth rate. These experimental findings may be explained by the hypothesis that we have three types of crystal surface sites; kinks, steps and terraces in or-

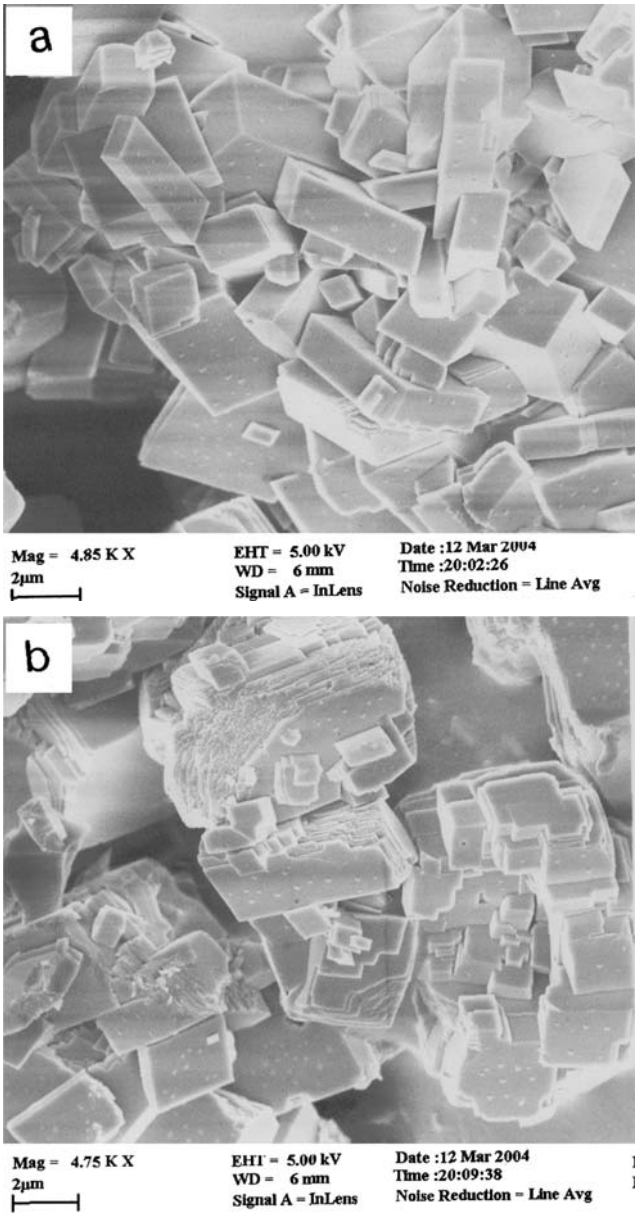

Fig. 1 Scanning electron micrographs of : (a) calcite seed crystals; (b) and (c) calcite grown in the presence of 2.4×10^{-4} M taurine, $Ca_t = C_t = 3 \times 10^{-3}$ M.

der of decreasing activity [30, 31]; and the further blocking of the less active growth sites. Note the laterally discontinuous planes of growth in photomicrographs (b) and (c).

The reproducibility of the measured rates was better than 2% (a mean of five experiments), typical for crystal growth rates measured at sustained supersaturation. Kinetic analysis both in the presence and in the absence of the aminoacid taurine showed that the rates of crystal growth of calcite strongly depended on the relative supersaturation $\sigma_c = \Omega_c^{1/2} - 1$ according to the phenomenological equation

$$R = k\sigma_c^n \qquad (2)$$

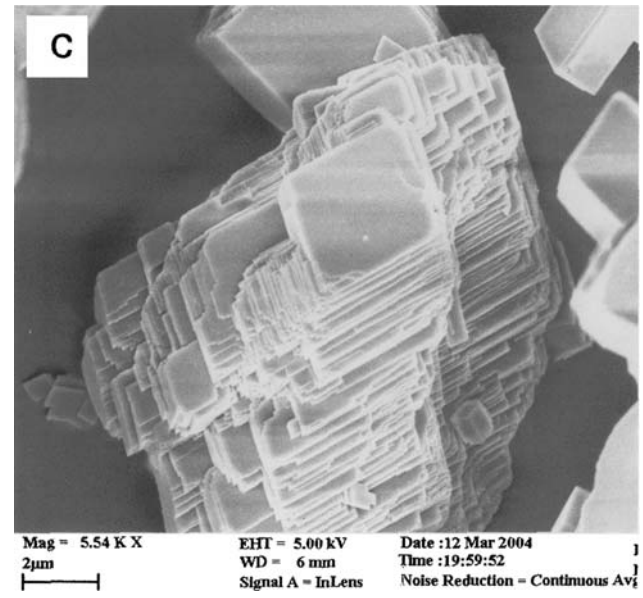

Fig. 1 *(cont.)*

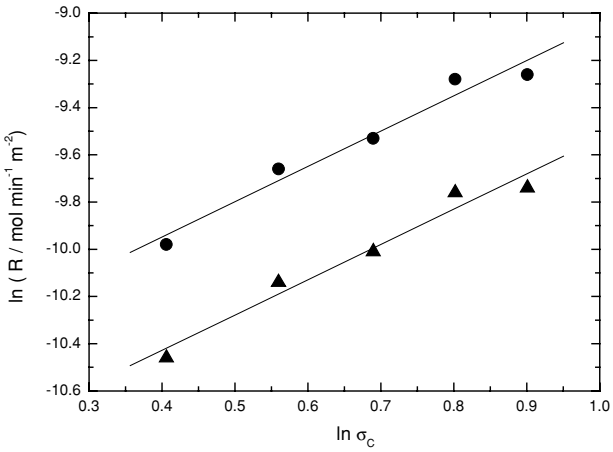

Fig. 2 Kinetics of calcite crystallization in the presence (●) and in the absence (▲) of 30 mg taurine.

In Eq. (2), k is the crystal growth rate constant, which is a function of the active growth sites on the seed crystals, and n the apparent order for the crystal growth of calcite.

Logarithmic plots of the rates as a function of σ_c yielded the straight lines shown in Fig. 2. As may be seen from this figure the same value $n = 2 \pm 0.2$, was obtained from the slopes of the curves both in the presence and in the absence of taurine pointing out to the same surface diffusion controlled, spiral growth mechanism. According to the SEM photomicrographs we assume high kink density and crystals in which the unit cells have several non-equivalent molecular positions way show strong cooperative interaction within the cell, inducing real potential barrier for one dimensional nucleation and non-linear dependencies of kink, step and face rates on supersaturation [32]. Finally, different amounts of

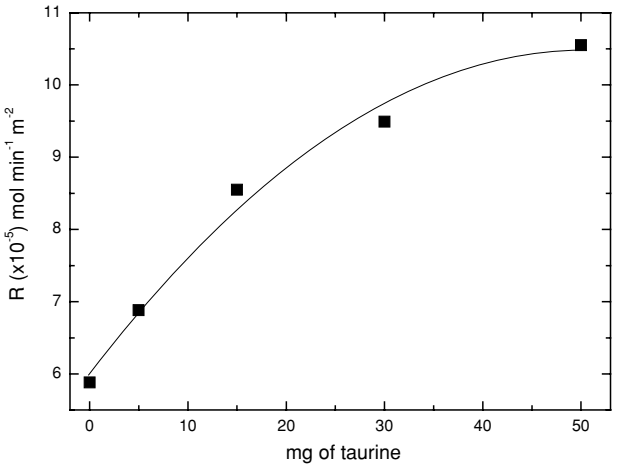

Fig. 3 The effect of taurine concentration on the crystal growth rates of calcite precipitation, $Ca_t = C_t = 3 \times 10^{-3}$ M.

taurine added to the supersaturated solutions resulted in an acceleration effect as much as 17–96 % on the crystal growth rate as shown in Fig. 3.

4. Conclusion

In conclusion, it can be said that the presence of taurine in the supersaturated solutions, lead to calcite crystals with a characteristic discontinuous planes of growth and poor habit, as compared to the rhombohedral morphology. There is an acceleration effect on the crystal growth rates 17–96 % for 4×10^{-5} – 4×10^{-4} m taurine concentration respectively. There is no effect on the mechanism or the particle size of the crystals growth. The apparent order was found to be 2 ± 0.2 typical for surface diffusion controlled spiral growth processes. As a general result, it is obvious from the '*in vitro*' experiments that an increase in the concentration of taurine in the blood plasma may lead to pathogenic deposition of calcium carbonate as pancreatic stones, gallstones on the atherosclerotic plaque.

Acknowledgements The authors thank the Greek foundation I.K.Y. for the postdoctoral fellowship of Dr. P. Malkaj.

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