Dissolution behavior of vaterite spherulite in solutions containing phosphate ions

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Vaterite is a crystalline polymorph of calcium carbonate that exhibits low stability in comparison with the other crystalline polymorphs of calcium carbonate. It often assumes a spherical shape. The physical properties of vaterite are utilized in advanced biomaterials such as drug delivery systems (DDSs). The potential application of vaterite in DDSs demands a comprehensive understanding of its dissolution rate as a function of PO4 concentration. Using in situ optical studies, we analyzed the dissolution behavior of vaterite in a simulated PO4-containing biogenic environment. The varying physicality (i.e., the pseudo-physiological conditions) of vaterite were investigated using high-resolution cryogenic transmission electron microscopy and small-angle X-ray scattering. In addition, we measured the PO4/Ca ratio in each part of vaterite spherulites using field-emission scanning electron microscopy in conjunction with energy-dispersive X-ray spectroscopy and inductively coupled plasma atomic emission spectrometry.

We categorized the vaterite spherulites into three parts—the shell, mantle, and core parts—on the basis of their dissolution behavior and PO4 response. The PO4 response to the dissolution dynamics increases toward the outer part, although the adsorption rate decreases in the presence of PO4 and PO4 fluorescence materials. The composition of the shell part was nearly identical to that of ideal vaterite, whereas the inner part exhibited a high C/Ca ratio and a poorly crystalline phase referred to as vaterite-like material. The dissolution rate of each part was 0.23–1.58 nm/s (shell), 0.63–3.19 nm/s (mantle), and 1.38–5.71 nm/s (core). The vaterite and vaterite-like materials were further identified according to their composition and particle size distribution.

1. Introduction

Calcium carbonate is a common mineral that is widely distributed over the Earth in materials ranging from sedimentary rocks to biological hard tissues.1–9 In nature, calcium carbonate manifests itself in an amorphous phase (ACC) or in one of three crystalline polymorphic forms: calcite, aragonite, or vaterite.10,11 These polymorphs are widely used in numerous technological fields, including agriculture, ceramic engineering, paper manufacturing, and biomedical engineering. These calcium carbonate polymorphs are extensively used in various engineering applications that utilize their physicality, which include low hardness, complete cleavage, and low coefficient of friction. In addition, they are used as a potential source of Ca because these polymorphs of calcium carbonate readily dissolve under acidic conditions.12–15

Among the various polymorphs of calcium carbonate, vaterite is a metastable phase with greater solubility and lower stability compared to calcite and aragonite.9,10 This physical property of vaterite is being utilized in various engineering applications, especially in biomaterial engineering. For example, vaterite is not only used as a Ca resource but also as a material for the synthesis of advanced capsules in drug delivery systems (DDSs). However, the real-time application of vaterite in biomedical engineering is limited by several challenges. During the dissolution of vaterite in a biological environment, some of the essential elements, such as phosphate, might affect the physicality and kinetics of vaterite. For instance, Katsifaras and Spanos10 reported that, under high pH conditions (~10), phosphate inhibits the dissolution of vaterite during a phase transformation process; they compared the X-ray diffraction (XRD) intensity of the calcite (104) reflection with that of the vaterite (112) reflection. Under simulated body environmental conditions, Sugita et al.16 demonstrated that even a small amount of phosphate (PO4/Ca ≈ 1/1000) could inhibit the formation of vaterite. As previously mentioned, phosphate is considered to strongly affect the dissolution of vaterite. In addition, the results of some studies have suggested that vaterite spherulites contain more unstable phases, such as amorphous-like phases, at the inner parts than at the outer parts.9,16
These physical and chemical inhomogeneities of vaterite might lead to serious medical conditions, similar to thalidomide, when used in advanced biomaterials such as DDSs. Therefore, the safe use of vaterite in biomedical applications demands a comprehensive understanding of the effect of phosphate on the dissolution behavior of vaterite.

Herein, we estimated the dissolution rate of each part of vaterite spherulites under various pseudo-physiological conditions. In a typical analysis, the dissolution rate was determined under various phosphate conditions using in situ optical measurements. Phase identification of each part of vaterite spherulites, such as the C/Ca ratio, was analyzed using field-emission scanning electron microscopy combined with energy-dispersive X-ray spectroscopy (FE-SEM/EDX) and high-resolution cryogenic transmission electron microscopy (cryo-HR-TEM). Furthermore, the crystallinity of the vaterite was quantitatively analyzed using XRD and small-angle X-ray scattering (SAXS). Our studies on the dissolution behavior, C/Ca ratio, and crystal features indicate that the vaterite spherulites are composed of three regions that are clearly distinguishable at high phosphate concentrations.

2. Experimental Method

2.1 Measurement of the dissolution rate of vaterite spherulite under various PO4 concentrations

2.1.1 Solution Preparation

Experimental reagents were purchased from Wako Pure Chemical Industries, Japan (CaCl2, NaHCO3, NaCl, and KH2PO4), Nakarai Tesque, Japan (tris(hydroxymethyl) aminomethane (Tris) and K2HPO4), and Iwaki Seiyaku, Japan (tris(hydroxymethyl) amino-triphosphate (FMN) (C17H21N4O9P)). The stock solution was prepared by completely dissolving CaCl2, NaCl, NaHCO3, K2HPO4, KH2PO4, FMN, and Tris in ultra-pure water to concentrations of 1 M, 1 M, 0.5 M, 0.5 M, 25 mM, 25 mM, and 125 mM, respectively. The pH of the resulting solution was adjusted to 8.0 by the addition of HCl.

2.1.2 Protocol for measuring the dissolution rate of vaterite spherulite under various PO4 concentrations

The protocol adopted for this experiment consisted of two steps: (1) the preparation of an in situ optical observation system and (2) the preparation of the solution for dissolution.

The in situ observation system consisted of an optical observation cell and a solution flow system. Figure 1 schematically illustrates the in situ observation system used in this study. The observation cell was composed of a glass slide and polypropylene tubes, which were positioned on the Pyrex glass slide coated with vaterite spherulites. The vaterite spherulites containing glass were prepared by dipping the glass slide into a solution with a composition of 40 mM CaCl2, 40 mM NaHCO3, and 100 mM Tris for 60 min. The glass slides were subsequently washed repeatedly in 99.5% ethanol and dried. The number of vaterite spherulites on the glass slide was adjusted to approximately 5–10 vaterite spherulites particles in the observation eyesight. The diameter of the vaterite spherulites was approximately 5 µm.

The observation cell was placed on an optical microscope (BX-60, Olympus, Tokyo, Japan) using silicon-gum tubes. The optical microscope was connected to a rotary pumping system, which facilitated the flow of dissolution solution into the observation cell at 2 mL/min. Sequence photographs were taken at 1-min intervals.

The dissolution solution used in this study consisted of 500 mM NaHCO3, 500 mM KH2PO4, and 1 M NaCl. The concentration of the solution was adjusted using 5 mM NaHCO3 solution and 0, 20, 50, 100, 200, 500, and 1000 µM K2HPO4 solutions. The ionic strength of the solution was adjusted by the addition of 1 M NaCl. The pH of the solution was maintained at ~8.6. All experiments were performed in a cleanroom under controlled temperature and pressure conditions of 22 ± 0.5°C and 1 atm.

In addition to simple dissolution experiments, we investigated the dissolution behavior of PO4-doped vaterite spherulites. Vaterite seeds precipitated onto a glass substrate were sealed into the cell, and supersaturated calcium-carbonate solution containing PO4 (2 mM CaCl2, 2 mM NaHCO3, and 0.25 mM KH2PO4, pH ~8.6, 25°C) was flowed at 2 mL/min for 2 h to dope PO4 into the spherulites. An undersaturated solution (2 mM NaHCO3 and 5 mM NaCl, pH ~8.6, 25°C) was then flowed to dissolve the spherulites. NaHCO3 was added to maintain the solution pH. The undersaturation of the solution with respect to calcium carbonates was 100%.

The length of the spherulites was measured in the three directions that were separated at an angle of 60°. The average of the three lengths was defined as the average diameter of the spherulites. At least 50 spherulites were analyzed under each solution condition. The acquired data were subsequently statistically analyzed.

2.1.3 Fluorescence microscopy analysis in phosphate-containing environments, including fluorescence materials (FMN)

The protocol adopted for this experiment is similar to that described in Section 2.2. The solution flow system was connected...
to a fluorescence optical microscope (Olympus BX-53, Olympus, Tokyo, Japan) equipped with a fluorescence optical filter set (Omega Optical Filter Set XF71, Omega Optical, USA), and the Pyrex glass slide in the observation cell was changed to a Si template.

FMN exhibits an excitation wavelength $\lambda_e$ of 510 nm and a fluorescence wavelength $\lambda_f$ of 540 nm. FMN, also known as riboflavin-5'-phosphate, is categorized as a phosphate derivative of vitamin B2. The reaction solution used in the flow system consisted of 5 mM NaHCO$_3$, 50 $\mu$M CaCl$_2$, and 20 $\mu$M FMN. This solution was pumped by a rotary pump into the vaterite observation cell at 2 mL/min for 1 h. Subsequently, the observation cell was washed with 5 mM NaHCO$_3$ for dissolution.

2.2 Phase characterization of each part of the vaterite spherulites

2.2.1 Sample preparation

For XRD and SAXS measurements, the precipitates of vaterite spherulites were prepared by mixing 100 mM of Tris, with 40 mM of CaCl$_2$, NaHCO$_3$, and 0.0 or 0.3 mM of KH$_2$PO$_4$, under constant stirring at 400–600 rpm for a reaction period of 10 and 120 min. Prior to the analysis, the resulting precipitates were washed in 99.5% ethanol and dried.

2.2.2 XRD measurements

The dried samples were placed on a Si non-reflective plate and were measured using XRD (RINT 40, Rigaku, Tokyo, Japan, $\lambda = 0.1506$ nm); the diffractometer was operated at an acceleration voltage of 50 kV and an amplitude of 300 kV using a Cu target. The 2-theta step was 0.01° per 1 s for each angle, and the entrance-slit size was 5 $\times$ 10 mm$^2$.

2.2.3 Small-angle X-ray scattering analysis

The dried precipitates were wrapped in propylene film, and the crystallite size distribution was measured using SAXS (R-AXIS combined SAXS holder, Rigaku, Tokyo, Japan; $\lambda = 0.15406$ nm); the instrument was operated at an acceleration voltage of 50 kV and an amplitude of 100 mA using a Cu target. The 2-theta step size was 0.01° per 0.5 s for each angle; the entrance-slit size was 5 $\times$ 10 mm$^2$.

2.2.4 Electron microscopy analysis

For electron microscopy analysis, the vaterite spherulites which synthesized in 100 mM of Tris, with 40 mM of CaCl$_2$, NaHCO$_3$, and 0.3 mM of KH$_2$PO$_4$ were mixed with cooled (~20° C) epoxy resin (Logitech 310, Logitech, Scotland, UK; EpoFix-20, Struers, Sweden) and left undisturbed until completely solidified. Subsequently, ultrathin (~200 nm) cross-sections were prepared using a microtome and positioned like bridges between the Si template for FE-SEM/EDX (FE-SEM: FEI XL30, FEI Company, Oregon, USA; EDX: EDAX Genesis, EDAX Japan, Tokyo, Japan) and SEM/EDX (SEM: JEOL JSM-6610LV/A/RA, LEOL, Tokyo, Japan; EDX: INCA x-spect detector, Oxford Instruments Co., Tokyo, Japan) observations at an acceleration voltage of 15 kV and an amplitude of 130 µA. The C/Ca ratios were measured at three different locations: core, middle region, and outer region. For comparison, standard calcium carbonate samples were prepared by mixing CaF$_2$, CaCO$_3$, and Na$_2$CO$_3$ at various ratios and were analyzed using EDX. In this study, 10 standard samples with different C/Ca ratios were prepared to obtain a reference curve for C/Ca ratios between 0.85 and 1.50. The measured C/Ca ratios were normalized using this reference curve.

The prepared ultra-thin (~200 nm) cross-sections were placed on a Cu micro-grid for TEM analysis. The analysis was performed using both a normal observation holder and a liquid-N$_2$ cryogenic holder (FE-TEM: Hitachi HF3300s, Hitachi, Tokyo, Japan, cryo-TEM: Tecnai 20, FEI, The Netherlands) at acceleration voltages of 300 kV (FE-TEM) and 200 kV (cryo-TEM). The operating temperature was approximately $-170^\circ$C.

2.2.5 Measurement of the PO$_4$/Ca ratio in precipitates using inductively coupled plasma atomic emission spectroscopy (ICP-AES)

The reacted solution was mixed with 100 mM of Tris, 40 mM of CaCl$_2$, and NaHCO$_3$, and 0.3 mM of KH$_2$PO$_4$. The solution volume was 300 mL.

We sampled calcium carbonate precipitates (~20 mL) at specific intervals to measure the changes in the phosphate concentration in the reaction solution. The sampling times were 10 min, 120 min, and 24 h. The sampled precipitates were centrifuged, washed several times using 99.5% ethanol, and finally dried. We added 1 M HCl to approximately 0.05 g of each sample and completely dissolved the sample. We subsequently added ultra-pure water to bring the total volume of the sample to 5 mL. We measured the PO$_4$ and Ca concentrations in the react solution using ICP-AES with Ar gas as the carrier gas (SII 7000, Seiko, Tokyo, Japan). We also measured the XRD patterns and calculated the ratios of vaterite and calcite according to the formulas of Katsifaras and Spanos$^{13}$ for estimating vaterite and calcite ratios on the basis of XRD peak intensities.

3. Results and discussion

3.1 Dynamics of the vaterite spherulite dissolution process and the dissolution rate as a function of PO$_4$ concentration

Figures 2(a) and 2(b) show the change in the diameter of the vaterite spherulites upon dissolution under various PO$_4$ concentrations. The typical dissolution behavior of the vaterite spherulite is as follows. During the dissolution of vaterite spherulites, the diameter of the particles decreased linearly. Irrespective of the PO$_4$ concentration, the dissolution process exhibited two inflection points of the dissolution rate. The first point appeared when the vaterite spherulites approximately 0.1–0.5 µm from the surface dissolved. Until this point, the dissolution rate was much lower in the outer part than in the inner parts. In fact, the dissolution behavior was non-uniform, with some fragments of the outer part remaining, even after the start of the dissolution of the inner parts. The inner parts exhibited a faster dissolution rate than the outer part. As the diameter of the vaterite spherulites reached approximately 1.5–3 µm, the second inflection point appeared. In addition, the dissolution rate accelerated and the vaterite spherulites could be readily divided into three parts on this basis: the outermost, intermediate, and core parts. In this study, the three parts of vaterite spherulites were designated as the shell (outermost), mantle (intermediate), and core, as shown in Fig. 2(d). We further measured and calculated the dissolution rate of each part [Fig. 2(c)].

With an increase in PO$_4$ concentration, the dissolution rates decreased [Figs. 2(a)–2(c)]. This tendency was stronger in the shell part than in the inner parts. The average dissolution rate of the shell in the phosphate-free solution was 1.58 nm/s and it was 0.28 nm/s in the solution containing 1000 µM PO$_4$, i.e., their ratio was 6.87:1. In contrast, the ratio of dissolution rate of the core part was 4.17:1 and that of the mantle was 5.10:1, respectively. Furthermore, in systems with a high concentration of PO$_4$ (greater than 200 µM), some of the vaterite spherulites exhibited separation among the shell, mantle, and inner parts prior to the dissolution of the shell. Finally, only the shell part remained in the spherulites, which suggests that the shell parts of the vaterite
spherulites exhibited greater PO₄ response to dissolution dynamics than the inner two parts (mantle and core). During separation of the shell and mantle, we measured the dissolution rates of both the shell and mantle (Table 1).

### 3.2 Morphological evolution of PO₄-doped vaterite spherulites during the dissolution process and the relationship to PO₄

Since biological beings have PO₄ as an essential molecule in their body fluid, the interaction between vaterite spherulites and PO₄ should be clarified when we used the vaterite spherulites as a bio-industrial material such as DDS. We estimated the dissolution behavior of PO₄-doped vaterite spherulites.

We gained further insight by visualizing the PO₄ response to dissolution dynamics of each part of the vaterite spherulites. Figures 3(a)-(c) show the dissolution behavior of the PO₄-doped vaterite spherulites. The morphology of PO₄-doped vaterite spherulites before dissolution [Fig. 3(a)] exhibited a

| Locating region | Core | Mantle | Shell |
|-----------------|------|--------|-------|
| Dissolution rate | 1.38–5.71 nm/sec | 0.63–3.19 nm/sec | 0.23–1.58 nm/sec |
| PO₄ response on dissolution process | Low | Relatively high | High |
| C/Ca rate from EDX measurement | 1.38 ± 0.03 | 1.28 ± 0.03 | 1.09 ± 0.09 |
| Crystalline structure from TEM and SAXS analysis | Many dislocations. | Small dislocations. | Small dislocations. |
| | Mono-modal crystalline distributions | Mono-modal crystalline distributions | Bi-modal crystalline distributions |

Table 1. Observed vaterite phases and their dissolution behavior under various PO₄ concentrations

Fig. 2. The dissolution behavior and rate of vaterite in solutions with various PO₄ concentrations. (a) Typical change in the diameter of particles during dissolution of vaterite spherulite under low-PO₄-concentration conditions; PO₄-free (red solid squares), 20 μM PO₄ (dark-blue solid circles), and 50 μM PO₄ (black open squares). The guided line signifies the two inflection points, which indicate the boundary of shell to the mantle and vice versa. (b) Typical change in particle diameter during dissolution of vaterite spherulites in 1 mM PO₄ (green open circles). For guidance, the results for PO₄-free conditions (red solid squares) are also represented. Even under high-PO₄ concentrations, the dissolution behavior exhibited no significant changes compared with that of the low-PO₄ system. (c) Dissolution rate vs. PO₄ concentration of the shell part (red squares), mantle part (blue squares), and core part (green squares). The dissolution rate exponentially decreases with increasing PO₄ concentration. (d) Schematic illustrating the structures of vaterite spherulites. Vaterite spherulites consist of three parts: shell part, or outermost part, with a thickness of approximately 0.2–1 μm; mantle part, which consists of tightly packed needle-like structures; and core part, which represents the center of spherulites and ranges from 1.5 to 3 μm.
radial structure from the core to the outermost part, without non-sequential points. Alternatively, the morphology of PO₄-doped vaterite spherulites during dissolution [Figs. 3(b) and 3(c)] showed boundary separation and the formation of a hollow structure. This result suggests that the PO₄ affects the shell part of vaterite spherulites more strongly than the inner parts; that is, PO₄ essentially affects the surface of the vaterite surface without intruding inside. In contrast, no PO₄ containing vaterite spherulites maintained a radial structure during dissolution process [Figs. 3(d)–3(f)].

Figure 3 shows a change in diameter of PO₄-doped vaterite spherulite behavior during the dissolution dynamics. (a, b, and c) Temporal images of the dissolution process of PO₄-doped vaterite spherulites at (a) 0 min (before dissolution), (b) 30 min, and (c) 60 min. The vaterite spherulites exhibited a hollow structure; the mantle part was dissolved first. Temporal images of the dissolution process of no PO₄ containing vaterite spherulites (reference) at (d) 0 min (before dissolution), (e) 30 min, and (f) 65 min. (g) Photograph of a normal reflected light image of vaterite spherulites. (h) Photograph of the fluorescence light image of vaterite spherulites. Red broken circles are provided as a guide for the entire scale of vaterite spherulites. The core part was brighter than the other parts. The concentration of FMN was greater in the core part than in the other parts. Scale = 5 μm (a–h).

Fig. 4. Change in the diameter of vaterite spherulites during dissolution in the solution containing 5 mM NaHCO₃. Data of PO₄-doped vaterite spherulite (core; green circle, shell; blue square) and of no PO₄ containing vaterite spherulite (red; whole diameter) were shown.

In this study, FMN was used as a marker of PO₄. The FMN adsorption of each part of vaterite spherulites was detected by in situ analysis. Figures 3(g) and 3(e) show photographs of FMN-doped vaterite spherulites in solution under normal light and fluorescent light, respectively. As evident in Fig. 3(h), the core region of the vaterite spherulites is much brighter than the outer parts. In particular, the shell region exhibited relatively less
NaHCO₃, and 0.3 mM of KH₂PO₄). During normal observations, we observed closely packed needle-like structures with lengths ranging from 2 to 5 μm. In the core region, we observed vaterite spherulites (100 mM of Tris, 40 mM of CaCl₂ and NaHCO₃, and 0.3 mM of KH₂PO₄) at 60 min. Red broken circles correspond to each HR-TEM image. The PO₄ concentrations were 0.23 ± 0.03 and 1.38 ± 0.02, respectively. We also observed needle-like structures almost reaching the outermost part, forming the surface of the vaterite spherulite.

3.3 Characterization of each part of vaterite spherulites

To individually characterize each part of the vaterite spherulites, we adopted optimum experimental conditions that resulted in the precipitation of vaterite spherulites with a hollow structure [Fig. 5(a)], which were specifically selected and subsequently analyzed by electron microscopy. For analysis, we prepared an ultrathin section for TEM observation. Figure 5(b) shows the inner structure of a vaterite spherulite, indicating the formation of a hollow structure. The core part consisted of spherical particles with diameters ranging from 50 to 100 nm. These particles were highly aggregated, forming a region of ~1–1.5 μm. Surrounded by the core region, we observed closely packed needle-like structures with lengths ranging from 2 to 5 μm and thicknesses of 100–200 nm. The needle-like structures were directly connected to the core region, without clear delineation of the boundary. The needle-like structures almost reached the outermost part, forming the surface of the vaterite spherulite. The boundary between the mantle and shell parts could not be distinguished clearly.

Figures 5(c)–5(e) show HR-TEM images of each part of the vaterite spherulites (100 mM of Tris, 40 mM of CaCl₂ and NaHCO₃, and 0.3 mM of KH₂PO₄). During normal observations, the electron beam significantly affected and collapsed the core part. Therefore, the core part was specifically observed under cryogenic conditions to minimize specimen damage during observation. All parts exhibited lattice fringes, which were identified as the crystalline phases, although the degree of crystallinity varied substantially, especially in the region between the core and shell. In the core part, the lattice fringes showed vaterite (111) and (120) faces. The core contained numerous dislocations, including point defects and blade dislocations, whereas the mantle and shell contained few dislocations compared to the core part [Figs. 5(d)–5(e)]. The HR-TEM images revealed minor differences between the mantle and shell parts. However, the FE-SEM/EDX analysis showed substantial differences in the chemical compositions. The C/Ca ratio of the shell part was 1.09 ± 0.08, whereas those of the mantle and core parts were 1.28 ± 0.03 and 1.38 ± 0.02, respectively. We also observed spherulites formed in no PO₄ containing solutions. The features of lattice fringes in each part were similar to those of spherulites formed in PO₄ containing solutions, however, the number of dislocations in core and mantle parts were smaller (Table 1).

We could not detect P ions in the calcium carbonate precipitates using domain measurement methods such as FE-SEM/EDX and X-ray photoelectron spectroscopy. The PO₄ concentration in the crystalline calcium carbonate during each time sequence was also measured using ICP-AES. We attempted to calculate the value of PO₄ in the core and outer parts. The PO₄/Ca ratio was 0.18% in the sample reacted for 10 min, 0.14% in the sample reacted for 120 min, and 0.11% in the sample reacted for 24 h, which was completely phase-transformed into calcite. We calculated the vaterite-to-calcite ratio to estimate the PO₄ concentration in the core region and outer part of the vaterite spherulites. The PO₄ concentrations were 0.23% in the core region and 0.15% in the outer region. We could not estimate the difference between the PO₄ concentrations in the core and mantle parts. However, the results of FMN experiments suggested that the core part contained more PO₄ than the mantle part.

The observations of the samples using HR-TEM resulted in...
the lattice fringes being fused by the focused electron beam. However, this behavior differed from that of the dislocations, such as point defects and edge dislocations, which appeared in the core and mantle regions. Therefore, these defects [Figs. 5(c) and 5(d)] are considered to have originated from the samples and not from the damage induced by the HR-TEM observations.

The electron microscopy, FE-SEM/EDX, and HR-TEM results suggested that the vaterite spherulite consisted of several materials with slightly different physical properties. Although these materials were identified as crystalline vaterite, certain parameters of these materials, such as chemical composition, were observed to differ. When vaterite dissolved under PO4-containing conditions, the vaterite spherulites retained their hollow structure.14 We hypothesized that the vaterite spherulites were formed radially from the core part and grew toward the shell. As a consequence, the reaction time was relatively short. Also, for the same reason, the sample was confirmed to exhibit physicality close to those of the core part. In addition, we considered that the shell part can be readily obtained because of its self-induced phase transformation to calcite. We further substantiated this hypothesis by analyzing four different vaterite samples that were obtained after various reaction durations.

Figure 6(a) shows the XRD patterns of samples collected at different stages of the reaction. The sample collected after a reaction time of 10 min consisted mostly of the core part, whereas that obtained after a reaction time of 120 min exhibited a hollow structure [Fig. 5(a)]. The diffraction peaks of the samples indicated the mixed formation of vaterite and calcite phases. We could not identify some of the minor peaks, such as the broad peaks at 40.0° and 60.5°, on the basis of the crystal structures of LeBail et al.26 However, these unidentified peaks were observed among all samples and with approximately the same relative intensities. We concluded that these peaks were vaterite crystal peaks that originated from crystal dislocations such as micro-twinning or from unknown structures. We did not detect a peak shift among any sample.

SAXS analysis revealed that the crystallite size distribution in the sample (100 mM of Tris, 40 mM of CaCl2 and NaHCO3, and 0.3 mM of KH2PO4) reacted for 10 min was only a few nanometers. In contrast, the SAXS analysis of the sample reacted for 120 min indicated the presence of particles with a bimodal distribution and a diameter of 10 nm, in addition to particle sizes with a distribution of a few nanometers [Fig. 6(b)]. Figure 6(c) shows the SAXS analysis of vaterite spherulites formed in no PO4 containing solution (100 mM of Tris, 40 mM of CaCl2 and NaHCO3) at 10 and 120 min after solution preparation. The crystallite size showed bimodal distributions for both samples. The diameters were a few nanometers and 50 nm for the sample at 10 min, and they were a few nanometers and ~320 nm for the sample at 120 min, which was larger than that observed for the sample formed in PO4 containing solution (Table 1).

According to the theory of Ostwald ripening, this theory corresponds to the size distribution and stability of crystallite particles. Therefore, we concluded that the shell part is more stable than the inner parts because the particle size distributions tended toward broad distributions because of the absence of disorder. We define the shell part as real vaterite, meaning that its physicality and chemical composition are most similar to crystals among all vaterite spherulites composing the materials. However, the inner parts differed from vaterite with respect to certain parameters such as chemical composition and crystallite distribution and were poorly crystalline because of the variation in the dissolution behavior and rate; we hence refer to these parts as a vaterite-like phase.

The present study reveals that PO4 could be stabilized vaterite. The FMN experiments revealed that PO4 intruded into the vaterite spherulites and stabilized. These results indicate that after vaterite forms in a biological system, it would not easily dissolve because of the relatively high concentration of PO4 ions, which inhibit dissolution. Some fish contain otoliths, which mainly consist of vaterite.17 Thus, the vaterite crystals that composed otoliths were growing as a consequence of the metabolism of biological beings. The present study suggests that PO4 stabilizers these tissues, which consist of metastable or unstable mineral
phases. In addition, once vaterite has unexpectedly formed in a biological system, it remains and may lead to diseases such as calculus stones. In fact, calculus stones sometimes consist of vaterite.\(^{18}\)

As previously mentioned, the physical differences of vaterite spherulites poses several problems, especially in an environment where PO\(_4\) is present, and hence needs to be resolved for these materials to be used in biomedical engineering applications. When vaterite is formed from solution, it does not precipitate directly but proceeds through some precursor stages. Gebauer et al.\(^{19}\) proposed prenucleation cluster theory to explain this phenomenon. The calcium and carbonate ions in solution form liquid-like ion pairs, which correspond to the final crystalline phase. Subsequently, the ions randomly aggregate as the ACC through the cluster-based reconstruction process finally resulting in the formation of crystalline phase.\(^{19-21}\) Here the ACC tends to have an essentially loose structure and is strongly affected by the solution conditions.\(^{19}\) In the present study, vaterite spherulites were precipitated from solution, analogous to the precipitation induced by changing the Ca ion concentration. Furthermore, the results suggested that the changes in solution conditions affected the ACC physicality, which induced further changes in the physicality of vaterite.\(^{19,20}\)

We further evaluated the dissolution behavior and physicality of vaterite spherulites under conditions where PO\(_4\) was present. Recently, other authors have proposed that the vaterite crystal structure is not a single structure but rather has many polytypes.\(^{24,25}\) Using ab initio calculations, Demichelis et al.\(^{25}\) demonstrated the difference in phase stability among the different vaterite crystal structures. A clear understanding of the crystal structure of vaterite is highly essential, especially when they are used as biomaterials. The systematic analyses performed in the present study suggest that the physicality of vaterite are not only decided by its crystal structure but also influenced by factors such as chemical composition and crystallite size distribution, e.g., difference between the core and shell parts of vaterite spherulites. In particular, these effects are highly influential when PO\(_4\) is present, such as in bioinorganic environments.

4. Conclusion

In response to the need for rapid advancements in biomaterials, especially advanced DDSs, we investigated the dissolution behavior of vaterite in bioinorganic environments and observed that the behavior of vaterite corresponds to its crystallographic features. Systematic studies indicated that the vaterite spherulites consist of different chemical compositions and crystallite features. We determined the phase stability of the spherulites by measuring their PO\(_4\) response. The results indicate that the more unstable phase exhibits relatively weak PO\(_4\) response with respect to dissolution rate and high PO\(_4\) (FMN) adsorption. In contrast, the most stable phase exhibits a strong PO\(_4\) response and low PO\(_4\) (FMN) adsorption. These results suggest that vaterite spherulites consist of vaterite and vaterite-like phases, which we could categorize according to their PO\(_4\) response.

To enable the use of vaterite as a biomaterial, its physicality should be estimated and understood. We first examined the physicality on the basis of the particle size probability trends, as determined by the SAXS analysis. The shell part, which corresponds to the solid part with weak PO\(_4\) response, was observed to contain particles with diameters of 10 nm. We refer to this portion as real vaterite. The second parameter was determined by EDX measurements, which revealed a chemical composition approximately equal to that of the theoretical vaterite structure and a C/Ca ratio of 1.00.

Acknowledgment We would like to thank Dr. Y. Sogo, Dr. M. Sorai, and Dr. A. Ito for assistance with the in situ optical studies. We also thank Dr. A. Oyane, Dr. T. Hari, and Ms. Y. Hara for assistance with the FE-SEM/EDX measurements and Dr. T. Nagai, Dr. T. Sakai, Ms. F. Hayashi, and Mr. T. Goto for TEM observations using the microtome equipment. We thank Dr. K. Nakamura for helpful discussions. This work was performed with a grant-in-aid by the Japan Society for Promotion of Science (JSPS) Research Fellowships for Doctoral Course Student, grant number 252283 and was supported in part by the Material Characterization Central Laboratory, Waseda University, Nano Processing Facility of the National Institute of the Advanced Industrial Science and Technology (AIST), and by the Nano Technology Platform, Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

References

1) I. Sunagawa, Crystals: Growth, Morphology and Perfection, Cambridge University Press, London (2005).
2) S. Weiner and P. M. Dove, in: Review in Mineralogy and Geochemistry Vol. 54 Biominalization, Mineralogical Society of America Geochemical Society, Washington (2003) pp. 1–29.
3) L. Breecic and A. E. Nielsen, J. Cryst. Growth, 98, 504–510 (1989).
4) L. Addadi, S. Raz and S. Weiner, Adv. Mater., 15, 959–970 (2003).
5) S. Mann, Biominalization Principles and Concepts in Bioinorganic Materials Chemistry, Oxford University Press, U.K. (2001).
6) H. R. Wenk and A. Bulahk, Minerals: Their Constitution and Origin, Cambridge University Press, London (2004).
7) A. Akiva-Tal, S. Kababa, Y. S. Balazs, L. Glazer, A. Berman, A. Sagi and A. Schmidt, Proc. Natl. Acad. Sci. U.S.A., 36, 14763–14768 (2011).
8) A. Sato, S. Nagasaka, K. Furihata, S. Nagata, I. Araik, K. Saruwatari, T. Kogure, S. Sakuda and H. Nagasawa, Nat. Chem. Biol., 7, 197–199 (2011).
9) Y. Nishikane, K. Kaito, S. Takahashi, C. M. Henderson, N. Suzuki and M. Kanno, Mar. Micropaleontol., 78, 84–95 (2011).
10) P. T. Sudheesh-Kumar, C. Ramya, R. Jayakumar, S. V. Nair and V. K. Lakshmanan, Colloids Surf., B, 108, 109–116 (2013).
11) S. Kim and B. P. Chan, Adv. Funct. Mater., 23, 10–25 (2013).
12) L. N. Plummer and E. Busenberg, Geochim. Cosmochim. Acta, 46, 1011–1040 (1982).
13) A. Katsifaras and N. Spanos, J. Cryst. Growth, 204, 183–190 (1999).
14) Y. Sugiuira, K. Onuma, Y. Kimura, K. Tsukamoto and A. Yamaokshi, Am. Min., 98, 262–270 (2013).
15) C. Zhong and C. C. Chu, Cryst. Growth Des., 10, 5043–5049 (2010).
16) M. Suzuki, H. Nagasawa and T. Kogure, Cryst. Growth Des., 6, 2004–2006 (2006).
17) G. Falini, S. Fermani, S. Vanzo, M. Miletic and G. Zaffino, Eur. J. Inorg. Chem., 1, 162–165 (2005).
18) D. J. Sutor and S. E. Wooley, Science, 159, 1113–1114 (1968).
19) D. Gebauer, A. Vökel and H. Colfen, Science, 322, 1819–1822 (2008).
20) D. Gebauer, P. N. Gunawidjaja, P. J. Y. Ko, Z. Bacsik, B. Aziz, L. Liu, Y. Hu, L. Bergstrom, C. W. Tai, T. K. Sham, M. Eden and N. Hedin, Angew. Chem., Int. Ed., 49, 8889–8891 (2010).
21) R. Demichelis, P. Raiteri, J. D. Gale, D. Quigley and D.
Gebauer, *Nature Commun.*, 2, 590–597 (2011).

22) J. D. Rodriguez-Blanco, S. Shaw and L. G. Benning, *Nanoscale*, 3, 265–271 (2010).

23) N. Kanzaki, G. Treboux, K. Onuma, S. Tsutsumi and A. Ito, *Biomaterials*, 22, 2921–2929 (2001).

24) L. Kabalah-Amitai, B. Mayzel, Y. Kauffmann, A. N. Fitch, L. Bloch, P. Gilbert and B. Pokroy, *Science*, 340, 454–457 (2013).

25) R. Demichelis, P. Raiteri, J. D. Gale and R. Dovesi, *Cryst. Growth Des.*, 13, 2247–2251 (2013).

26) A. LeBail, S. Ouhenis and D. Chateigner, *Powder Diffraction*, 26, 16–20 (2011).