Three-dimensional Periodic and Fractal Precipitation in Metal Ion–Deoxycholate System: A Model for Gallstone Formation

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It was recently suggested that the periodic and fractal precipitation of calcium salts and proteins in cholesterol gallstonesis closely related to the process of stone formation. The present study is to mimic the process of gallstone growth in vitro. We demonstrate for the first time that a three-dimensional structure of periodic rings (Liesegang ring) and/or fractal patterns can be produced by metal ion and deoxycholate precipitation, which are similar to gallstones. Precipitation in a periodic pattern occurs more often than in a fractal structure. Using X-ray diffraction, polarized light microscope, Fourier transform-infrared (FT-IR), and extended X-ray absorption fine structure (EXAFS), we studied the pattern formation mechanisms of both types of structures. The results indicate that the rate of nucleation in the crystal growth is a crucial element to affect the pattern formation. The pattern selection between periodic and fractal type depends on the crystal growth speed and the rate of nucleation.

Introduction

Concentric rings and fractal patterns are often found in cross-sections of gallstones. But despite some intensive studies,[1–5] their pattern formation mechanism is not clear. We have previously reported that the metal ion–deoxycholate(DC)-diffusion system is an ideal model to mimic pattern formation of gallstones in vitro, and we studied the system in glass test tubes and agar plates, where the reaction–diffusion process could be considered as being one- and two-dimensional, respectively.[6,7] These systems are also widely used in the studies of other periodic and fractal precipitation patterns.[8–14] However, the pattern formation in these 1- and 2-D systems cannot mimic the real process completely, because the gallstones are actually formed in vivo through a complex and prolonged diffusion and precipitation process in three dimensions. In this paper, we succeeded in developing a three-dimensional model, in which we let metal ions and DC ions diffuse into each other in an agar–gel sphere and react to form concentric rings and fractal patterns. We found that the rules of pattern formation in three-dimensional systems are not the same as those in one- and two-dimensional experiments.

Experimental Section

Two typical three-dimensional models were prepared, as shown in Figure 1. In the first case, the metal ions were put in the center, surrounded by a spherical gel (Figure 1a); in the second case the metal ions were put outside of gel sphere (Figure 1b). The detail experimental methods are described as follows.[15]

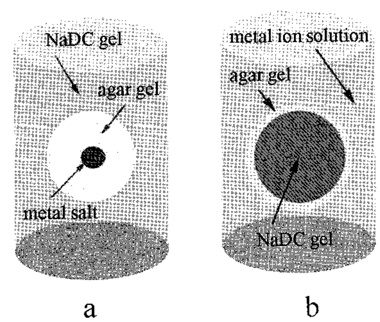

Figure 1. Schematic experimental set up of method 1, (a) and method 2(b).

Method 1. Hot agar solution was first poured into a small beaker, then a granular crystal of the metal salt, molded into a nearly spherical shape, was placed in the agar solution just before the latter converts into a gel. Owing to the viscosity of the agar solution, when the time of adding the crystal is carefully adjusted, the granular crystal can stay in the middle part of the gel. After the sol converts into gel, it was cut into a sphere with the salt crystal at the center. Placing the gel in boiling water for several minutes smoothed its surface. Twenty milliliters of agar solution containing 0.2 mM NaDC was placed

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TABLE 1: The Experimental Condition and the Resultant Pattern

	one-dimensional	two-dimensional	three-dimensional (model 1)	three-dimensional (model 2)
$[MCl_2]^a$	0.1 − 0.01 M	0.3 − 0.01 M	salt	0.1 − 0.01 M
[NaDC]	0.01 M	0.01 M	0.01 M	0.01 M
Cu^{2+}	periodic	periodic	periodic and fractal	periodic and fractal
Co^{2+}	fractal	fractal	periodic and fractal	periodic and fractal
Ni^{2+}	fractal	fractal	periodic and fractal	periodic and fractal
Mn^{2+}	fractal	fractal	periodic and fractal	periodic and fractal
Zn^{2+}	fractal	fractal	periodic and fractal	periodic and fractal
$Cu^{2+} + Ca^{2+}$	periodic	periodic	periodic and fractal	periodic and fractal

[a] M = Cu, Co, Ni, Mn, Zn.

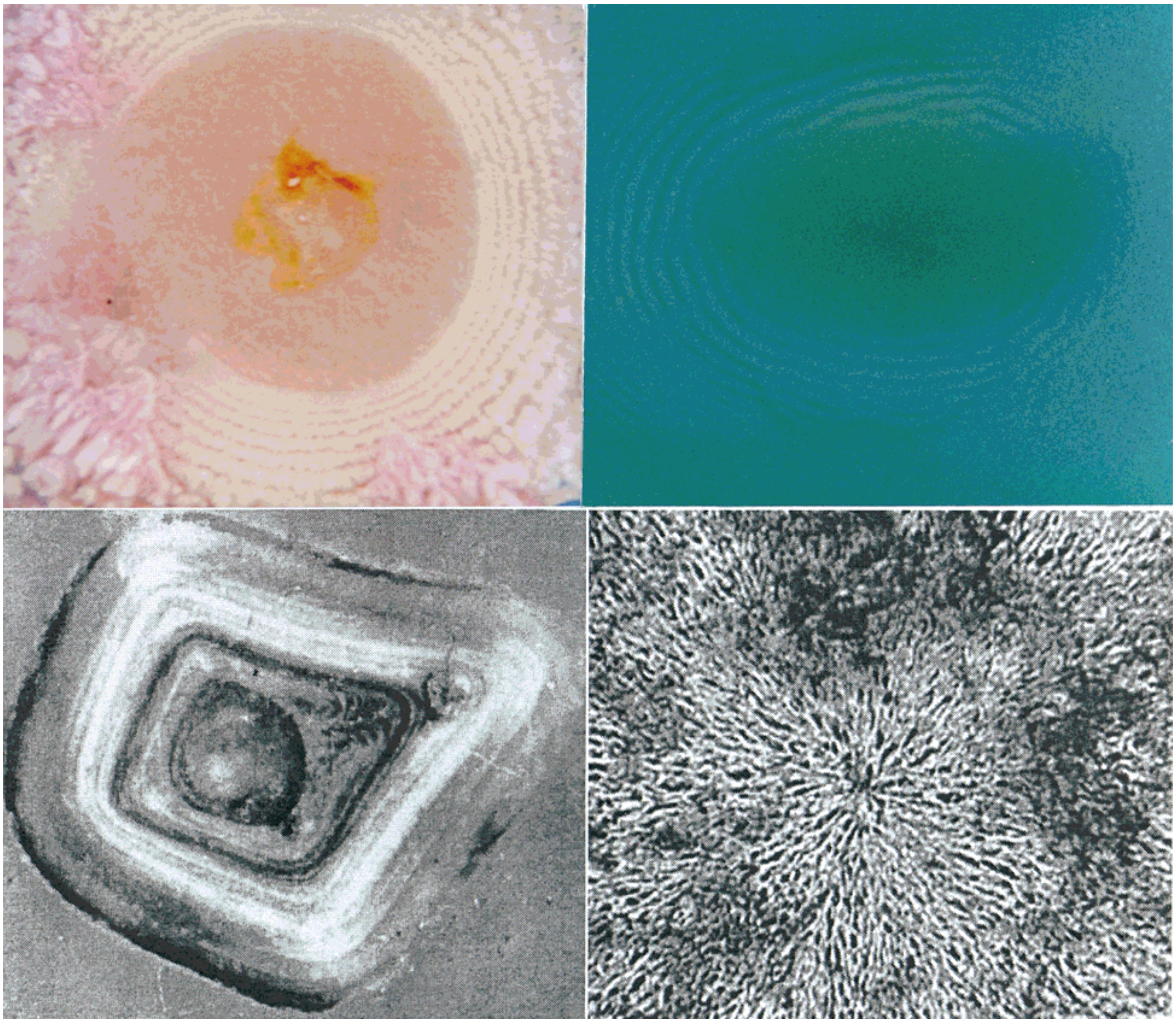

Figure 2. The precipitation patterns produced in three-dimensional metal ions-deoxycholate-gel diffusion systems (a,b), and examples of gallstones obtained from human body (c,d). (a) Three-dimensional Co^{2+} ion system. Prepared by using method 1, a granular salt of $CoCl_2$ was put in center position initially, and [NaDC] = 0.01 M (in outside position). (b) Three-dimensional $Cu^{2+} + Ca^{2+}$ ions system. Prepared by using method 2, $[Cu^{2+}] = [Ca^{2+}] = 0.01$ M (in outside position), [NaDC] = 0.01 M (in center position).

into another beaker (50 mL), and the gel sphere prepared earlier was placed in the sol before the sol converts into the gel. The beaker is then sealed and incubated at room temperature for a month.

Method 2. Agar solution containing 0.2 mM NaDC was poured into a beaker to make a gel. The gel was cut into a 4 cm diameter sphere (NaDC sphere) and was placed in boiling water for while to make its surface smooth. Then some blank agar solution was prepared and poured into another beaker, and the prepared NaDC sphere was dropped into the agar solution just before it converts into the gel. The dropping time is very well controlled such that the NaDC sphere will stop at the middle of the gel. The complex gel was taken out and was sculpted into a 6 cm diameter sphere, which was then boiled again to make its surface smooth. The complex sphere was put into a solution of metal ions and kept it at room temperature for a month.

Results

Many combinations of NaDC and metal ion concentrations have been explored in the experiments. In most case, no pattern formed after 1 or 2 months. For example, in method one, all experiments using from very dilute to very concentrated metal

TABLE 2: Main Frequency of FT-IR Spectrum

	$V_{OH}(cm^{-1})^a$	$V_{OH}(cm^{-1})^b$	$V_{CH}(cm^{-1})^a$	$V_{CH}(cm^{-1})^b$	$V^{as}_{COO}(cm^{-1})^a$	$V^{as}_{COO}(cm^{-1})^b$	$V^{s}_{COO}(cm^{-1})^a$	$V^{s}_{COO}(cm^{-1})^b$
$Mn(DC)_2$	3624 3351	3624 3346	2938 2863	2938 2863	1603 1572 1556	1603 1572 1553	1413	1414
$Co(DC)_2$	3624 3368	3625 3367	2932 2905 2861	2932 2905 2861	1552 1524	1553 1524	1413	1413
$Ni(DC)_2$	3623 3371	3625 3371	2933 2863	2931 2862	1561 1541 1518	1561 1541 1518	1412	1413
$Cu(DC)_2$	3402	3405	2938 2865	2937 2865	1613 1564	1597 1561	1416	1416
$Zn(DC)_2$	3624 3358	3624 3358	2932 2905 2860	2932 2908 2859	1602 1556 1545	1602 1553 1544	1411	1413

[a] Data from synthesized complexes. [b] Data from pattern precipitates.

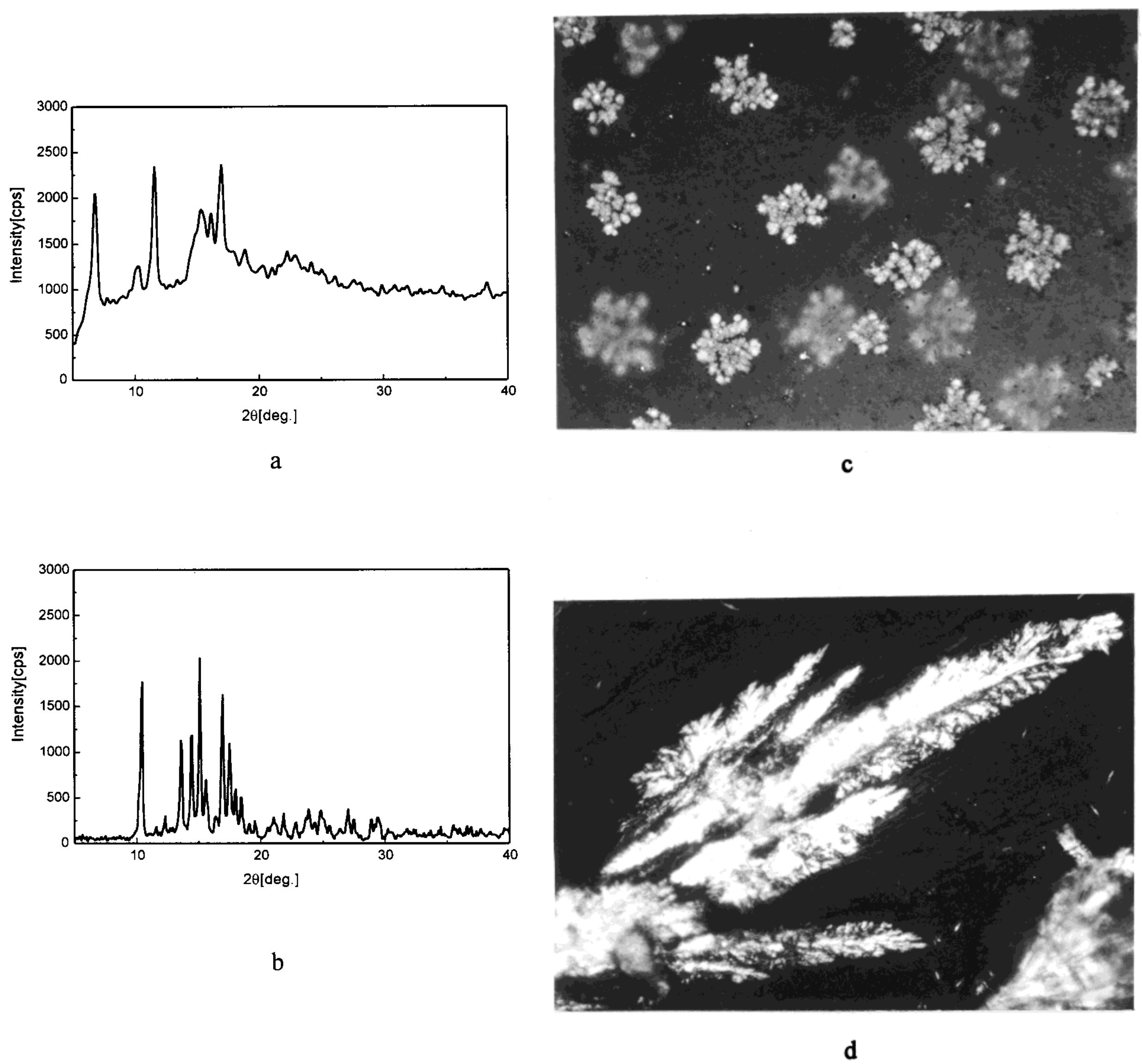

Figure 3. The spectra of X-ray diffraction (a,b) and the pictures of polarized light microscope of periodic and fractal precipitation (c,d). (a) The spectrum of $Cu(DC)_2$ precipitate, appeared in periodic pattern. (b) Spectrum of $Co(DC)_2$ precipitate, appeared in fractal pattern. (c) Periodic precipitation under the polarized light microscope. (d) Fractal pattern under the polarized light microscope.

ion solutions failed, until we used a granular crystal of the metal salt. The final successful experimental conditions and the kinds of patterns obtained are listed in Table 1, together with the results of one- and two-dimensional experiments for comparison.

The cross-section pictures of the complex spheres observed in the experiments are shown in Figure 2a,b, together with patterns in gallstones for comparison, see Figure 2c,d. In the experiments, the condition in Figure 2b is more similar to actual gallstones. Comparing the results of one- and three-dimensional systems, we found that the latter prefers to produce periodic precipitation. For example, no matter how the concentrations of metal ions and NaDC are adjusted, Co^{2+}, Ni^{2+}, Mn^{2+}, and Zn^{2+} ions can only produce fractal patterns in one-dimensional experiments, while they can produce periodic precipitation in three-dimensional systems.

To investigate the composition of precipitate patterns and the effect of agar gel, we characterized the precipitates in the patterns by FT-IR and EXAFS.[16] Since the precipitates have special colors, which are very similar to the corresponding complexes, we also characterized the synthesized complexes for comparison. The results of FT-IR and EXAFS are summarized in Tables 2 and 3. Comparing the precipitates in patterns and their corresponding synthesized complexes, we found that their FT-IR and EXAFS data are very similar. These results indicate that the chemical composition in the patterns is just its corresponding complexes; thus, the effect of agar gel is negligible.

TABLE 3: EXAFS Data

	R[a]	R[b]	δ (R−R[b])	N[a]	N[b]	δ (N−N[b])	Q^2 [a]	Q^2 [b]
$Mn(DC)_2$	2.16	2.16	0	4.9	5.4	−0.5	0.009	0.008
$Co(DC)_2$	2.07	2.07	0	5.7	6.0	−0.3	0.01	0.009
$Cu(DC)_2$	1.95	1.94	0.01	6.2	5.0	1.2	0.007	0.005

[a] Data from synthesized complexes. [b] Data from pattern precipitates.

To understand the mechanism of pattern formation, and the reason the system can produce two different kinds of patterns, we characterized the microstructures in the periodic and fractal patterns by X-ray diffraction and polarized light microscope. We first performed a special experiment, in which the patterns appear in the agar film and can be observed under the polarized light microscope easily. The experimental method is similar to that described in ref 13, but instead of an ordinary test tube, a specially made rectangular thin glass container ($6 \times 3 \times 2$ mm^3) was used. The X-ray diffraction spectra are shown in Figure 3a,b. The result indicates that both periodic and fractal patterns have polycrystalline structures, so that the fractal growth of crystal is a basic process occurred both in fractal and in periodic pattern formation. This observation is confirmed by Figure 3c,d, which are the pictures of patterns under the polarized light microscope. Comparing parts c and d of Figure 3, we observe that the microstructures of the two patterns have significant differences: the periodic one has numerous clusters, indicating the precipitation has many centers of nucleus, while the fractal pattern has only a few.

Discussion

To summarize the experimental results, we propose the following pattern formation and selection hypotheses. Both periodic and fractal patterns are from the aggregation of numerous crystal fractal clusters. The rule for choosing which of two patterns will be followed depends on the competition of the speed of crystal growth and the rate of nucleation. High crystal growth prefers the fractal patterns, while high nucleation rate prefers periodic structures. At the present, there are two theories concerning the precipitation pattern formation: the theory based on reaction−diffusion equations for the periodic precipitation (Liesegang rings)[17] and the theory of diffusion limited aggregation (DLA) model for the fractal growth separately.[18] Our experiments call for a unified theory to explain precipitation pattern formation.

The metal ions used in the present work are found to be important in gallstones. In one- or two-dimensional experiments, only Cu^{2+} and $Cu^{2+} + Ca^{2+}$ can form periodic structure (see Table 1); for the system with other ions, such as Co^{2+}, Ni^{2+}, Mn^{2+}, or Zn^{2+}, a periodic pattern was never observed. In the three-dimensional experiments presented here, the periodic patterns were found in all the above-mentioned system (see Table 1). The unified theory should account for this new observation.

The bile salts were found to exist in pigment gallstones and cholesterol gallstones. Our previous works have found that in the presence of NaDC, the main components of pigment stones, such as calcium bilirubinate, copper bilirubinate, and $Ca(CO_3)_2$, could form periodic precipitation in one-dimensional tests,[19] while without NaDC the system never form periodic structure. Further three-dimensional experiments on this system are on the way in our laboratory.

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References and Notes

(1) Wu, J.-G.; Zhou, X.-S.; Xu, Z.; Shen, T.; Xu, Y.-Z.; Li, W.-H.; Xu, D.-F.; Soloway, R. D.; Wentrup-byrune, E.; Xu, Z.-H.; Shi, J.-S.; Shen, G.-R.; Deng, S.-Q.; Li, X.-F.; Shi, N. *Biospectroscopy* **1997**, *3*, 381.

(2) Li, X.-F.; Soloway, R. D.; Huang, W.-D.; Wu, J.-G.; Huang, Y.; Huang, W.-Z.; W.-Z.; Zhu, Z.-X.; Zhou, N.-F.; Xu, D.-F. *Clin. Res.* **1993**, *41*, 158A.

(3) Wu, J.-G.; Xu, D.-F.; Liu, F.; Zhou, X.-S.; Zhang, Y.-F.; Zhou, N.-F.; Hong, N.-K.; Soloway, R. D. *Gastroenterology* **1990**, *98*, 256A.

(4) Lonsdale, Kathleen *Nature* **1968**, *217*, 56.

(5) Muller, S. C.; Kai, S.; Ross, J. *Science* **1982**, *216*, 635.

(6) Peng, Q.; Wu, J.-G.; Soloway, R. D.; Hu, T.-D.; Huang, W.-D.; Xu, Y.-Z.; Wang, L.-B.; Li, X.-F.; Li, W.-H.; Xu, D.-F.; Xu, G.-X. *Biospectroscopy* **1997**, *3*, 195.

(7) ; Xu, G.-X. *Mikrochim. Acta Suppl.* **1997**, *14*, 345.

(8) Pearson, J. E. *Science* **1993**, *261*, 189.

(9) Lee, K. J.; McCormick, W. D.; Ouyang, Q.; Swinney, H. L. *Science* **1993**, *261*, 192.

(10) Henisch, H. K. *J. Cryst. Growth* **1986**, *76*, 279.

(11) Brechet, Y.; Kirkaldy, J. S. *J. Chem. Phys.* **1989**, *90*, 1499.

(12) Mueller, K. F. *Science* **1984**, *225*, 1021.

(13) Das, Ishwar; Cland, Sudha; Pushkarna, Anal. *J. Phys. Chem.* **1989**, *93*, 7435.

(14) Wolform, S. *Nature* **1984**, *311*, 419.

(15) All the chemicals used in this work, such as sodium deoxycholate (NaDC), agar, $CaCl_2$, $CoCl_2$, $NiCl_2$, $MnCl_2$, $CuCl_2$, and $ZnCl_2$ were A. R. reagents from the Biejing Chemical Factory. Agar was dissolved in hot water or mixed solvents (e.g., alcohol and water), and gel was formed when the solution was cooled to room temperature.

(16) FT-IR measurement were recorded on Nicolet 750 FT-IR spectrometer, in the region 4000∼650 cm^{-1}, with Kbr pellet; EXAFS was undertaken in BSRF station, with the 4W1B beam line and 20−50 mA Current.

(17) Ostwald, W. *Der, Lehrrbuch Allgemeinen Chemie*; Engelmann: Leipzig, 1897.

(18) Meakin, P. *J. Theor. Biol.* **1986**, *118*, 101.

(19) Wu, J.-G.; Xu, D.-F.; Soloway, R. D. *Gastroenterology* **1990**, *98-(5)*, 249.