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The effect of acetaminophen on the crystal growth of calcium carbonate

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Abstract The effect of acetaminophen, a well known analgesic and fever-reducing medicine, on the calcium carbonate crystal growth was investigated under plethostatic conditions. The calcification rates measured was reduced by 9.1–63.2% in the presence of acetaminophen. Kinetic analysis according to a Langmuir-type adsorption isotherm lead to the calculation of an affinity constant $K_{aff} = 8.33 \times 10^2$ dm^3 mol^{-1}. The apparent order found from kinetic data was two suggesting a surface diffusion controlled spiral growth mechanism.

1 Introduction

Calcium carbonate is the most abundant mineral in nature and is found as different polymorphs, consisting, in order to increasing solubility of calcite, aragonite, vaterite, calcium carbonate monohydrate and calcium carbonate hexahydrate [1]. Under physiological conditions, the results of calcium carbonate deposition in biological systems can be seen in the formation of mollusk shells, egg shells, the exoskeleton of arthropods, pearls ad corals [2, 3]. There are however, also pathological aspects of biomineralization, including the formation of the human atherosclerotic aorta (9% carbonate mineral) [4], kidney stones, pancreatic calcification and gall stones [5]. High levels of bicarbonate concentrations and high pH values as well as total calcium concentrations of 2.4–9.7 mM are secreted by the pancreas and biliary tract [6]. Chemical analysis ad crystallographic studies showed that calcium carbonate was the major constituent ranged from 3.7% to 91.6% and averaged 77.8%) in pigment gallstones as well as in calcium carbonate gallstones (called limybile) [7–9]. The calcite carbonate polymorph found was calcite at 62.5%.

Acetaminophen {N-(hydroxyphenyl)acetamide}; One of the most common mediations found in households; is an analgesic and fever-reduced medicine similar in effect to aspirin. Acts by interfering with the synthesis of prostaglandins and other substances necessary for the transmission of pain impulses [10]. Over many years, it has been used countless times by many people and it has proven to be safe and effective medication. Acetaminophen exhibit moderate toxicity [11] with typical oral dose 0.5–2 g daily. Maximum plasma levels 6 h after administration without incidence of liver failure are reported to be 100 μg/ml [12]. Havy use, however, has been linked to liver and renal effects [13]. Despite the rather widespread use of acetaminophen as drug, very little is known concerning their influence on several pathological cases such as pancreatic calcification, kidney stones formation, gallstones and intravasal depositions.

In the present work, an attempt was made to evaluate the antimineralization activity of acetaminophen in the calcium carbonate system by the constant composition method [14, 15]. The conditions of the experiments

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selected in the present work were such that the supersaturated solution employed were stable for periods up to two days, their stability verified by the constant pH and calcium concentration. The low degree of supersaturation is a better representation of the physiological environment, where the free calcium concentration is rather low [6]. The supersaturated solution was seeded with synthetically prepared calcite crystals in the presence of acetaminophen and the calcium carbonate crystallization was followed.

2 Experimental procedure

All the experiments were done at 25 ± 0.1 °C, in a thermostated double-walled Pyrex vessel. Calcium carbonate supersaturated solutions of 0.2 dm^3 total volume were prepared from calcium nitrate, sodium bicarbonate and potassium nitrate stock solutions, (Merck proAnalysi) as described in detail elsewhere [16]. Acetaminophen (Merck proAnalysi) was added at various concentrations as indicated in Table 1. The arrangement was such that the air volume over the aqueous phase was kept at a minimum, so that the partial pressure of the carbon dioxide may be considered to be constant [15]. The pH in all experiments here was adjusted at 8.50 by the addition of standard potassium hydroxide solution (Merck, titrisol). Following verification of the stability of the supersaturated solution 100 mg of calcite synthetically prepared [15] was added to the solution. The BET specific surface area of calcite determined by N_2 adsorption (Perkin Elmer sorptometer 212D) was found to be 3.2 $m^2\ g^{-1}$.

Precipitation reaction in all cases started immediately after the introduction of the seed crystals in the crystallization medium. The pH change (0.003 pH units), concomitant with the formation of calcium carbonate triggered the addition of titrants, with the stoichiometry of calcium carbonate from the coupled burettes of an appropriately modified pH stat (Metrohm, 614). The concentration of the titrant in the two burettes was calculated as follows :

$$\text{titrant 1} : (Cx + 2x)\ (Ca(NO_3)_2),$$

$$\text{titrant 2} : Cx(Na_2CO_3) + 2x(NaHCO_3) + 2y(KOH),$$

where x is the molar concentration of calcium nitrate or sodium bicarbonate in the working solution and y the amount of potassium hydroxide required for the pH adjustment in the working solution. For maintenance of the ionic strength constant an amount $2C$ of inert electrolyte (potassium nitrate) was added in the working solution where C is a constant (expressing how many times the titrants are more concentrated than the working solution). In our experiments, C was chosen as 10. The choice of the best value for C requires preliminary experiments.

Random sampling during the course of reaction verified that the solution supersaturation was kept constant [15]. Employing a constant solution composition has the advantage of determining the reaction rates very accurately, since the initial conditions are kept constant for a large part of crystallization reaction. The samples withdrawn during the reaction were filtered through membrane filters (Millipore 0.1 μm); the filtrates were analyzed for calcium by atomic absorption spectroscopy (Varian 1200) and the solid residues by powder X-ray diffraction (Phillips PW

Table 1 crystallization of calcite on calcite seed crystals in the presence of acetaminophen, AC_t; pH 8.50; 25 °C; Total calcium (Ca_t) = total carbonate (C_t).

Ca_t (10^{-3} mol dm^{-3})	Ionic strength (10^{-2} mol dm^{-3})	$\Delta G_{calcite}$ (kJ mol^{-1})	AC_t (10^{-4} mol dm^{-3})	R (10^{-6} mol min^{-1} m^{-2})
3	7.19	–3.08	–	25.3
2.75	6.6	–2.90	–	17.4
2.5	6.0	–2.72	–	13.4
2	4.8	–2.27	–	8.3
3	7.19	–3.08	1.654	23.0
3	7.19	–3.08	3.308	19.1
3	7.19	–3.08	4.962	18.4
3	7.19	–3.08	6.616	16.5
3	7.19	–3.08	8.270	14.4
3	7.19	–3.08	9.924	11.6
3	19	–3.08	11.578	10.2
3	7.19	–3.08	13.232	9.3
2.75	6.6	–2.90	9.924	8.0
2.5	6.0	–2.72	9.924	6.1
2	4.8	–2.27	9.924	3.8

1830/1840 using Cukα RADIATION Ni filter), scanning electron microscopy (SEM-EDXS, JEOL JSM 5200 and LEO supra 35 VP), FT-IR spectroscopy (Perkin Elmer 16-PS Ft-IR using KBr pellets) and thermogravimetric analysis (TGA Du Pont 910). The rate of calcium carbonate crystallization was taken from the plots of titrant addition as a function of time, normalized for the total surface area of the seed crystals.

3 Results and discussion

The experimental conditions are summarized in Table 1. The solution speciation was computed from the appropriate equilibria between calcium and carbonate species, mass-balance equations for calcium and carbonate and the electroneutrality condition by successive approximations for the ionic strength [17] For the estimate of the activity coefficients the Davies equation [18] was used. The solid phases wee found to be calcite from the examination of the powder X-ray diffraction spectra (hkl: 102, 104, 110, 113, 202, 108, 116, 212, 214, 300) [18] and of the FT-IR spectra (bands : 1,800, 1,420,876, 714 cm^{-1}) [19, 20]. The thermogravimetric analysis exclude the formation of hydrated calcium carbonate salts. Well grown calcite crystals in the presence of acetaminophen may be seen in the scanning electron micrographs in Fig. 1 [20, 21]. The driving force fro crystallization may be expressed in terms of the change in Gibbs free energy of transfer from unstable supersaturated to saturated solutions (e.g., thermodynamic equilibrium):

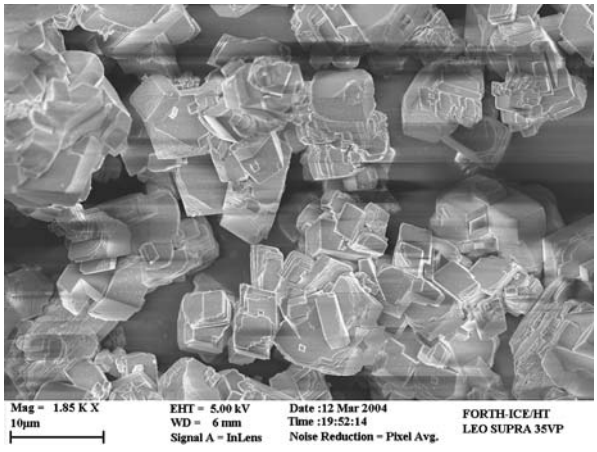

Fig. 1 Scanning electron micrograph of calcite calcification on calcite seed crystals in the presence of 9.924×10^{-4} mol dm^{-3} acetaminophen (Table 1)

$$\Delta G_{\text{calcite}} = -\frac{R_g T}{\upsilon} \ln \frac{\text{IP}}{K_s^o} = -\frac{R_g T}{2} \ln \Omega \quad (1)$$

In Eq. (1) IP is the ionic product of the precipitating salt, $K_s^o$the thermodynamic solubility product at 25 °C ($K_s^o = 3.311 \times 10^{-9}$) [22], υ the number of ions in the formula unit of the precipitating calcium carbonate (e.g. 2), R_g the gas constant, T the absolute temperature and Ω the supersaturation ratio.

From experiments performed with different amounts of seed crystals in he range between 50 and 150 mg it was revealed that the crystal growth rate of calcite on calcite seed crystals was proportional to the amount of seed crystals initially added. This indicates that the crystallization process occurs exclusively on the surface of the seed crystals without phenomena of spontaneous or secondary precipitation. Changes in the stirring rate between 100 and 600 rpm had no effect on the crystal growth kinetics, suggesting that the rate-determining step of the process is not diffusion from the bulk solution to the crystal surface [23]. The mean size of the calcite crystal particles into the working solution was aobut 10 µm as measured with a particle size analyzer (Laser Particle Counter ILI-1000) and therefore the criterion used before for the eludication of the rate determining step is valid [23, 24].

Crystal growth rates were found to be proportional to the relative solution supersaturation, σ, with respect to calcite, defined as [24, 25]

$$\sigma = \frac{(\text{IP})^{1/2} - (K_s^o)^{1/2}}{(K_s^o)^{1/2}} = \Omega^{1/2} - 1 \quad (2)$$

$$R = ks\sigma^{\text{n}} \quad (3)$$

Where R is the crystal growth rate, k, the rate constant, s, a function of the number of the active growth sites on the crystal surface, and n the apparent order of the crystallization reaction. Logarithmic plots according to Eq. (3), yielded straight lines in the presence and in the absence of acetaminophen (Fig. 2)From the slope of the linear plots a value of $n = 2$ was obtained, suggested a surface diffusion controlled spiral growth mechanism [25]. In the presence of acetaminophen no changes observed in the calcite overgrown morphology, as verified from scanning electron microscopy studies (Fig. 1).

As may be seen from Table 1 and Fig. 2 when acetaminophen is present in the supersaturated solution the rate of calcite crystal growth decreased significantly. The presence of a foreign compound in

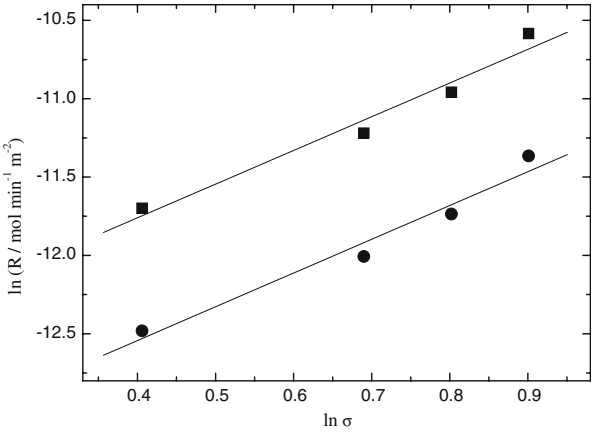

Fig. 2 Dependence of the rates of calcite crystallization on the relative supersaturation in the presence (•) and in the absence (■) of 9.924×10^{-4} mol dm^{-3} of acetaminophen

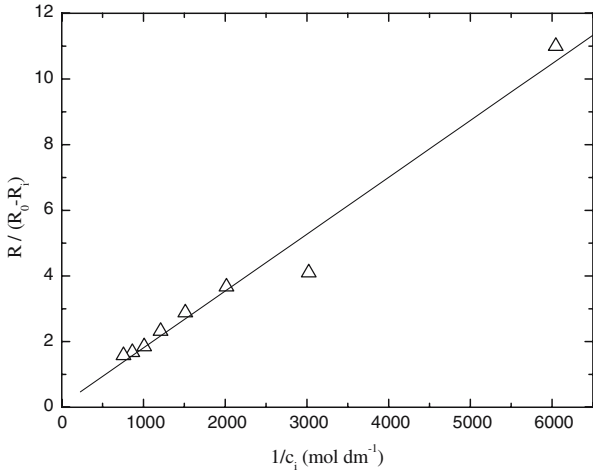

Fig. 3 Kinetics of calcite crystal growth in the presence of various concentrations of acetaminophen according to the kinetic model based on a Langmuir-type adsorption (ΔG = –3.08 kJ mol^{-1})

the supersaturated solution in which a crystal growth process is taking place, very often results in the interaction of the solute species have functional groups such as carboxyl and/or amino groups with the seed crystals. They may adsorb reversibly on the crystal surface, which contains centers of positively and negatively charged ions and therefore the solute species may be accommodated on the surface. It has been suggested that the adsorption of inhibitor molecules at active growth sites on the crystal surfaces accounts for the reduction of the crystal growth rate. Thus, the growth centers are blocked and the adsorbed molecules prevent the crystal lattice species from incorporation in the crystal.

Assuming that the basic principles of the Langmuir adsorption model are valid (i.e. the adsorption energy is the same for the all adsorption sites, absence of interaction between the adsorbed molecules and monolayer coverage of the surface), then at equilibrium the rates of adsorption and desorption of the solute on the surface are equal,

$$k_{\text{ads}}.(1-\theta)c_{\text{eq}}. = k_{\text{des}}.\theta, \tag{4}$$

where $k_{\text{ads.}}$ and $k_{\text{des.}}$ are the specific rate constants for adsorption and desorption, respectively, θ, the fraction of the crystal surface active growth sites occupied by the adsorbed molecules, and $c_{\text{eq.}}$ The equilibrium solution concentration of the additive. The kinetic results may be interpreted by the Langmuir formalism and therefore the growth rates depend on the surface coverage θ [26],

$$R_{\text{i}} = R_{\text{o}}(1-\theta), \tag{5}$$

Where R_{i} and R_{o} are the crystal growth rates in the presence and in the absence of the additive. Combination of Eqs. (4) and (5) gives

$$\frac{R_o}{R_o - R_i} = 1 + \frac{1}{k_{aff.}}\frac{1}{c_{eq.}} \tag{6}$$

In Eq. (6), $K_{\text{aff.}}$ Is the affinity constant (equal to $k_{\text{ads.}}/k_{\text{des.}}$) and it is a measure of the affinity of the adsorbate for the adsorbent. As may be seen in Fig. 3 a straight line was obtained for acetaminophen, suggesting the validity of the assumed model. The affinity constant, as determined from the slope of the linear plot of $R_{\text{o}}/(R_{\text{o}}–R_{\text{i}})$ against $1/c_{\text{eq.}}$according to Eq. (6) is 8.33×10^2 dm^3 mol^{-1}. Similar results were obtained for metallocene dichlorides [27].

In conclusion, acetaminophen may be a useful drug not only as analgesic but in many other cases of pathological calcification (pancreative calcification, kidney stones formation, gallstones and intravasal depositions) It is effective at concentrations 165.4 μmol dm^{-3} (Table 1) significantly lower than the uper safety plasma level of 600 μmol dm^{-3} [12] and decrease the calcification rate ''in vitro'' by 9.1–63.2% (Table 1).

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