The Condition of pH Level Where Calcium Carbonate Crystallizes

Abstract

The shells of most mollusks, including shellfish, are composed of crystalline or amorphous calcium carbonate, which consists of calcium ions present in their body fluid. However, the conditions and processes of crystallization are not known as of yet, so we carried out the research on them.

Among several conditions of crystallization, we focused on the concentration and the pH level of the fluid during crystallization, which is considered to be particularly important. Then we examined these conditions by conducting several series of experiments using artificially reproduced simulated body fluids. The results clearly indicated that the pH level and the concentration of body fluid had some effect on the crystallization, and suggested that inorganic ions other than calcium ions may inhibit crystallization of calcium carbonate

1. Research Background and Objectives

The main component of the shellfish exoskeleton is crystallized calcium carbonate, which is formed by the crystallization of calcium ions in the body fluid secreted from the shellfish body, but the detailed conditions and processes of crystallization are still unknown. In the exoskeleton of pearl oyster, which is a type of shellfish, there are two types of calcium carbonate crystals with different crystal structures, aragonite and calcite, and a small amount of calcium carbonate amorphous, but the formation conditions of each crystal are also unknown.

There are two commonly accepted hypotheses as to what triggers the crystallization of calcium carbonate in the shellfish exoskeleton: first, that the pH of the body fluid increases rapidly during crystallization, and second, that the concentration of the body fluid increases rapidly during crystallization.

Regarding the first hypothesis, the following series of reactions are in equilibrium in shellfish body fluids: (1) the ionization equilibrium of water, (2) the first-step ionization equilibrium of carbonic acid, (3) the second-step ionization equilibrium of carbonic acid, and (4) the dissolution equilibrium of calcium carbonate.

$$H^{+} + OH^{-} \rightleftharpoons H_2O \quad (1)$$

$$H_2CO_3 \rightleftharpoons H^{+} + HCO_3^{-} \quad (2)$$

$$HCO_3^{-} \rightleftharpoons H^{+} + CO_3^{2-} \quad (3)$$

$$Ca^{2+} + CO_3^{2-} \rightleftharpoons CaCO_3 \quad (4)$$

As the pH in the body fluid increases, or in other words the concentration of OH^{-} increases, the chemical equilibrium shown above shifts according to Le Chatelier's principle. First, (1) is biased to the right, and then (2) is biased to the right due to the decrease in H^{+}.

This increases HCO_3^- , so (3) is biased to the right, and the increase in CO_3^{2-} causes (4) to be biased to the right, resulting in the crystallization of calcium carbonate $CaCO_3$.

For the second hypothesis, we focused on (4). Ca^{2+} being present in the shellfish body fluid, and as the fluid concentration increases, the concentration of Ca^{2+} increases. If (4) is skewed to the right, calcium carbonate precipitates.

Based on these hypotheses, we aimed to elucidate the following two questions: First one is about the specific pH increase required for crystallization in the body of mollusks, and second one is about the pH level required for crystallization change with increasing body fluid concentration.

Clarifying the actual situation regarding the formation of inorganic crystals in vivo could be applied to the suppression of stone formation in the human body, and this research is socially significant.

2. Research Methods

Three experiments were conducted in the following way.

2-1. Experiment 1

The purpose of Experiment 1 is to elucidate the pH increase necessary for crystallization to occur in the shellfish body. The experimental approach is described below.

Ⅰ. Creation of Pseudo-body Fluids

Originally, we had considered creating an aqueous solution that completely reproduced the body fluid of shellfish, but we decided that the organic matter in animal body fluid is too complex to reproduce, so we decided to reproduce only the inorganic components. Therefore, the effect of organic matter in body fluid on the crystallization of calcium carbonate was not considered in this study.

Since it is known from previous studies that the inorganic composition of shellfish body fluid is almost the same as that of seawater, the composition of the simulated body fluid we created, in other words the ratio of each inorganic ion concentration, was made to be almost the same as that of seawater. As mentioned above, it has been proposed that the concentration of simulated body fluid may increase rapidly during crystallization, but we did not consider it in this series of experiments. In other words, the simulated body fluid in Experiment 1 is a reproduction of seawater in both composition and concentration. If the composition and concentration of the shellfish body fluid is the same as that of seawater at the time of crystallization, what level of pH increase is necessary for calcium carbonate crystallization?

Sodium chloride, magnesium chloride hexahydrate, sodium sulfate, calcium chloride dihydrate, and potassium chloride are added as solutes in order to ensure that the solution contains appropriate amount of the inorganic ions, calcium, sodium, magnesium, potassium, chloride, sulfate, and carbonate ions, which are in particularly high concentrations in seawater, and finally, distilled water was added as a solvent to adjust the volume to 100 mL. The composition of the solutions used in Experiment 1 is shown below. (Table 1)

Table 1	mass / g	molar concentration / $mol \cdot L^{-1}$
sea water	1.000×10^{2}	10^{-1}
$NaCl$	2.24	3.83×10^{-2}
$MgCl_2 \cdot 6H_2O$	4.9×10^{-1}	2.4×10^{-2}

Na_2SO_4	2.4×10^{-1}	1.7×10^{-3}
$CaCl_2\cdot 2H_2O$	1.4×10^{-1}	1.0×10^{-3}
KCl	7.0×10^{-2}	1.0×10^{-3}
$(NH_4)_2CO_3$	40.0	5.13×10^{-2}
H_2O	96.81	5.38

Ⅱ. pH Elevation in the solution

In the experiment, the ammonium carbonate gas method was used to raise the pH of the simulated body fluid, in accordance with previous research. In this method, ammonium carbonate solid and solution are sealed in the same container, and the pH of the solution is increased by the dissolution of ammonia generated from ammonium carbonate into the solution. In the experiment, 40.0 g of ammonium carbonate was used and sealed together with the solution as shown in the following picture. A 1000-mL beaker with a Parafilm lid was used as the sealed container.

Ⅲ. Measurement of pH

We measured pH with a pH meter. The pH meter was inserted into the solution, the system was sealed, and the pH was measured and recorded every 2 minutes 30 seconds after sealing. (Figure 1)

Figure 1

Ⅳ. Confirmation of Crystallization

When calcium carbonate crystals were formed, the surface of the solution became cloudy (Figure 2). This cloudiness was considered to indicate that crystallization had occurred, and the pH at the time of crystallization was recorded.

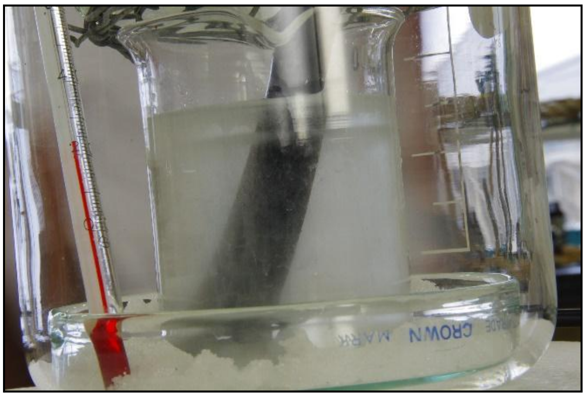


Figure 2

2-2. Experiment 2

The purpose of Experiment 2 is to determine how the pH required for crystallization changes as the body fluid concentration increases. The methodology of the experiment is described below.

Ⅰ. Creation of Simulated Body Fluids

Four types of simulated body fluid solutions were prepared whose composition was equal to that used in Experiment 1, but with different concentrations. The concentrations of the solutions used were 0.67, 1.00, 1.23, and 1.50 times those used in Experiment 1. Their compositions are shown below (Tables 2, 3, 4, and 5).

Table 2	mass / g	molar concentration / $mol \cdot L^{-1}$
sea water	1.000×10^{2}	
$NaCl$	1.49	2.55×10^{-2}
$MgCl_2 \cdot 6H_2O$	3.3×10^{-1}	1.6×10^{-3}
Na_2SO_4	1.6×10^{-1}	1.1×10^{-3}
$CaCl_2 \cdot 2H_2O$	1.0×10^{-1}	7.0×10^{-4}
KCl	5.0×10^{-2}	6.0×10^{-4}
H_2O	97.88	

Table 3	mass / g	molar concentration / $mol \cdot L^{-1}$
sea water	1.000×10^{2}	
$NaCl$	2.24	3.83×10^{-2}
$MgCl_2 \cdot 6H_2O$	4.9×10^{-1}	2.4×10^{-2}
Na_2SO_4	2.4×10^{-1}	1.7×10^{-3}
$CaCl_2 \cdot 2H_2O$	1.4×10^{-1}	1.0×10^{-3}
KCl	7.0×10^{-2}	1.0×10^{-3}

H_2O	96.81	

Table 4	mass / g	molar concentration / $mol \cdot L^{-1}$
sea water	1.000×10^2	
$NaCl$	2.74	4.69×10^{-2}
$MgCl_2 \cdot 6H_2O$	6.0×10^{-1}	3.0×10^{-3}
Na_2SO_4	3.0×10^{-1}	2.1×10^{-3}
$CaCl_2 \cdot 2H_2O$	1.8×10^{-1}	1.2×10^{-3}
KCl	9×10^{-2}	1.2×10^{-3}
H_2O	96.09	

Table 5	mass / g	molar concentration / $mol \cdot L^{-1}$
sea water	1.000×10^2	
$NaCl$	3.36	5.74×10^{-2}
$MgCl_2 \cdot 6H_2O$	4.8×10^{-1}	2.4×10^{-3}
Na_2SO_4	3.7×10^{-1}	2.6×10^{-3}
$CaCl_2 \cdot 2H_2O$	1.6×10^{-1}	1.2×10^{-3}
KCl	1.1×10^{-2}	1.5×10^{-3}
H_2O	96.09	

Ⅱ. pH Elevation in the solution

As in Experiment 1, the ammonium carbonate gas method was used.

Ⅲ. Measurement of pH

The pH required for crystallization was measured for each solution using the same technique as in Experiment 1.

Ⅳ. Confirmation of Crystallization

The same checks were made as in Experiment 1

2-3. Observation

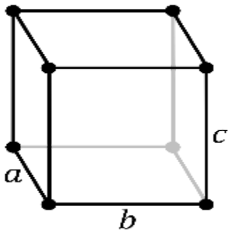
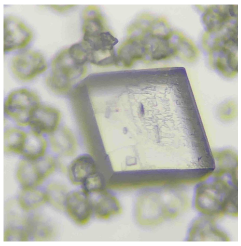
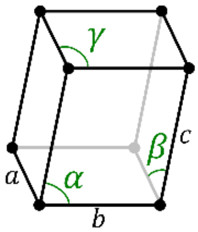
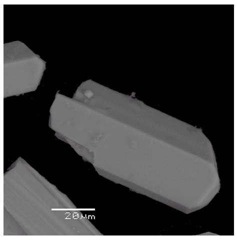
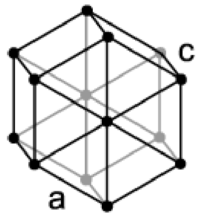
In Experiments 1 and 2, the crystal structures produced were examined as observations. The specific methods of the experiments and points of interest in the observations are described below.

Ⅰ. Observation Method

In the observation, the generated crystals were observed using an optical microscope. Magnification was 60x, 150x, and 600x, and the resulting photographs described below were observed at 150x.

Ⅱ. Crystal Structure of Calcium Carbonate

In the observations, attention was paid to the crystal structure of the obtained calcium carbonate. There are three different crystal structures of calcium carbonate, as shown in the following table (Table 6), and in addition to these crystal forms, there is also an amorphous form.

Table 6	 	 	 
	aragonite	calcite	Vaterite
Crystal structure	Rectangular crystal system	Tetragonal	Hexagonal
Angle of the crystal plane	90°	72°	60°/ 90°

3. Hypothesis

The hypotheses for each experiment and discussion are as follows.

2-1. Experiment 1

Because it is originally an in vivo phenomenon, it is unlikely that the pH at the time of crystallization will be strongly basic (8.5 or higher) which would be very harmful to living organisms.

2-2. Experiment 2

As the body fluid concentration increases, the increase in pH required for crystallization is expected to be smaller, based on the aforementioned theory of chemical equilibrium. In other words, the crystallization pH is expected to approach the neutral value of 7.00 as the body fluid concentration increases.

2-3. Observation

Since the composition of the simulated body fluid is expected to be close to that of the actual fluid, aragonite and calcite, which are actually present in the body of shellfish such as pearl oyster, are expected to be produced.

4. Result

The results for each experiment and observation are as follows.

2-1. Experiment 1

The graph below (Figure 3) shows the relationship between the time from the start of the measurement and the pH of the system.

It can be seen that the behavior is generally consistent in several trials.Taking the average of the pH at the time of crystallization, we obtain a pH of 9.34.

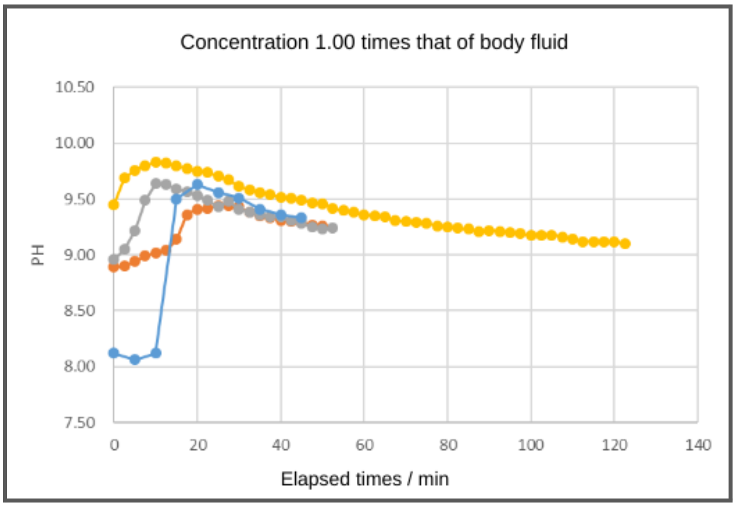


Figure 3

2-2. Experiment 2

The following graphs show the relationship between the time from the start of the measurement and the pH of the system for each concentration of the simulated bodyfluid (Figures 4, 5, 6, and 7).

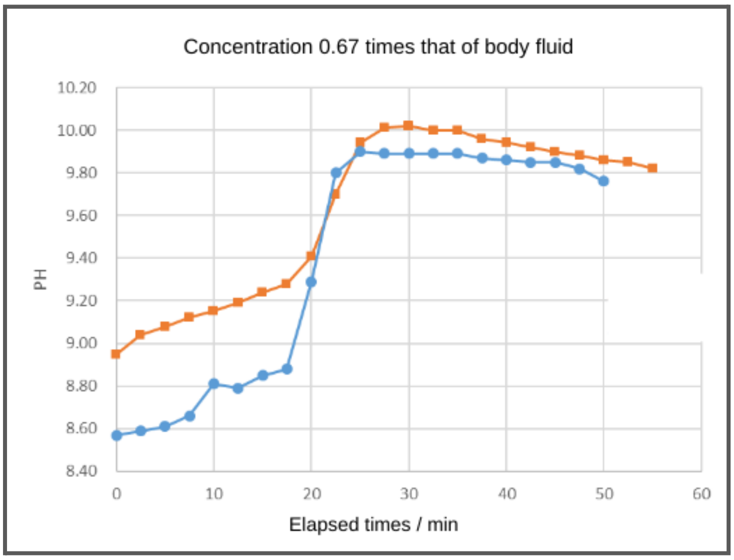


Figure 4

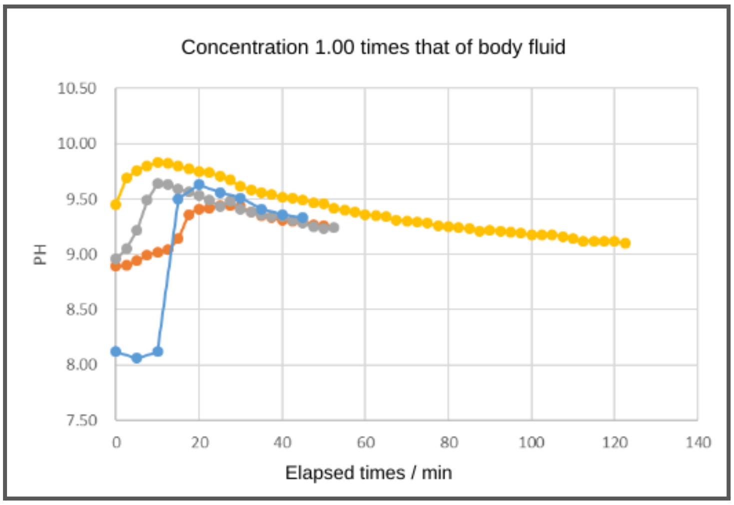


Figure 5

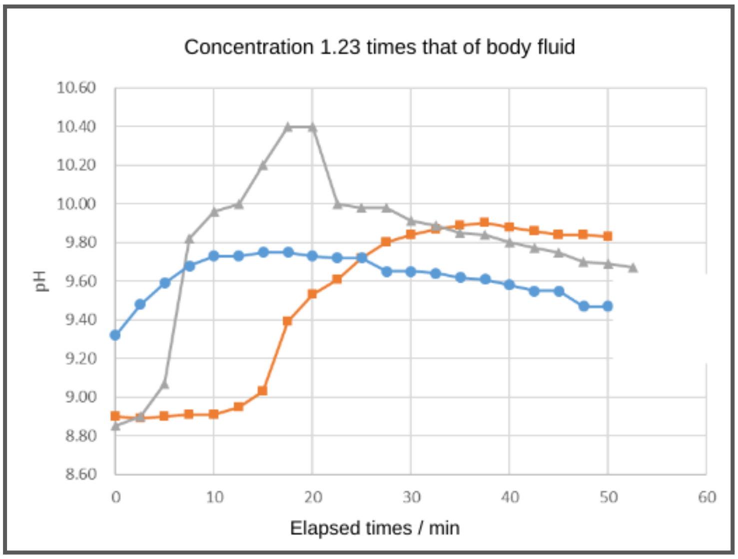


Figure 6

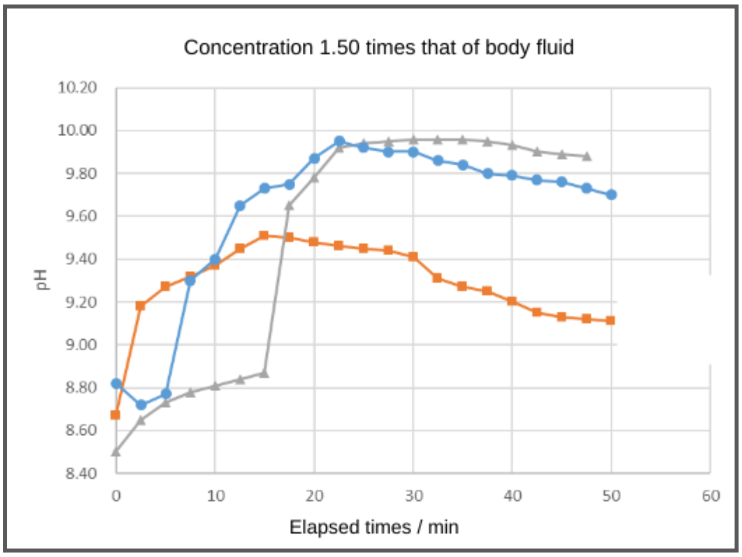


Figure 7

It can be seen that for each concentration, the behavior is generally consistent across multiple trials.

The relationship between the concentration of the solution and the average pH at crystallization for each concentration is plotted in the graph below (Figure 8).

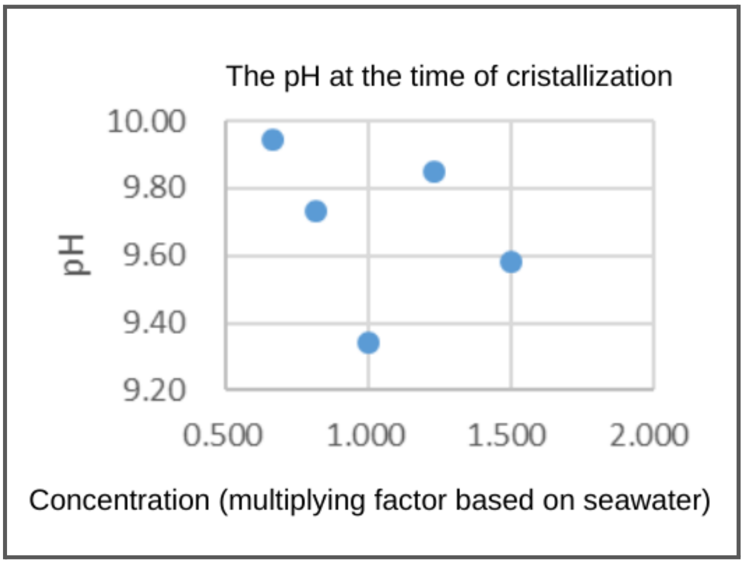


Figure 8

2-3. Observation

Observation of the actual crystals observed in both Experiment 1 and Experiment 2 under an optical microscope revealed that the crystals produced were round in shape both on the surface and at the bottom of the beaker. The crystals at the top of the beaker were relatively small in size, and as one approached the bottom, these smaller, round-shaped crystals clustered together to form larger ones (Figures 9 and 10).

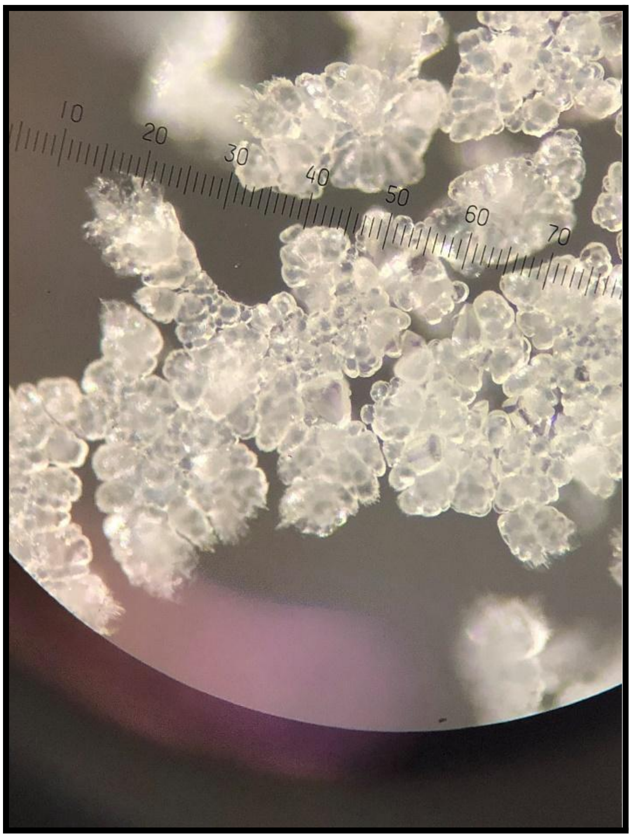


Figure 9

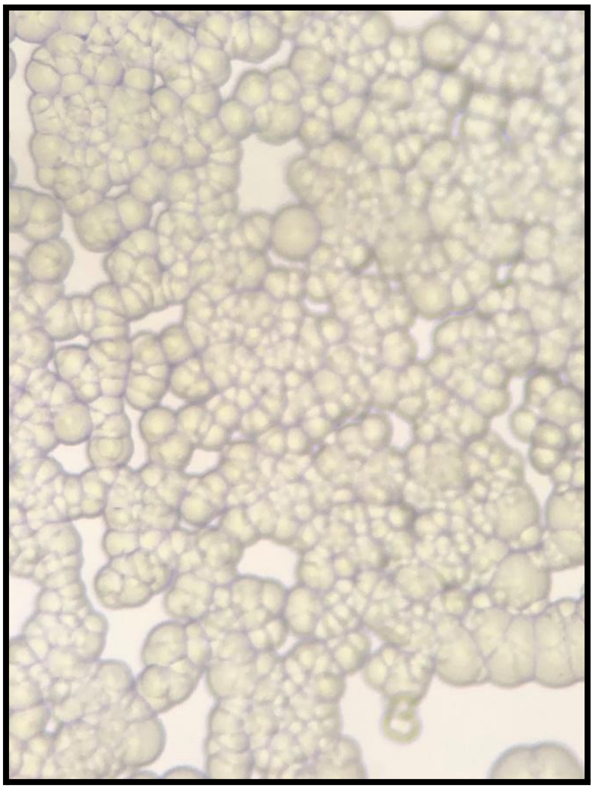


Figure 10

5. Discussion

2-1. Experiment 1

The average crystallization pH was 9.34, but the following indeterminacy in this experiment should be considered. Although the pH of the system gradually increases in the ammonium carbonate gas method, even if the system had truly reached the pH required for crystallization, the true pH required for crystallization would be lower than the pH at which crystallization was visually confirmed because it takes long for crystals to grow until they can be visually confirmed.

From this, it can be concluded from Experiment 1 that if there is no increase in the concentration of body fluid during crystallization in the shellfish body, the pH of the body fluid would only need to increase to a level of at most 9.34 for crystallization to occur.

2-2. Experiment 2

Considering the theory of chemical equilibrium by focusing on Ca^{2+} in solution, one would expect the pH required for crystallization to approach neutral as the solution concentration increases, but the results show that this is not the case for all concentration ranges. Specifically, as the solution concentration increases above a certain value, the pH required for crystallization is found to increase.

The reason for this is discussed below. When the body fluid concentration increases, the concentration of Ca^{2+} of course increases, but the concentration of other inorganic ions also increases at the same time. From this, we hypothesized that inorganic ions other than Ca2+ may somehow inhibit the crystallization of calcium carbonate.

As mentioned above, it has been reported that certain organic compounds inhibit the crystallization of calcium carbonate, but there is no such report on inorganic ions.

2-3. Observation

Aragonite is orthorhombic and calcite is hexagonal, but since the crystals produced in both Experiment 1 and Experiment 2 were round in shape, these are not considered aragonite or calcite. These may be amorphous or vaterite.

In a preliminary experiment conducted prior to Experiment 1, a calcium chloride solution was used instead of the simulated body fluid used in Experiment 1. The crystals formed at this time were clearly angular calcite and aragonite (Figure 11).

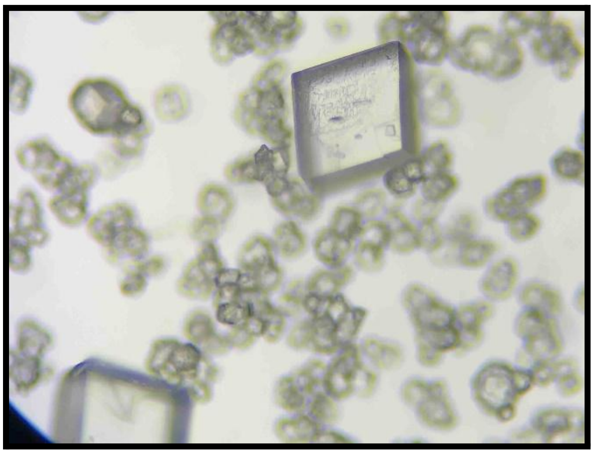


Figure 11

Therefore, it is possible for the inorganic ions in the simulated body fluid to have some effect on the form of crystallization, causing the calcium carbonate to become indeterminate or vaterite.

6. Conclusion

Assuming that there is no increase in body fluid concentration during crystallization in the shellfish body, we found that the pH of the body fluid should increase to a level of at most 9.34 for crystallization to occur.

We also found that the greater the body fluid concentration, the less likely crystallization is to occur, and that inorganic ions other than calcium ions may inhibit the crystallization of calcium carbonate.

In this study, the artificial crystallization of calcium carbonate was reproduced, and vaterite and amorphous were observed in the crystal structure of calcium carbonate, which indicates that inorganic ions may have an effect on the crystal structure of calcium carbonate.

7. Future Issues

We would like to conduct experiments with different concentrations of only inorganic ions other than calcium ions to clarify the inhibition of crystallization by inorganic ions. We would also like to clarify the crystal structure and inorganic ion concentration.

8. References

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