JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 13 (2002) 69–73

The crystallization of calcium carbonate on sodium cholate

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Recent studies in the bibliography showed that calcium carbonate was the major constituent (77.8%) in gall stones, and the polymorph calcite was at 62.5% of the cases examined. The kinetics of crystallization of calcite on sodium cholate has been studied using the constant composition technique. Analysis of the initial rates as a function of the solution supersaturation, according to the classical nucleation theory, yielded a value of 33 mJ m^{-2} for the surface energy of the growing phase and a five-ion cluster, forming the critical nucleus. The apparent order for the calcite crystallization was found to be 4.5 ± 0.7 indicative of a surface nucleation mechanism. The formation of calcite may be initiated through the interaction of Ca^{2+} ions with the negative end of the C = 0 bond of the sodium cholate molecule.

1. Introduction

Calcium carbonate polymorphs are abundant in our world, in geological deposits, in microorganisms, in plants and in animal kingdom [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 and corals. The formation of calcium carbonate polymorphs (mainly vaterite, aragonite and the most stable calcite) is also important in several pathological cases, such as pancreatic calcification [2], kidney stones formation [3], gallstones [4] and intravasal depositions.

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 [5–7]. Chemical analysis and crystallographic studies showed that calcium carbonate was the major constituent (ranged from 33.7% to 91.6% and averaged 77.8%) in pigment gallstones as well as in calcium carbonate gallstones (called limybile) [8, 9]. The calcite carbonate polymorph found was calcite at 62.5%.

The aim of the present work is to investigate the possibility of calcium carbonate overgrowth on sodium cholate and to study the mechanism of calcium carbonate formation, by the constant composition method [10–12]. This method is particularly suited for studying the formation of a new phase on a substrate, since the initial conditions of precipitation are maintained. Initial rates and induction periods may be precisely measured even at low degrees of supersaturation, where the extent and the rate of precipitate formation are very low.

The conditions of the experiments selected in the present work were such that the supersaturated solutions 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 low due to complexation with the macromolecules in biological fluids. The solutions were seeded with sodium cholate and the calcium carbonate overgrowth was followed. Despite the fact that a number of studies concerning nucleation and growth of calcium carbonate have been done at low supersaturations [13–21], there is still considerable uncertainty regarding the mechanism of formation of calcium carbonate on foreign substrates, especially those found in the physiological environment.

2. Experimental procedure

All experiments were done at $25 \pm 0.1\,^{\circ}C$, 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 stock and potassium nitrate solutions, as described in detail elsewhere [22]. 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 [12]. The pH in all experiments here was adjusted at 8.50 by the addition of standard potassium hydroxide solution. Following verification of the stability of the supersaturated solutions 100 mg of sodium cholate (Sigma, from Ox or Sheep Bile) was added to the solution. The BET specific surface area of sodium cholate was determined by N_2 adsorption (Perkin Elmer sorpometer 2112D) was found to be 6.9 $m^2\,g^{-1}$. No change in solution pH or in calcium concentration was observed, indicating lack of precipitation. Precipitation, however started following the lapse of well defined, though relatively long, induction periods,

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reproducible with 5% accuracy. 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:

titrant 1: $(Cx + 2x)(Ca(NO_3))_2$,
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 [12]. 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 the precipitation reaction, and also the possibility to follow the growth of the overgrowing phase, sufficiently large to be characterized by physiochemical methods. The samples withdrawn during the reaction were filtered through membrane filters (Gelman, 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 1830/1840 using Cuka radiation Ni filter), scanning electron microscopy (Jeol GSM 5200) and FT-IR spectroscopy (Perkin-Elmer 16-PC FI-IR using KBr pellets). The induction periods were measured as the time lapsed between the addition of sodium cholate in the supersaturated solutions and the onset of titrant additions. The rate of calcium carbonate formation was taken from the plots of titrant addition as a function of time, normalized for the total surface area of the sodium cholate used as substrate.

3. Results and discussion

The experimental conditions are summarized in Table I. The solution speciation in all experiments was calculated from the proton dissociation and ion pair formation constants for calcium and carbonate, the mass balance and electroneutrality conditions by successive approximations for the ionic strength [23]. The driving force for the crystal growth of the calcium carbonate polymorphs is the change in Gibbs free energy, ΔG, for the transfer from the supersaturated solution to equilibrium

$$\Delta G_x = -\frac{R_g T}{2} \ln \frac{IP}{K^0_{S,x}} \tag{1}$$

In Equation 1 IP is the activity product $(Ca^{2+})(CO_3^{2-})$, $K^0_{S,x}$ the thermodynamic solubility product of polymorph x, (see Table II), R_g the gas constant and T the absolute temperature.

The supersaturation with respect to each polymorph, Ω_x, is defined as

$$\Omega_x = \frac{IP_x}{K^0_{S,x}} \tag{2}$$

and the relative solution supersaturation, S, is defined by

$$S_x = \frac{(IP)_x^{1/2} - (K^0_{S,x})^{1/2}}{(K^0_{S,x})^{1/2}} = \Omega_x^{1/2} - 1 \tag{3}$$

The overgrowth of the crystalline material started only after the lapse of well defined induction periods as it can be seen from Table I. The reproducibility with respect to the induction periods was $\pm$ 5% and the subsequent rates of crystallization was $\pm$ 2%. The solid phases were found to be calcite [26] from the examination: (a) of the powder X-ray diffraction spectra, the crystalline sample exhibit the characteristic reflections for calcite phase such as (102), (104), (110), (113), (202), (108), (116), (212), (214) and (300); (b) of the FT-IR spectra (adsorption band assigned to calcite, 1800, 1420, 876 and 714 cm^{-1} [12, 20]); (c) Thermogravimetric analysis (TGA). TGA analysis exclude the existence of hydrated calcium carbonate salts; and (d) SEM photographs as shown in Fig. 1. Well grown calcite crystal on sodium cholate substrate may be seen in these scanning electron micrographs.

As can be seen in Table I, the induction periods are inversely proportional to the relative solution supersaturation. The subsequent rates of crystallization were found to increase with supersaturation. Doubling or tripling the amounts of sodium cholate introduced in the supersaturated solution had no effect on the initial rates normalized per unit of surface area of the substrate. It should be noted that the rates we have used in the kinetics analysis of our experiments were obtained from the slopes of the curves of titrant addition (reflecting the amount of solid precipitating) at time zero. This is justified by the fact that the amount precipitated

TABLE I Crystal growth of calcite on sodium cholate at sustained supersaturation, 25 °C, pH 8.50, 0.5 mg of sodium cholate/ml, total calcium (Ca_t) = total carbonate $^*C_t$)

Exp. no.	Ca_t (10^{-3} mol dm^{-3})	Ionic strength (10^{-2} mol dm^{-3})	$\Delta G_{calcite}$ (KJ mol^{-1})	$\Delta G_{arag.}$ (KJ mol^{-1})	$\Delta G_{vat.}$ (KJ mol^{-1})	ΔG_{mono} (KJ mol^{-1})	τ (sec)	R (10^{-9} mol s^{-1} m^{-2})
sxs-2	3	7.19	− 3.08	− 2.34	− 1.45	− 1.40	4809	26.9262
sxs-4	2.75	6.6	− 2.90	− 2.16	− 1.28	− 1.23	13822	11.0412
sxs-6	2.5	6.0	− 2.72	− 1.97	− 1.09	− 1.04	11345	6.7938
sxs-11	2	4.8	− 2.27	− 1.53	− 0.64	− 0.59	24482	2.5186

TABLE II Thermodynamic solubility products of calcium carbonates at 25 °C

Calcium carbonate polymorph x	$K^0_{s,x}$	References
Calcite	3.311×10^{-9}	24
Aragonite	4.613×10^{-9}	24
Vaterite	1.222×10^{-8}	24
Calcium carbonate monohydrate	1.279×10^{-8}	25, 16

continuously increased, thus changing the total surface area.

The amount of sodium cholate added had no effect on the induction period, a fact proving the absence of secondary nucleation [26]. Also, changes in the stirring rate (between 60 and 350 rpm) had no effect on the kinetics parameters. It may therefore be suggested that calcite overgrowth was induced by the substrate by heterogeneous nucleation [26, 27]. Similar considerations obtained for the mineralization of other substrates [15–17, 20, 21].

Calcite overgrowth was observed following the lapse of induction periods, reflecting the time required for the development of the critical nucleus. The dependence of the induction period, τ, on the initial free calcium concentration $[Ca^{2+}]_0$ is given by [28]

$$\tau = k_r [Ca^{2+}]_0^{1-p} \tag{4}$$

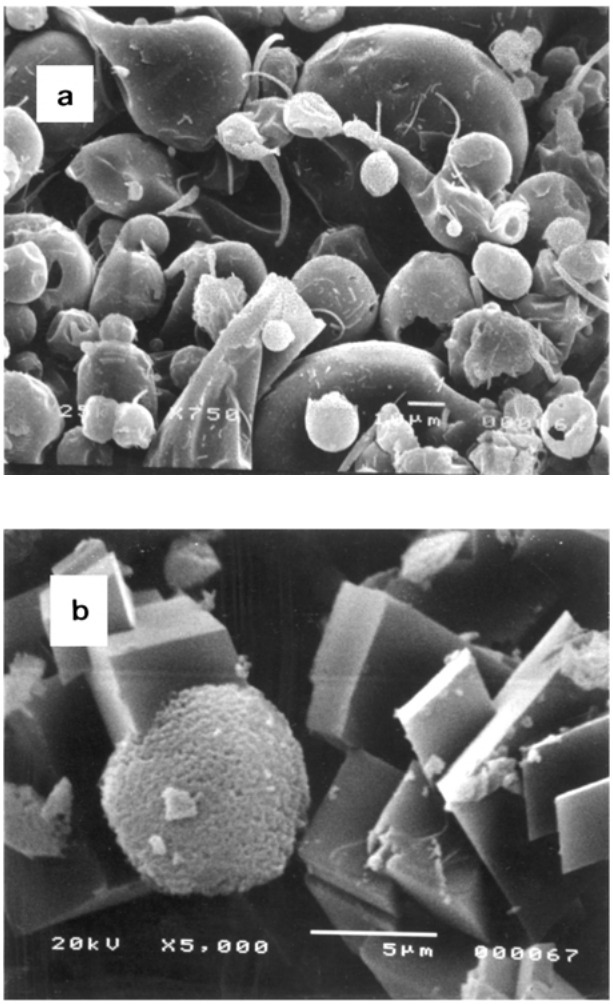

Figure 1 Scanning electron microscopy of (a) sodium cholate substrate; (b) calcite crystals on sodium cholate.

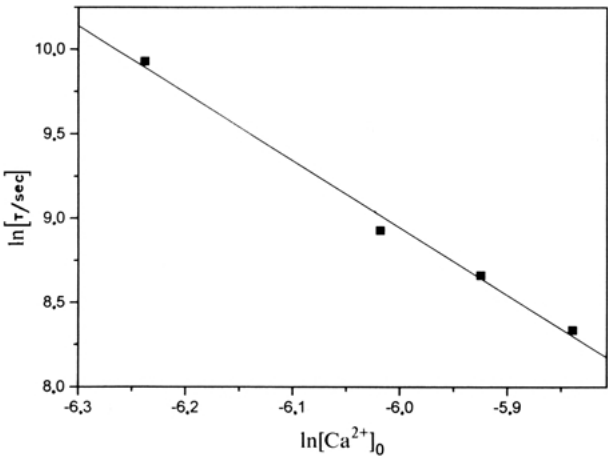

Figure 2 Induction periods, preceding the overgrowth of calcite on sodium cholate, as a function of the initial calcium concentration in solution.

In Equation 4, k_r is a constant and p the number of ions forming the critical nucleus. Although the meaning of the size of the critical nucleus has been subjected to considerable criticism, it may be a useful parameter for the comparison of various substrates for the deposition of sparingly soluble salts, a process which although quantitatively is not in agreement with the classical nucleation theory, qualitatively it may be described in the same terms [27, 29]. Thus a value of $p = 5$ was obtained for the number of ions in the critical nucleus.

The size of the critical nucleus may be estimated from the slope of plots of the logarithm of initial rates, R, as a function of the logarithm of the initial free calcium concentration [30]

$$\frac{d \ln R}{d \ln [Ca^{2+}]_0} = n^* \tag{5}$$

Thus plotting Equation 5 a value of $n^* = 5$ was estimated.

Using nucleation rate equations derived from the classical homogeneous nucleation theory, interfacial energy for calcite overgrowth on sodium cholate was calculated [27],

$$R = K_{\text{het}} \exp\left[- \frac{\beta V_m^2 \gamma^3 f(\theta)}{k^3 T^3 (\ln \Omega_c)^2} \right] \tag{6}$$

In Equation 6 V_m is the molecular volume of the precipitated calcite, γ the interfacial energy, Ω_c the supersaturation ratio, $f(\theta)$ a factor expressing the compatibility between the salt formed and the substrate ($f(\theta) = \frac{(2+\cos\theta)(1-\cos\theta)^2}{4}$) [20, 21]. K_{inet} is constant, and β is the shape factor ($\beta = 32$ assuming a cubic shape nuclei). From the slope of the plot $\ln R$ against $1/(\ln \Omega_c)^2$ a value of 33 mJ m^{-2} was obtained for the $\gamma f(\theta)$ for the growing calcite. Similar values for the overgrowth of calcite on foreign substrates are shown in Table III. The dependence of the induction periods on supersaturation may also be used for the estimate of the surface energies, plotting lnt as a function of $1/(\ln \Omega_c)^2$ [32, 33].

$$\ln \tau = \ln B + \frac{A V_m^2 \gamma^3 f(\theta)}{k^3 T^3 (\ln \Omega_c)^2} \tag{7}$$

TABLE III Surface energy, $\gamma f(\theta)$, for the overgrowth of calcite on foreign substrates

Substrate	$\gamma f(\theta)$/mJ m^{-2}	Ref.
Collagen	58	13
Chitin	27	20
Elastin	25	21
Cellulose	46	31
Sodium cholate	33	a

[a]This work.

In Equation 7 B is a constant and $A = 0.4\,\beta$. A plot of t according to Equation 7, shown in Fig. 3, results in a straight line from the slope of which a value of 41 mJ m^{-2} for the $\gamma f(\theta)$ of the growing phase was estimated.

A value of 230 mJ m^{-2} for the surface energy was obtained by using the empirical relationship [34, 35]:

$$\gamma = \alpha^2[11.6 - 1.12\ \ln([Ca^{2+}]_\infty)] \times 10^{-21}\ \mathrm{J\,m^{-2}} \quad (8)$$

In Equation 8, $[Ca^{2+}]_\infty$ is the solubility of calcite at 25 °C [16] and α is the molecular diameter, usually calculated as $\alpha = V_m^{1/3}$, $\alpha = 3.13 \times 10^{-10}$ m. The high value predicted by Equation 8 pertains to homogeneous nucleation conditions in contrast to our experiments, where the new phase is grown on a foreign substrate with a definite number of active growth sites. Logarithmic plots of the rates of calcite formation on sodium cholate, R, as a function of the relative solution supersaturation S_c, yielded a straight line as shown in Fig. 4. From the slope of the line an apparent order of $n = 4.5 \pm 0.7$ was calculated. This value may be interpreted as indicative of a surface nucleation mechanism [36]. A consideration of the polarity of the C=0 bond in sodium cholate molecule, in which the negative charge is shifted towards the oxygen atom, suggests that the formation of calcite may be initiated through the interaction of Ca^{2+} ions with the end of the C=0 bond [18, 20, 21].

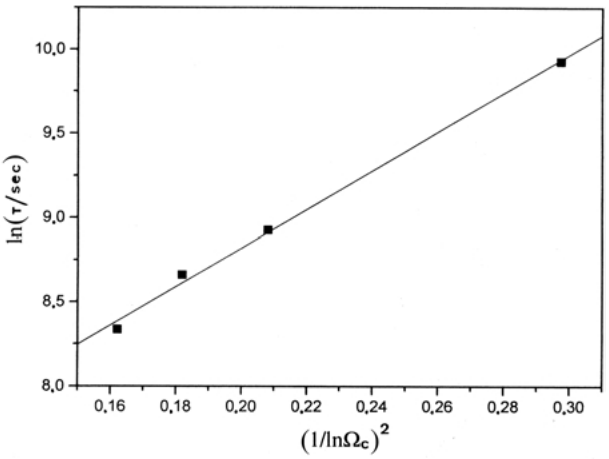

Figure 3 Induction period as a function of the solution supersaturation according to the classical nucleation theory (Equation 7).

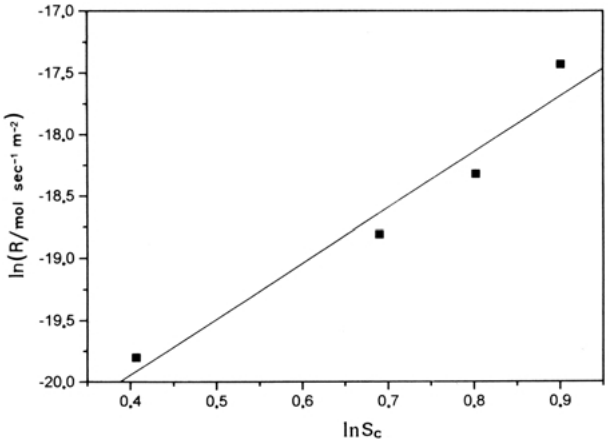

Figure 4 Rate of crystallization of calcite on sodium cholate as a function of the relative solution supersaturation.

References

1. K. SIMKISS, *Am. Zool.* **24** (1984) 847.
2. H. SARLES, *Gastroenterology* **66** (1974) 604.
3. H. J. VERINE, *Bull. Com. Pathol.* **5**(3) (1973).
4. H. S. KAUFMAN, T. H. MAGNUSON, H. A. PITT, P. FRASCA and K. D. LILLEMOE, *Hepatology* **19**(5) (1994) 1124.
5. D. J. SUTOR and L. I. WILKIE, *Clinica Chimica Acta* **79** (1977) 119.
6. D. GLEESON, K. A. HOOD, G. M. MURPHY and R. H. DOWLING, *Gastroenterology* **102**(5) (1992) 1707.
7. D. I. HAY, S. K. SCHLUCKEBIER and E. C. MORENO, *Calcif. Tissue Int.* **39** (1986) 151.
8. K. SAITO, H. OMORI, S. KANNO, Y. HIRATA, T. OKADA, S. MORI and K. NAKADATE, *Gastroenterologia Japonica* **21**(2) (1986) 162.
9. S. D. STRICHARTZ, M. Z. ABEDIN, M. S. ABDOU and J. J. ROSLYN, *Am. J. Surgery* **155**, (1988) 131.
10. M. B. TOMSON and G. H. NANCOLLAS, *Science* **200** (1977) 1059.
11. P. G. KOUTSOUKOS, Z. AMJAD, M. B. TOMSON and G. H. NANCOLLAS, *J. Am. Chem. Soc.* **102** (1980) 1553.
12. T. F. KAZMIERCZAK, M. B. TOMSON and G. H. NANCOLLAS, *J. Phys. Chem.* **86** (1982) 103.
13. E. DALAS and P. G. KOUTSOUKOS, *Langmuir.* **4** (1988) 907.
14. E. DALAS, P. V. IOANNOU and P. G. KOUTSOUKOS, *ibid.* **6** (1990) 535.
15. E. DALAS and P. G. KOUTSOUKOS, *J. Colloid Int. Sci.* **127**(1) (1989) 273.
16. E. DALAS, J. KALLITSIS and P. G. KOUTSOUKOS, *J. Crystal Growth* **89** (1988) 287.
17. M. M. REDDY and G. H. NANCOLLAS, *J. Colloid Int. Sci.* **36** (1971) 166.
18. E. DALAS, *J. Mater. Chem.* **1**(3) (1991) 473.
19. E. DALAS, *J. Mater. Sci. Lett.* **11** (1992) 1408.
20. F. MANOLI, S. KOUTSOPOULOS and E. DALAS, *J. Crystal Growth* **182** (1997) 116.
21. F. MANOLI and E. DALAS, *ibid.* **204** (1999) 369.
22. E. GIANNIMARAS and P. G. KOUTSOUKOS, *J. Colloid Int. Sci.* **116** (1987) 423.
23. G. H. NANCOLLAS, ''Interactions in Electrolyte Solutions'' (Elsevier, Amsterdam) 1966.
24. N. L. PLUMMER, T. M. L. WIGLEY and D. C. PARKKHURST, *Am. J. Sci.* **278** (1978) 179.
25. H. HULL and A. G. TUTRBULL, *Geochim. Cosmochim. Acta* **37** (1973) 685.
26. N. WATABLE, *Progr. Crystal Growth Characterization* **4** (1981) 182.
27. J. NYVLT, O. SOHNEL, M. MATUCHOVA and M. BROUL, ''The Kinetics of Industrial Crystallization'' (Elsevier, Amsterdam, 1985) pp. 68, 284.

28. J. W. MULLIN, ''Crystallization'', 2nd ed., CRC Press, Cleveland, 1972, p. 219.
29. A. E. NIELSEN and J. CHRISTOFFERSEN, in ''Biological Mineralization and Demineralization'', edited by G. H. Nancollas (Springer-Verlag, Berlin, 1982).
30. A. E. NIELSEN, ''Kinetics of Precipitation'' (Pergamon, Oxford, 1964) p. 18.
31. E. DALAS, P. G. KLEPETSANIS and P. G. KOUTTSOUKOS, *J. Colloid Int. Sci.* **224**(1) (2000) 56–62.
32. J. GARSIDE, in ''Biological Mineralization and Demineralization'', edited by G. H. Nancollas (Springer-Verlag, Berlin, 1982) p. 23.
33. A. E. NIELSEN, in ''Crystal Growth'', edited by H. S. Preiser (Pergamon, Oxford, 1967) p. 419.
34. A. E. NIELSEN, *Pure Appl. Chem.* **53** (1981) 2025.
35. A. E. NIELSEN, *J. Crystal Growth* **67** (1984) 289.
36. H. E. L. MADSEN, *ibid.* **80** (1987) 450.

Received 19 April 2000
and accepted 7 March 2001