

Presence and Dehydration of Ikaite, Calcium Carbonate Hexahydrate, in Frozen Shrimp Shell

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Ikaite, calcium carbonate hexahydrate, has by means of X-ray diffraction analyses of frozen samples been identified as the mineral component of the white spots formed in the shell of frozen shrimp during storage. When the shrimp thaw and the shell material is dried and kept at room temperature, ikaite rapidly transforms into a mixture of anhydrous calcium carbonate forms. X-ray diffraction analyses and Raman spectra of synthetic ikaite as well as the dehydration product confirm the assignments, and the rate constant for dehydration is approximately $7 \times 10^{-4} \text{ s}^{-1}$ at ambient temperature. Differential scanning calorimetry showed that dehydration of synthetic ikaite is an entropy-driven, athermal process and confirms that a single first-order reaction is rate-determining. Ikaite is found to be stable in aqueous solution at temperatures below 5 °C and in the shell of frozen shrimps but decomposes on thawing to form anhydrous calcium carbonates.

Keywords: *Shrimp; ikaite; calcium carbonate hexahydrate; differential scanning calorimetry; NIR FT-Raman; X-ray diffraction*

INTRODUCTION

White spots may form in the shell of pink shrimp, *Pandalus borealis*, during frozen storage at a rate depending on several factors including the nature of any prefreezing treatment of the shrimp, production time, and storage temperature (Mikkelsen et al., 1997a,b). Dried white spot material isolated from shrimp shells has previously been shown to consist of calcium carbonate in the two crystal forms calcite and vaterite in a matrix of amorphous α -chitin (Mikkelsen et al., 1997b). The weight ratio of α -chitin (poly-(1 \rightarrow 4)- α -D-N-acetylglucosamine) and calcium carbonate was constant (0.34:0.66), while the ratio of vaterite and calcite was found to depend on prefreezing treatments of the shrimp with different chemicals (Mikkelsen et al., 1997b). We previously proposed that α -chitin plays an important role in calcium carbonate precipitation in the frozen shrimp, and that calcium carbonate might have precipitated in the shrimp shell as a hydrate like ikaite, which subsequently dehydrated during prolonged storage or during the employed sample preparation procedure (Mikkelsen et al., 1997b).

Ikaite, calcium carbonate hexahydrate, was first found in nature in 1962 (Pauly, 1963) in the Ika Fjord, Greenland. Here, underwater ikaite columns grow at the low-temperature conditions when seep water with high pH and rich in phosphate leaks from underwater springs and mixes with seawater (Buchardt et al., 1997). At ambient conditions ikaite is unstable and decomposes

into anhydrous calcium carbonate forms (calcite and/or vaterite) and water (Johnston et al., 1916; Krauss and Schriever, 1930; Marland, 1975). Thus, geochemical records of certain calcium carbonate forms are suggested to be indicative of ancient low-temperature conditions appropriate for ikaite formation (Jansen et al., 1987; Sherman et al., 1989; Council and Bennett, 1993). However, while ikaite is unstable relative to other calcium carbonate forms under many conditions, it has been found to be relatively stable at 25 °C at a pressure of about 5 kbar (Marland, 1975) and when kept under oil (Johnston et al., 1916). In contrast, ikaite is more soluble than the anhydrous forms of calcium carbonate at temperatures above 0 °C, and contrary to the anhydrous forms, the solubility of ikaite increases with increasing temperature (Bishof, 1993). Phosphate favors ikaite formation, as it suppresses the growth of anhydrous calcium carbonate forms (Brooks et al., 1950) but does not interact with the crystallization of ikaite (Bishof et al., 1993). For this reason laboratory procedures for ikaite synthesis (Johnston et al., 1916; Krauss and Schriever, 1930; Dickens and Brown, 1970) usually include a "calcite inhibitor", e.g., magnesium cations, sodium polyphosphate (Dickens and Brown, 1970), Calgon (Brooks et al., 1950), or amino trismethylene-phosphonic acid (Gal et al., 1996).

Gal et al. (1996) recently evaluated available data on the formation and solubility of ikaite and other calcium carbonate forms and suggested that calcium carbonate precipitation is initiated by formation of ikaite, which subsequently transforms into vaterite and other anhydrous forms. Inhibition of calcite precipitation was further described as an interaction between the inhibitor and a hydrated calcium carbonate complex, $\text{CaCO}_3 \cdot n\text{H}_2\text{O}$

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(aq), in solution rather than by adsorption of the inhibitor to crystal nuclei.

Raman spectroscopy has proven very useful in characterizing carbonate minerals, as the CO bonds with a substantial degree of double bonding are polarizable and give rise to characteristic and intense Raman bands (Griffith, 1969; Griffith, 1970). The weaker cation-oxygen bonds holding the crystal lattice together are not as polarizable and do not interfere with the carbonate vibrations. Therefore, the spectra are relatively simple, as is seen for the Raman spectra of the nonhydrated calcium carbonates, calcite, vaterite, and aragonite, which have been thoroughly characterized (Griffith, 1969; Griffith, 1970; Kinard, 1980; Mikkelsen et al., 1997b). As a change in polarizability is required for a vibration to be Raman active, Raman spectroscopy is, unlike infrared spectroscopy, insensitive to the weakly polarizable vibrations of water and is therefore a useful tool in characterizing the effect of moisture upon crystal conformation, as has been demonstrated for D-glucose (Corbett et al., 1991). Accordingly, Raman spectroscopy may, despite the inherent low signal-to-noise ratio, also be expected to be an ideal analytical method for monitoring the dehydration of ikaite.

The present study has two aims: (i) to provide evidence for the proposed calcium carbonate hydrate precursor of calcite and vaterite found in white spot material from shrimp shell (Mikkelsen et al., 1997b) and (ii) to investigate the thermophysical conditions for the transformation of calcium carbonate hexahydrate, ikaite, into nonhydrated forms using X-ray diffraction analysis, Raman spectroscopy, and differential scanning calorimetry.

EXPERIMENTAL PROCEDURES

Pink shrimp, *Pandalus borealis*, freshly caught by trawlers in Davis Strait, were kept in frozen storage ($\sim -20^\circ\text{C}$) for 1 year during which time numerous white spots formed in the shell. Samples of the white spot material were removed by scalpel and transferred to capillary tubes with an inner diameter of 0.5 mm and a length of 10 mm. The capillary tubes containing 1–2 mg of the white material were sealed and kept frozen ($< -18^\circ\text{C}$) until analyzed by X-ray diffraction analysis as described below.

Synthetic ikaite crystals were precipitated as described by Dickens and Brown (1970); 4.6 g of Na_2CO_3 (pro analysis from Merck, Germany) and 0.5 g of $\text{Na}_5\text{P}_3\text{O}_{10}$ (pro analysis from Sigma, St. Louis, MO) were dissolved in 150 mL of water (Millipore-Q quality) and, during stirring, a solution of 5.6 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (pro analysis from Merck, Germany) dissolved in 100 mL water was added. The gellike solution was left at $3\text{--}5^\circ\text{C}$ for several months during which time crystals of ikaite formed and grew. The crystals, 1–2 mm in size, were carefully picked up, and if not otherwise indicated in the text, the ikaite crystals were washed twice in water, dried with acetone, and ground to a fine powder in a mortar and never stored for more than 36 h at temperatures above -18°C until the time of analysis. Calcite (pro analysis from Merck, Germany) and vaterite precipitated as described earlier (Mikkelsen et al., 1997b) were used as reference compounds.

X-ray diffraction (XRD) analyses of frozen white spot material from shrimp shell were conducted with a STOE STADI P diffractometer (Stoe, Darmstadt, Germany) using $\text{Cu K}\alpha_1$ radiation (45 kV, 40 mA) and cooling with liquid nitrogen. The samples, prepared in capillary tubes as described above, were scanned at -30°C from 5 to $45^\circ 2\theta$ at a rate of $0.04^\circ 2\theta \text{ min}^{-1}$. The same instrument was used for thermostated XRD analyses of synthetic ikaite, washed twice with cold water and left on a filter paper for a few seconds to remove water and placed in capillary tubes with an inner diameter of 1 mm and

a length of 10 mm, which were sealed before analysis. The samples of synthetic ikaite were scanned at -1°C from 14 to $45^\circ 2\theta$ at a rate of $1.2^\circ 2\theta \text{ min}^{-1}$. In a series of increasing temperature from -1 to 21°C samples of synthetic ikaite were scanned from 30 to $37^\circ 2\theta$ at a rate of $1.2^\circ 2\theta \text{ min}^{-1}$. The instrument was stabilized for 10 min at -1°C before the first scan, the temperature was increased 1°C at $0.1^\circ\text{C min}^{-1}$, the instrument was stabilized at the new temperature for 10 min, etc. until a final scan was run at 21°C 10 h after the first scan. All other XRD analyses of synthetic ikaite were conducted in an open sample holder at room temperature with a Siemens D5000 diffractometer and a PSD-50M position sensitive detector (M. Braun GmbH, Germany) applying Fe filtered $\text{Co K}\alpha$ radiation (40 kV, 40 mA). Samples were scanned at a rate of $2^\circ 2\theta \text{ min}^{-1}$, except for kinetic experiments in which the samples were scanned from 35 to $45^\circ 2\theta$, a range with characteristic ikaite and vaterite peaks, at a scan rate of $0.96^\circ 2\theta \text{ min}^{-1}$.

Near-infrared Fourier transform Raman (Raman) analyses were carried out with a Perkin-Elmer System 2000 interferometer equipped with an Nd:YAG laser emitting at 1064 nm with a laser power of 600 mW. Data were collected with an InGaAs detector and stored as Raman shifts between 3600 and $\sim 150 \text{ cm}^{-1}$. The spectra were all recorded at 8-cm^{-1} resolution and averaged over four scans. The 180° backscattering arrangement was used and no correction for the spectral response of the instrument was applied. Samples of ikaite powder, prepared as described above, and ikaite crystals washed and dried by a similar procedure, were placed in the microsample holder, and Raman spectra were obtained at intervals of 1 min. Measurements were performed at room temperature. The time series Raman data were evaluated by using principal component analysis (Wold et al., 1987) and the Simplisma method (Windig, 1997). Spectral data were collected and converted to ASCII by using the software Spectrum (Perkin-Elmer, Palo Alto, CA) and the chemometric calculations were performed by using Matlab (Mathworks, Inc., Natick, MA) installed with the PLS_Toolbox (B. Wise and N. B. Gallagher, Eigenvector Technologies, Mason, WA).

Differential scanning calorimetry (DSC) was conducted with a DSC 820 from Mettler Toledo (Schwerzenbach, Switzerland) based on the heat flux principle and cooled with liquid nitrogen. The furnace was purged with dry air. Calibration of heat flow and temperature was performed with indium as standard (mp 156.6°C , $\Delta_{\text{fus}}H = 28.5 \text{ J g}^{-1}$, Mettler-Toledo Calibration Kit, ME-119442), and the calibration was checked with gallium (mp 29.8°C , $\Delta_{\text{fus}}H = 80.1 \text{ J g}^{-1}$). Samples, 1–4 mg, of synthetic ikaite were weighed into $40\text{-}\mu\text{L}$ open aluminum pans (Mettler Toledo, ME-27331). An empty aluminum pan was used as reference in all runs. For dynamic measurements, samples were inserted at -5°C , heated to 120°C with a heating rate of 1, 2, 4, 7, 10, 15, or $20^\circ\text{C min}^{-1}$, cooled to 20°C , and reweighed immediately after the scan. For isothermal measurements, samples were inserted at -5°C , and the temperature was raised to the isothermal temperature in the interval $15\text{--}25^\circ\text{C}$ at a rate of $10^\circ\text{C min}^{-1}$. The samples were kept at the isothermal temperature for 120 min, cooled to -5°C , reweighed, and then measured dynamically with $10^\circ\text{C min}^{-1}$ as described above. The endothermic peaks were integrated by using the evaluation software (STAR system, version 4) of the DSC 820.

RESULTS AND DISCUSSION

Figure 1 shows X-ray diffractograms of synthetic ikaite at -1°C and of white spot material from shrimp shell prepared at -20°C and kept frozen until analysis at -30°C . The similarity of these diffractograms clearly demonstrates that the calcium carbonate component present in the frozen white spot material is ikaite. Several substances are present in shrimp shell, some of which may restrain nucleation of calcite and favor formation of ikaite, which is also favored by the low

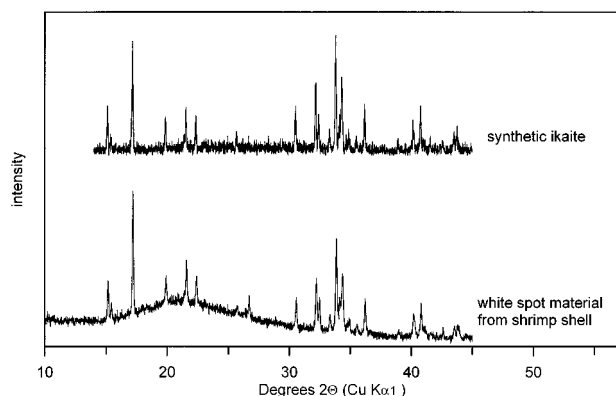


Figure 1. X-ray diffractograms of synthetic ikaite analyzed at $-1\text{ }^{\circ}\text{C}$ (scan rate $1.2^{\circ}\ 2\theta\ \text{min}^{-1}$) and of white spot material from frozen shrimp shell, prepared and analyzed at $-20\text{ }^{\circ}\text{C}$ (scan rate $0.04^{\circ}\ 2\theta\ \text{min}^{-1}$). Samples were kept in closed capillary tubes during analysis. Intensity values of synthetic ikaite are scaled by a factor of 15 relative to intensity values of white spot material. All reflections are due to ikaite (JCPDS-ICDD, 1995).

temperature during freezer storage. Calcite and vaterite found together with chitin in white spot material when analyzed at ambient temperature (Mikkelsen et al., 1997b) can thus be concluded to be formed from ikaite during sample preparation in the decomposition reaction



involving the loss of 6 hydrate waters corresponding to a theoretical weight loss of 51.9%. The weight ratio of chitin and calcium carbonate in the dried white spot material from shrimp shell was invariably 0.34:0.66 (Mikkelsen et al., 1997b), and the weight ratio of chitin and ikaite in the white spots in the frozen state must thus have been 0.20:0.80. The ratio of vaterite and calcite was found to vary among samples with different prefreezing treatments of the shrimp (Mikkelsen et al., 1997b), although it cannot be precluded that these differences also could be due to small variations in sample preparation conditions such as room temperature, relative humidity, and particle size. It is accordingly of interest to study the transformation process of ikaite into anhydrous forms. For this purpose, ikaite was synthesized in the laboratory, as described under Experimental Procedures. Ikaite crystals kept at $5\text{ }^{\circ}\text{C}$ in the mother solution or in deionized water were stable and did not transform into anhydrous forms within several months, as verified by XRD analysis.

The Raman spectrum of ikaite, which to the best of our knowledge has not been presented before, is shown in Figure 2A. It represents a simple and typical calcium carbonate spectrum with a weak contribution from water ($3500\text{--}3000\text{ cm}^{-1}$). The most intense peak at 1070 cm^{-1} in the ikaite spectrum, due to the totally symmetric CO stretch, is characteristic for carbonates (Table 1). Only one secondary carbonate peak, namely, the in-plane band at 719 cm^{-1} , can safely be identified. The second most intense band in the ikaite spectrum is due to lattice vibrations and found at 203 cm^{-1} with secondary peaks at 220 and 278 cm^{-1} , which may be compared with lattice vibrations centered at 281 cm^{-1} in calcite, at 282 and 207 cm^{-1} in aragonite, and at 300 and 267 cm^{-1} in vaterite (Mikkelsen et al., 1997b).

When synthetic ikaite is dried and ground as described under Experimental Procedures and kept at

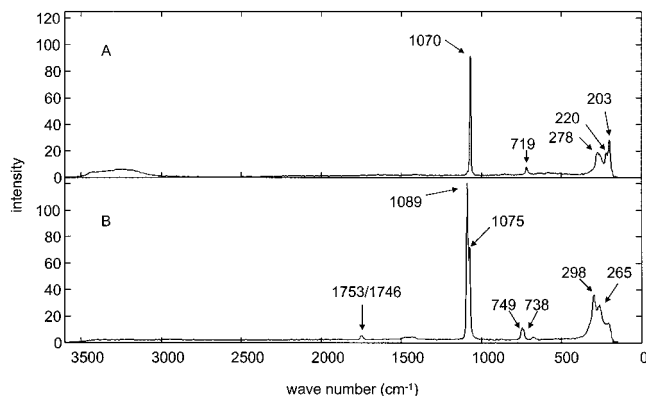


Figure 2. Raman spectra of synthetic ikaite (A) and the end product after dehydration of ikaite (B) in the range $3600\text{--}0\text{ cm}^{-1}$ Raman shifts.

Table 1. Characteristic Raman Bands (Raman Shifts in cm^{-1}) of Calcium Carbonates Indicated by Wavenumbers, ν_n^a

carbonate	lattice	ν_1	ν_2	ν_3	ν_4
CO_3^{2-} ^b		1063	879	1415	680
calcite ^c	281	1086		1436	712
vaterite ^c	300/267	1090/1077	(874)	(1477/1419)	750/738
aragonite ^d	282/207	1086	854	1462	705
ikaite ^c	278/220/203	1070		(1417)	719

^a Wavenumbers of dual and triple peaks are separated by a slash. Wavenumbers in parentheses are ill-defined and very weak peaks. ^b From Griffith, 1969; Griffith, 1970. ^c The present study. ^d From Mikkelsen et al., 1997b.

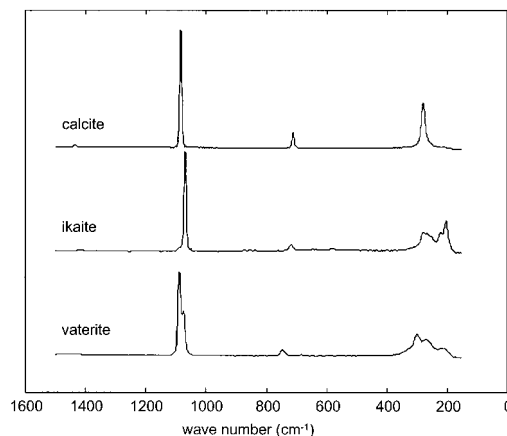


Figure 3. Raman spectra of calcite, ikaite, and vaterite in the range $3600\text{--}150\text{ cm}^{-1}$ Raman shifts.

room temperature, it rapidly decomposes. From its Raman spectrum the end product (Figure 2B) is unambiguously identified as vaterite (Figure 3), as also demonstrated by XRD. In the dehydrated product the strongest and totally symmetric carbonate stretch of ikaite, which is seen as the sharp band at 1070 cm^{-1} , splits into a dual peak at the wavenumbers 1089 and 1075 cm^{-1} , characteristic for vaterite (Table 1). The single in-plane carbonate peak at 719 cm^{-1} has similarly split into a dual peak at 749 cm^{-1} with a shoulder at 738 cm^{-1} , also characteristic for vaterite. When examining the lattice vibrations of the dehydrated product it is observed that the ikaite peaks located at 278 and 203 cm^{-1} have moved to 298 cm^{-1} with a less structured secondary peak at 265 cm^{-1} , with a band shape identical to that of vaterite (Figure 3). Finally, a weak but well-structured dual band is emerging at 1753 and 1746 cm^{-1} (Figure 2). This band was erroneously assigned to "the

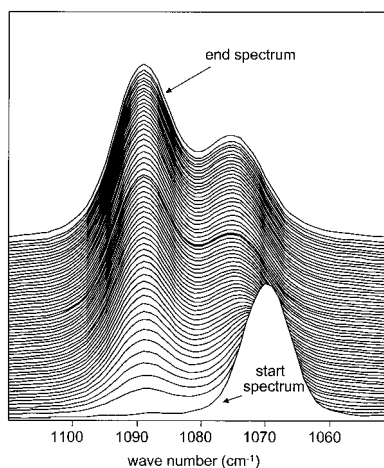


Figure 4. Time series of Raman spectra in the range 1110–1050 cm^{-1} Raman shifts during the dehydration process of ikaite at ambient temperature. The spectrum in the front with a single peak at 1070 cm^{-1} is the ikaite spectrum, and the end spectrum in the rear with peaks at 1089 and 1075 cm^{-1} is identified as vaterite.

presence of an unknown trace component” in our previous paper describing white spot material deposited in shrimp shells (Mikkelsen et al., 1997b). Scrutiny of the Raman spectrum of pure vaterite reveals the presence of this dual band positioned in a spectral region typical for an isolated carbonyl stretch. This band could perhaps be overtone components of ν_2 ($2 \times 879 = 1758 \text{ cm}^{-1}$).

To examine the dehydration process of ikaite in more detail the Raman spectrum of the substance was recorded as a function of time at 1-min intervals, leaving the material untouched in the sample holder. Figure 4 shows a time series of the strongest and totally symmetric CO stretch during the dehydration process. As apparent from the figure, the transformation of ikaite into vaterite is a continuous process in which the ikaite peak at 1070 cm^{-1} initially is gradually shifted toward higher wavenumbers to merge with the vaterite peak centered at 1075 cm^{-1} , which builds up along with the strongest vaterite peak at 1089 cm^{-1} . Since no transient peak can be identified, the process gives no indications of formation of any intermediate calcium carbonate hydrates during the dehydration process of ikaite.

From a recorded time series of such spectral information it is, in principle, possible by means of a two-dimensional data evaluation method to extract the underlying phenomena (latent variables) and their contributions (concentrations) without making assumptions about the number of bands and their shape. In this study we have applied two different chemometric techniques: principal component analysis (PCA) and the pure variable Simplisma method developed by Windig (1997) for analysis of Raman data, a method which is related to curve resolution algorithms such as alternating regression. The PCA method is a simple and efficient method for describing the systematic (noise-free) variations in a complex data structure and is especially well-suited when dealing with highly collinear variables, as is the case in most spectroscopic techniques. The multidimensional data set is resolved into orthogonal components whose linear combinations approximate the original data set in a least squares sense. In PCA, the original data matrix (\mathbf{X}) (samples \times spectral variables) is decomposed into a score matrix (\mathbf{T}) and a loading matrix (\mathbf{P}), and the residuals are collected in a matrix (\mathbf{E}): $\mathbf{X} = \mathbf{TP}^T + \mathbf{E}$. Only a limited number of principal

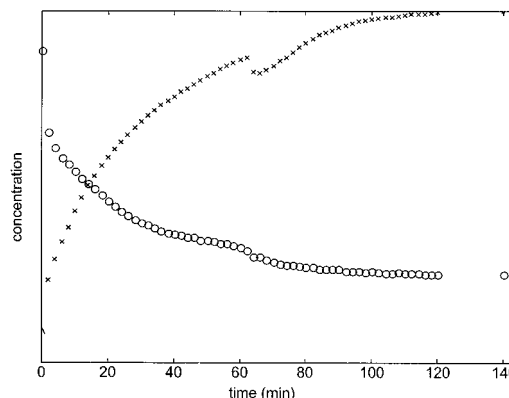


Figure 5. Decreasing ikaite (\circ) and increasing vaterite (\times) concentrations during dehydration of ikaite at ambient temperature given in arbitrary units, as calculated from the Simplisma concentration matrix from a time series of Raman data (see Figure 4).

components (PC's) equal to the chemical rank of the \mathbf{X} -matrix are relevant in describing the systematic information in \mathbf{X} . The loading vectors for the principal components can be considered as pure hidden spectra that are common to all the measured spectra. What makes the individual raw spectra different are the amounts (scores) of hidden spectra (Engelsen and Nørgaard, 1996). The fully cross-validated (“leave one out” validation) PCA results clearly demonstrate that the chemical rank of the spectral time series (120 spectra corresponding to 2-h measurements) is equal to two with an explained systematic variance of 99.9% (99% + 1%). This could indicate a simple $A \rightarrow B$ type of reaction with a weak contribution of a nonadditive matrix effect such as, e.g., wavenumber shift. The spectral time series was subsequently submitted to a two-component resolution by the Simplisma method. In this approach the systematic (noise free) variation is decomposed according to $\mathbf{X}_{\text{sys}} = \mathbf{X} - \mathbf{E} = \mathbf{C} \times \mathbf{S}$, where \mathbf{X} (samples \times spectral variables) is the original spectral matrix, \mathbf{E} (samples \times spectral variables) is the residual noise matrix, \mathbf{C} (samples \times 2) is the concentration matrix, and \mathbf{S} (2 \times spectral variables) is the pure spectra matrix. The result of the Simplisma analysis in the form of the concentration matrix \mathbf{C} is shown in Figure 5. The kink after 1 h of reaction was a characteristic feature, also in repetitions of the experiment, and is probably due to a minor collapse in the sample caused by the structural rearrangement of the calcium carbonate. By least-squares fitting of these concentration profiles to a first-order kinetic term, $I(t) = A \exp(-kt)$, a characteristic rate constant, k , for the dehydration process at room temperature was calculated to be approximately $8 \times 10^{-4} \text{ s}^{-1}$. Application of a similar procedure on the time series of X-ray diffractograms obtained during the dehydration process at room temperature resulted in a first-order rate constant of approximately $5 \times 10^{-4} \text{ s}^{-1}$. There is good agreement between the two different methods indicated by the average first-order characteristic rate constant of $7 \times 10^{-4} \text{ s}^{-1}$.

Figure 6 shows a dynamic DSC scan of synthetic ikaite. The peak temperature, T_m , for the decomposition reaction was found to increase with increasing scan rate from 1 to 10 $^\circ\text{C min}^{-1}$ and to reach a maximum value of approximately 65 $^\circ\text{C}$ for scan rates above 10 $^\circ\text{C min}^{-1}$, as shown in the insert of Figure 6. The activation energy, E_a , for such a dehydration reaction (eq 1) may, according to Kissinger (1957), be derived from the

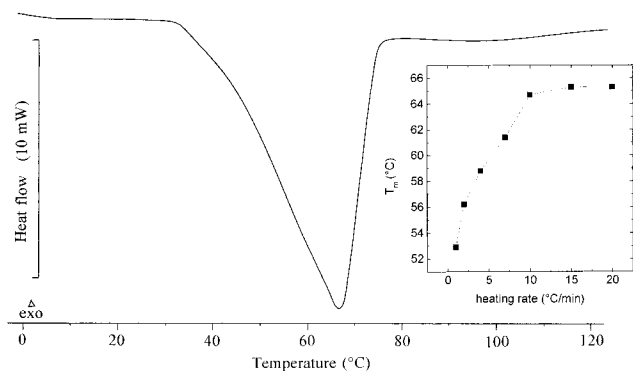


Figure 6. DSC thermogram of heating 1.24 mg of dry synthetic ikaite from 0 to 120 °C at a rate of 10 °C/min. Insert: Peak temperature, T_m , as a function of heating rate for otherwise identical conditions.

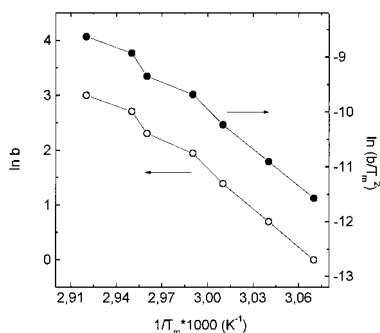


Figure 7. Ozawa plot (○) and Kissinger plot (●) of dry synthetic ikaite heated at rates of 1, 2, 4, 7, 10, 15 and 20 °C min⁻¹. See eqs 2 and 3 in the text.

following equation regardless of reaction order:

$$d(\ln(b/T_m^2)) = -E_a/R d(1/T_m) \quad (2)$$

where b is heating rate, T_m is peak temperature, and R is the gas constant. A similar but more general equation has been proposed by Ozawa (1970):

$$\ln b = C - E_a/RT_m \quad (3)$$

where C is a constant. A Kissinger plot, $\ln(b/T_m^2)$ versus $1/T_m$, and an Ozawa plot, $\ln b$ versus $1/T_m$, are both shown in Figure 7. Very similar activation energies for the ikaite decomposition reaction were obtained by the two methods, namely, $E_a = 176$ kJ mol⁻¹ and $E_a = 180$ kJ mol⁻¹, respectively. Combining the resulting estimate for the enthalpy of activation ($\Delta H^\ddagger = E_a - RT$), $\Delta H^\ddagger = 176$ kJ mol⁻¹ with the activation free energy, as calculated from the rate obtained from Raman spectroscopy and X-ray diffraction analyses (at 25 °C, $\Delta G^\ddagger = -RT(\ln k(s^{-1}) - \ln(Nh/RT)) = 91$ kJ mol⁻¹), an estimate of the entropy of activation $\Delta S^\ddagger = 285$ J mol⁻¹ K⁻¹ is obtained. In the transition state, a significant increase in disorder is driving the process toward dehydration, despite the very high enthalpy of activation, as evidenced by this rather large entropy of activation. It should be noted that for H₂O, $S^\ddagger(l) - S^\ddagger(s)$ at 0 °C is only 22 J mol⁻¹ K⁻¹ compared to $\Delta S^\ddagger = 48$ J (mol of H₂O)⁻¹ K⁻¹ for the transition state of dehydration of ikaite, entailing a highly structured bonding of water in the ikaite crystals, which is already lost in the transition state of dehydration (Espenson, 1981).

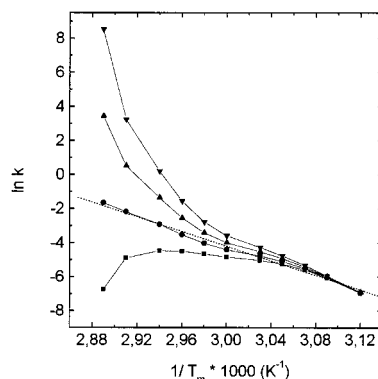


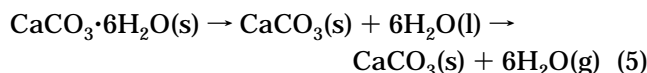
Figure 8. Arrhenius plots for decomposition of ikaite during a DSC run for assumed reaction order of 0, 1, 2 and 3, respectively, according to the method of Torfs *et al.* (1984). Dry synthetic ikaite, 1.24 mg, was heated at 10 °C min⁻¹ from 0 to 120 °C. □, 0 order; ●, 1st order; ▲, 2nd order; ▼, 3rd order.

The order of reaction for the dehydration of ikaite (eq 1) can be derived by the method of Torfs *et al.* (1984). The rate constant, k , is calculated at various temperatures, T_i , within the decomposition endotherm according to

$$k = (dH_i/dt)/(\Delta_{tot}H \times (\Delta_{rest}H/\Delta_{tot}H)^n) \quad (4)$$

where dH_i/dt is the heat flow at the temperature T_i , $\Delta_{tot}H$ is the total heat of the reaction, $\Delta_{rest}H$ is the reaction heat as evolved above the temperature T_i , and n is the reaction order. Estimated rate constants, k , were calculated for $n = 0, 1, 2$, and 3 at various temperatures, T_i , within the temperature interval of the decomposition. From Arrhenius plots, $\ln(k)$ as a function of $1/T_i$, the reaction order of the decomposition can be derived as the n -value yielding the best fit for a linear curve, as illustrated in Figure 8. For all experiments, the decomposition of ikaite was recognized as a first-order reaction in agreement with the kinetic fits of the Raman and XRD time series.

The decomposition of ikaite may be presented as a two-step reaction



In the first step, the crystal rearranges and water moves from the crystal lattice to the crystal surface, and in the second step, water evaporates. The total heat of decomposition, $\Delta_{tot}Q$ (J/g of ikaite), is the sum of the heat of rearrangement, $\Delta_{re}Q$ (J/g of ikaite), and the heat of vaporization of water, $\Delta_{vap}Q$ (J/g of ikaite)

$$\Delta_{tot}Q = \Delta_{re}Q + \Delta_{vap}Q \quad (6)$$

For each DSC scan, $\Delta_{tot}Q$ was calculated as the integral of the endotherm divided by sample weight. The heat of water vaporization is temperature dependent and was calculated from the literature values of $\Delta_{vap}H$ (kJ g⁻¹) at the peak temperature, T_m , combined with the weight loss, W_{water} (g), and the sample weight. The heat of rearrangement expressed per gram ikaite was thus calculated as

$$\Delta_{re}Q = \Delta_{tot}Q - (\Delta_{vap}H(T_m)HW_{\text{water}})/W_{\text{ikaite}} \quad (7)$$

$\Delta_{re}H$ was found to be 0.02 (± 0.06) kJ g⁻¹, indicating that

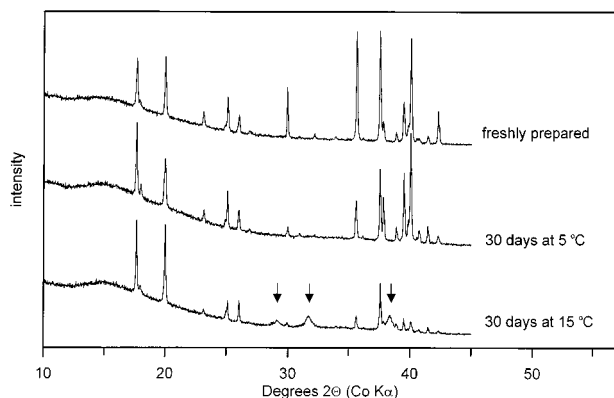


Figure 9. X-ray diffractograms of synthetic ikaite (dried and ground as described under the Experimental Procedures) freshly prepared, after one month storage at 5 or 15 °C, respectively. The reflections marked with arrows are due to vaterite. Unmarked reflections are due to ikaite (JCPDS-ICDD, 1995).

rearrangement of ikaite into a dehydrated calcium carbonate form is an athermal reaction.

In experiments designed to investigate the stability of ikaite at different conditions, dry, ground ikaite was kept at atmospheric conditions at 5 and 15 °C in open containers. From XRD analyses (Figure 9), ikaite was found to be stable for at least 30 days at 5 °C, whereas at 15 °C some vaterite had formed after 30 days. The stability of ikaite at 5 and 15 °C is thus in great contrast to the lack of stability at room temperature. Synthetic ikaite placed in sealed capillary tubes and analyzed by XRD at increasing temperatures from -1 to 21 °C showed no decomposition during the time required for the experiment, i.e., 10 h. A diffractogram obtained after further 24 h at 21 °C indicated no changes in the crystal structure, while a diffractogram obtained after 1 week at 21 °C showed complete decomposition of ikaite into vaterite with weak traces of calcite. When these results are compared with the DSC results, aside from the temperature, the relative humidity may be identified as the most important factor controlling the rate of the decomposition of ikaite.

As the dehydration process involves movement of water molecules from the crystal lattice to the crystal surface, it is expected that the reaction rate increase with decreasing distance required for water to move. Accordingly, decreasing size of crystals is expected to increase the rate of dehydration, an effect which may be further substantiated by the increasing total surface area for decreasing crystal size, which facilitates evaporation of water. The procedures of ikaite preparation and reaction conditions used in the present study resulted in formation of vaterite as the only dehydration product, with a few exceptions in which very small amounts of calcite appeared. No obvious reasons for calcite formation were identified, although variations in the particle size after grinding of ikaite may yield different dehydration product distributions. Particle size being an important factor in determining the product(s) of dehydration of ikaite may also be indicated from the presence of a relatively large fraction (10–20%) of calcite in the dehydration product of calcium carbonate in the white spot material from shrimp shell (Mikkelsen et al., 1997b), as the white spot material was less homogeneous.

In conclusion, the white spots formed in shrimp shell during freezer storage of raw shrimp are due to pre-

cipitation of ikaite, calcium carbonate hexahydrate. Ikaite is stable when kept in aqueous solution below 5 °C and in frozen shrimps but decomposes to anhydrous calcium carbonate at ambient conditions in an entropy-driven, athermal process. The decomposition reaction shows first-order kinetics with a high energy of activation and a large positive entropy of activation, corresponding to a significant decrease in structure in the transition state of dehydration.

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