

# Pressure-induced association of oleic acid (OA) under varying temperature studied by multiple-perturbation two-dimensional (2D) IR correlation spectroscopy

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**Abstract**

Pressure-induced association of oleic acid (OA) under varying temperature was studied by multiple-perturbation two-dimensional (2D) IR correlation spectroscopy. Pressure-dependent ATR-IR spectra of OA were measured by imposing the static pressure ranging up to 12 MPa. Then transient variations of the IR spectra were captured over the temperature range of 20 to 40 °C. Multiple-perturbation 2D correlation analysis, coupled with the ATR-IR measurements, reveals association dynamics of OA molecules caused by the applied pressure. The compression first generates the liquid crystalline structure by aggregating the disordered dimer units. Further compression induces the development of the dimer via the assembling of the isolated monomers. In addition, the variation of the partial asynchronous correlation intensities induced by the change in temperature reveals the way the association kinetics is influenced by temperature. The increase in the temperature essentially increased segmental motion of the alkyl chain, which in turn restricts the generation of the liquid crystalline structure based on the tightly packed dimer units.

Keywords: Two-dimensional correlation spectroscopy; Multiple-perturbation two-dimensional correlation spectroscopy; Infrared; Oleic acid, Self-assembling

## 1. Introduction

Self-assembly of fatty acid molecules into polymer-like materials through the use of noncovalent interactions has received growing attention for the potential utility from fundamental research and practical technological points of view [1-8]. Oleic acid (OA) has been widely studied to provide insight into the molecular structure and self-assembling. For example, Iwahashi et al. showed that OA probably has three kinds of liquid structures depending on temperature [9-12]. In the temperature range from melting point at 15 °C to about 30 °C, the liquid structure consists of clusters having a quasismectic liquid crystal structure, while the structure in the temperature range between 30 °C and 55 °C is composed of clusters with a less ordered structure. Above 55 °C, OA appears to be an isotropic liquid. In addition, the association dynamics of isolated OA monomer undergoing the self-assembly process into the development of quasismectic liquid crystalline structure has been studied in our previous studies. The spectroscopic studies have revealed that the self-assembling process includes the formation of dimer based on hydrogen bonding of carboxyl group and aggregation of dimer units into the tightly packed quasismectic liquid crystals.

The spontaneous development of the liquid crystalline structure of OA is essentially related to intermolecular interactions, such as the degree of hydrogen bonding of the

carboxyl group and segmental movements of carbon atoms along the alkyl chain [13-17]. Thus, probing the nature of the association dynamics under well-controlled perturbations, in turn, provides an interesting opportunity to control the self-assembling of OA molecules. In this study, pressure- and temperature-dependent behavior of OA was explored by IR spectroscopy. Namely, compression-induced structure variations of OA under varying temperature were monitored by means of ATR-IR spectroscopy. Spectroscopic study of OA under well-controlled multiple perturbations makes it possible to derive adequate molecular level insight into the underlying mechanisms of the system. For example, during the compression, OA molecules substantially go through several transitions depending on the applied pressure. Such variation may also be influenced by the temperature. Thus, the detailed analysis of the pressure- and temperature-dependent ATR-IR spectra may in turn provide useful information on the nature of the liquid crystalline structure.

Pressure- and time-dependent IR spectra of OA were analyzed by a multiple two-dimensional (2D) correlation technique to elucidate time-dependent variations influenced by changes in pressure [18-23]. The basic concept of multiple-perturbation 2D correlation spectroscopy is a subset of generalized 2D correlation spectroscopy techniques [24-26]. It is used to probe the mixed dynamic responses of a system

caused by more than one type of stimuli [18-23]. In the multiple-perturbation 2D correlation scheme, a sample is perturbed by at least two different types of perturbations to induce mixed responses. For example, a spectroscopic measurement carried out with two independent perturbations e.g., pressure and temperature will generate three-way data with the  $(i,j,k)$ th element denoting the spectral value at the  $i$ th pressure, the  $j$ th temperature, and the  $k$ th wavenumber. Unfortunately, systems stimulated by multiple perturbations are generally much more complex and harder to interpret intuitively. A reasonable approach to such a three-way dataset would be to keep one perturbation variable constant, and then apply standard 2D correlation analysis to the data now reduced to a two-way array matrix. In multiple-perturbation 2D correlation scheme, partial 2D correlation spectra are defined as synchronous and asynchronous 2D correlations over one perturbation variable, while keeping the other perturbation variable constant. Namely, 2D correlation spectra generated from the two-way slice correspond to the partial synchronous and asynchronous correlation spectra for one particular perturbation variable, providing the ease of detecting pertinent features arising from complex perturbation systems.

Substantial changes in the IR spectral feature were readily captured during the application of the pressure and temperature to the system. Details of spectral changes

associated with the multiple stimuli were fully analyzed with the multiple-perturbation 2D correlation method. The 2D correlation spectra revealed the seemingly complicated of the self-assembly system influenced by the temperature as well pressure.

## 2. Methods

### 2.1. Partial 2D correlation

Partial 2D correlation spectrum is the simplest form of multiple-perturbation 2D correlation analysis [20]. Figure 1 illustrates a graphical representation of the partial 2D correlation analysis. In conventional 2D correlation analysis, system is stimulated with one perturbation and the dynamic response is captured as a function of the perturbation. Thus the data obtained results in so-called two-way data matrix. On the other hand, multiple-perturbation experiment, stimulating the system with more than one perturbation, generates three-way data array. A reasonable approach to such a three-way dataset would be to keep one perturbation variable constant, and then apply standard 2D correlation analysis to the data, now reduced to a two-way array matrix. There are many different options in obtaining two-way slices from three-way data arrays. For example, assume that a set of spectra  $A(v,p,q)$  of a system under multiple

perturbations are collected as a function of an appropriate spectral variable, like wavenumber  $\nu$ . The two additional variables,  $p = 1, 2, \dots, P$  and  $q = 1, 2, \dots, Q$ , represent different perturbation variables, e.g., time, temperature, concentration, and so on. The reference spectrum and dynamic spectra over  $p$  for a fixed  $q$  are given by

$$\bar{A}_p(\nu, q) = \frac{1}{P} \sum_{p=1}^P A(\nu, p, q) \quad (1)$$

$$\tilde{A}_p(\nu, p, q) = A(\nu, p, q) - \bar{A}_p(\nu, q) \quad (2)$$

Partial synchronous and asynchronous correlation spectra are obtained as

$$\Phi_p(\nu_1, \nu_2, q) = \frac{1}{P-1} \sum_{p=1}^P \tilde{A}_p(\nu_1, p, q) \cdot \tilde{A}_p(\nu_2, p, q) \quad (3)$$

$$\Psi_p(\nu_1, \nu_2, q) = \frac{1}{P-1} \sum_{p=1}^P \tilde{A}_p(\nu_1, p, q) \cdot \tilde{A}_p^\#(\nu_2, p, q) \quad (4)$$

where  $\tilde{A}_p^\#(\nu_2, p, q)$  represents the Hilbert-Noda transformation<sup>18</sup> given by

$$\tilde{A}_p^\#(\nu_2, p, q) = \sum_{r=1}^P N_{pr} \tilde{A}_p(\nu_2, r, q) \quad (5)$$

$$N_{pr} = \begin{cases} 0 & \text{for } p = r \\ 1/(r-p)\pi & \text{otherwise} \end{cases} \quad (6)$$

Partial synchronous and asynchronous correlation spectra over  $q$  for a fixed  $p$  are also given in the similar manner. The partial synchronous correlation intensity  $\Phi_p(\nu_1, \nu_2, q)$  represents the overall similarity or coincidental nature between two signal variations  $\nu_1$  and  $\nu_2$  over  $p$  for a fixed  $q$ . The partial asynchronous correlation intensity  $\Psi_p(\nu_1, \nu_2, q)$ , on the other hand, represents the out-of-phase nature of the signals over  $p$  for a fixed  $q$ . There are many different options in the selection of  $q$ . Thus, by

exploring the variation of the synchronous and asynchronous correlation intensities between  $\nu_1$  and  $\nu_2$  depending on each  $q$ , it becomes possible to sort out the effect of  $q$ -perturbation on alternation induced by  $p$ -perturbation.

### 3. Experiment

#### 3.1. Materials

OA and ethanol was purchased from Wako Pure Chemical Industries, Ltd, (Osaka, Japan) and used without further purification. The initial mole fraction of OA in the binary mixture solution was 0.02.

#### 3.2. Pressure- and temperature-dependent ATR-IR spectra

A schematic illustration of the experimental set-up is shown in Fig. 1. A Graseby Specac Golden Gate diamond ATR accessory with pressure control (Specac. Ltd., Orpington, UK) was aligned in the external sample compartment of a PerkinElmer Spectrum 100 spectrometer equipped with a TGS detector. A silicon rubber heater (OM heater, Aichi, Japan) was placed around the diamond internal reflectance element (IRE).



The 5  $\mu\text{L}$  binary mixture solution of OA and ethanol was deposited on a horizontal ATR plate. The sample was exposed to open atmosphere to undergo spontaneous evaporation of the ethanol from the system and only thin layer of the OA remained behind. Then, a static pressure was applied with a torque wrench by varying compression level (0, 4, 6, 8, 10, 12 and 14 MPa) to collect IR spectra with  $4\text{ cm}^{-1}$  resolution by co-adding 16 scans. The pressure was released before each spectral measurement. The measurements of the pressure-dependent IR spectra were carried out under the temperature: 20, 25, 30, 35 and 40  $^{\circ}\text{C}$ .

## 4. Results and discussion

### 4.1. Pressure- and temperature dependent IR spectra of OA

Conformational transitions of OA result in the systematic alteration of vibrational spectroscopic features. For example, Fig. 3(A) represents the pressure-dependent ATR-IR spectra of the OA collected at 20  $^{\circ}\text{C}$ . Peaks observed in this region are specific to C=O vibrational modes of carboxyl group of OA [12,17]. A major peak observed at around  $1700\text{ cm}^{-1}$  represents the contribution from dimer of OA. On the other hand, the development of a minor peak at  $1740\text{ cm}^{-1}$  is attributed to monomer. The emergence of the monomer band is especially important since it indicates the

possible surface interaction between OA and IRE. In a pure liquid state, OA spontaneously forms stable dimer form due to the hydrogen bonding of carboxylic group [12-17]. On the other hand, almost all the OA dimer is dissociated in the mixture solution mainly occupied by ethanol, and the dissociated OA and ethanol form a complex because of the hydrogen bonding [12-17]. Thus, the development of the monomer band reveals that, when the dissociated OA is deposited on the IRE, some portion of the isolated OA monomer essentially interacts with the IRE due to the possible surface interaction, and eventually the monomers remain behind despite the evaporation of ethanol.

The IR spectra substantially reflect difference in the way OA molecules exist in the system. Thus, the detailed analysis of the spectral intensity variation induced by the pressure may provide useful intimation on how OA molecules undergo the alternation depending on the structures. For example, in Fig. 3(A), one can find that monomer band at  $1740\text{ cm}^{-1}$  shows gradual decrease in the spectral intensity with the increase in pressure. On the other hand, the major peak observed at around  $1700\text{ cm}^{-1}$  slightly increases with the pressure. It is thus likely that the relatively low level compression applied in this study essentially induces the decrease in the isolated monomer and the increase in the coagulated structure. In fact, Iwahashi et al. reported that OA tends to

form a specific self-assembled model, which provides most condensed packing form of the dimers of OA due to the segmental movements of carbon atoms along the alkyl chain [12]. Consequently, the above observation reveals that the application of the pressure results in the coagulation of the monomers to develop the quasismectic liquid crystals in which the dimer units are tightly packed together and have only short-range positional order.

Fig. 3(B) represents the pressure-dependent ATR-IR spectra of the OA collected at 40 °C. It is obvious that the sample heated at 40 °C also undergoes seemingly similar structure variation during the observation period, namely coagulation of the monomers and development of the quasismectic liquid crystals. The changes in the spectral feature shown in Fig. 3(A) and (B) reasonably reflect the structural alternation of the OA by the applied pressure. Thus, the detailed analysis of the spectra, in turn, reveals not only structural change induced by the pressure but also effect of temperature on the association dynamics. It is, however, rather difficult to identify from such simple stacks of spectra the subtle changes of spectral features, such as development of shoulders and peak position shift, and their complex relationships with respect to pressure and temperature, i.e., sequential order of intensity changes. Especially, in three-way data analysis, this task becomes even more acute in elucidating the difference

in the pressure-dependent behavior among stacks of the spectra. For example, it is obviously difficult to sort out the difference in the way the spectral feature changes between Fig. 3(A) and (B). This is true even for a skilled spectroscopist who may eventually discover such hidden features directly from one-dimensional spectra. Thus, the application of 2D correlation analysis becomes useful to elucidate such subtle but pertinent information present in the three-way data.

#### 4.2. 2D correlation analysis of pressure-induced variation of OA

Figure 4(A) represents partial synchronous correlation spectrum calculated from the pressure-dependent IR spectra collected at 20 °C. In the 2D correlation spectrum, negative correlation regions are shaded. Note that partial 2D correlation analysis in this case was carried out along the applied pressure direction, while keeping the level of temperature constant, e.g., at 20 °C. In Fig 4(A), auto-correlation peaks develop at 1740, 1714 and 1690  $\text{cm}^{-1}$  along the diagonal line, suggesting the predominant spectral intensity variation induced by the applied pressure. One can also find the generation of cross peaks at 1714 and 1690  $\text{cm}^{-1}$  along the 1740  $\text{cm}^{-1}$  coordinate. The negative peak between 1740 and 1690  $\text{cm}^{-1}$  indicates that spectral intensity changes at 1740 and 1690  $\text{cm}^{-1}$  occur in the opposite direction, reflecting the development of the crystalline

structure at the expense of the monomer. More importantly, the positive cross peak at 1740 and 1710  $\text{cm}^{-1}$  suggest the presence of a specific component showing the decrease in the quantity during the compression. The development of the cross peak probably reveals that the transition from the monomer to the crystalline structure essentially goes through an intermediate state. In fact, the band observed at 1710  $\text{cm}^{-1}$  is assignable to the dimers with disordered orientation [17]. It is interesting to point out that the variation of such dimer band is not readily identified in the simple stack of the spectra (Fig. 3(A)) because of the highly overlapped peaks. Consequently, it is demonstrated that the difference in the structure can be advantageously utilized to differentiate between overlapped bands in the 2D correlation analysis. The observation above reveals that the compression leads to the decrease in the monomers and dimers. Such decrease is compensated with the development of the liquid crystals in which the dimers are tightly packed together. It is likely that the development of the liquid crystals occurs via the packing of the dimer units during the compression.

Fig. 4(B) represents the corresponding partial asynchronous correlation spectrum. It is noted that the partial asynchronous correlation spectrum shows a cross peak between 1740 and 1690  $\text{cm}^{-1}$ . The appearance of the asynchronous correlation peak indicates that the change in the crystal band predominantly occurs before that in the monomer

band. Another notable observation in the partial asynchronous correlation spectrum is the appearance of a cross peak between 1714 and 1690  $\text{cm}^{-1}$ , revealing that the intensity changes of the crystalline bands occurs before that of the monomer band. Consequently, sequential order becomes as: liquid crystal  $\rightarrow$  dimer  $\rightarrow$  monomer.

The development of cross peaks between the dimer and liquid crystal becomes especially important. For example, if the decrease in the dimer is completely compensated with the development of the liquid crystalline structure, one should expect to see only the development of a negative synchronous correlation peak but no asynchronous correlation [24-26]. Thus, the appearance of the asynchronous correlation peak, in turn, indicates the presence of additional contribution delaying the variation of the dimer band. It is thus very likely that a change in the pressure produces irregular and disproportionate compositional change in OA, resulting in complex spectral changes. In fact, the development of the synchronous and asynchronous correlation peaks between the monomer and dimer bands suggests that the compression eventually results in the decrease in the monomer and corresponding variation of the dimer. In other words, it indicates that the decrease in the dimers by the evolution of the crystalline structure is partially compensated with the association of the monomers. This provides the apparent delay in the rate of the reduction of the

dimer. Accordingly, the variation of the monomer should substantially lag behind that of the liquid crystal. Altogether, an even clearer picture can be drawn as shown in Fig.

5. The compression first generates the crystalline structure by aggregating the dimers.

Further compression induces the development of the dimer via the assembling of the isolated monomers, providing the delay in the decrease of the dimer content.

#### 4.3. *Effect of temperature on association dynamics*

We have so far explored the pressure-induced variation of the OA molecules at 20 °C. It is to be noted here that the association dynamics of the molecules may also be influenced by the change in the temperature. Thus, it becomes important to probe the variation of the 2D correlation feature caused by the increase in the temperature. Figure 6 represents (A) partial synchronous and (B) partial asynchronous correlation spectrum, respectively, calculated from the set of pressure-dependent IR spectra of the OA measured at 40 °C. The entire patterns observed in the synchronous and asynchronous correlation spectra result in similar to those measured at 20 °C, suggesting that the association dynamics is mostly governed by the seemingly similar mechanism.

In partial 2D correlation analysis, it is often useful to plot the correlation intensity of specific peak along  $q$ -perturbation (i.e., temperature) direction to extract subtle but

worthwhile variation. For example, Fig. 7 shows partial asynchronous correlation intensities of the cross peaks at the coordinates: (1740, 1690) and (1714, 1690), respectively. The asynchronous correlation intensity between the monomer and dimer bands can be interpreted as a measure of the predominant reduction of the disordered dimers. The gradual decrease of the asynchronous correlation intensity between the monomer and dimer suggests that the increase in temperature substantially works to decrease in the dissimilarity in the pressure-induced variation. On the other hand, that between the dimer and crystalline bands represents the degree for the predominant development of the ordered liquid crystalline structure. The increase in the asynchronous correlation intensity with temperature can be interpreted as the increase in the dissimilarity in the pressure-induced variation between the dimer and crystal. In other words, the increase in the temperature essentially restrict the generation of the crystalline structure despite the development of the dimer via the association of the isolated monomers. The delay in the development of the crystalline structure then should result in the greater dissimilarity between the dimer and crystal. Now it becomes obvious to consider the effect of the change in temperature on the association dynamics. It is very likely that the increase in the temperature predominantly increases the segmental motion of the alkyl chain. The variation of the segmental motion in turn



disturbs the development of the crystalline structure consisting of tightly packed dimers. In fact, this result agrees well with the fact that quasismectic liquid crystal of OA gradually goes through the transient transition to develop isotropic liquid consisting of disordered dimer units. Consequently, the observation above effectively reveals that the association kinetics of the OA induced by the applied pressure is substantially influenced by the change in temperature due to the segmental motion of the alkyl chain.

## 5. Conclusion

Multiple-perturbation 2D correlation analysis, coupled with ATR-IR measurements under varying pressure and temperature, yields an interesting result on the structure variation of OA. The synchronous and asynchronous 2D correlation spectra reveal the evolutionary development of the liquid structure caused by the multiple perturbations.

The intensity increase of the IR band at  $1690\text{ cm}^{-1}$  due to the formation of liquid crystalline structure consisting of tightly packed dimer units occurs at the earlier stage of the compression. The steady decrease of the band intensity at  $1714\text{ cm}^{-1}$  associated with the dimer continues a latter stage. Further compression eventually results in the predominant decrease of the band intensity at  $1740\text{ cm}^{-1}$  attributed to the contribution

from the isolated monomer. In all, these results reveal the seemingly complicated variation of OA induced by the pressure. Namely, the compression first generates the crystalline structure by aggregating the disordered dimer units. Further compression induces the development of the dimer via the assembling of the isolated monomers.

In addition, the detailed analysis of the variation of the partial asynchronous correlation intensities induced by the change in temperature reveals the way the association kinetics is influenced by temperature. The variation of the correlation intensities indicates that the increase in the temperature essentially restrict the generation of the liquid crystalline structure due to the increased segmental motion of the alkyl chain.

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**Figure captions**

Figure 1. Schematic illustration of 2D correlation analysis for two-way and three-way data sets.

Figure 2. Schematic illustration of pressure- and temperature dependent ATR-IR measurement.

Figure 3. Pressure-dependent IR spectra of OA measured at (A) 20 °C and (B) 40 °C.

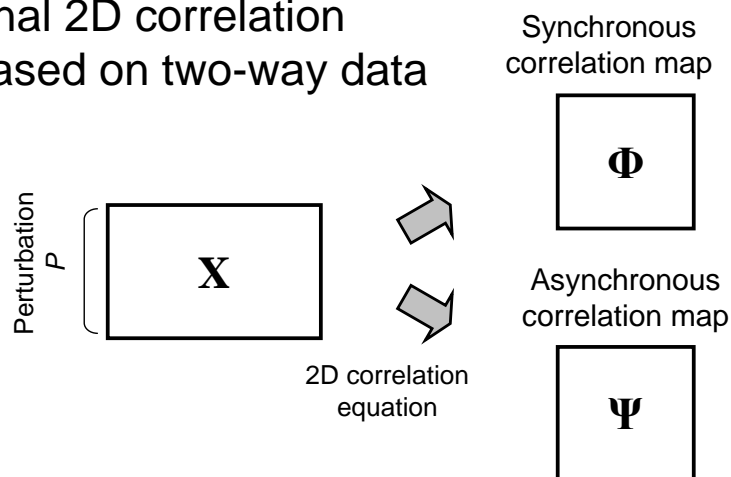
Figure 4. (A) Partial synchronous and (B) asynchronous correlation spectra calculated from pressure-dependent IR spectra of OA collected at 20 °C.

Figure 5. Schematic growth process of liquid crystals during compression.

Figure 6. (A) Partial synchronous and (B) asynchronous correlation spectra calculated from pressure-dependent IR spectra of OA collected at 40 °C.

Figure 7. Partial asynchronous correlation intensities of the cross peaks at the coordinates (1740, 1714) and (1714, 1690).

### Conventional 2D correlation analysis based on two-way data



### Multiple-perturbation 2D correlation analysis based on three-way data

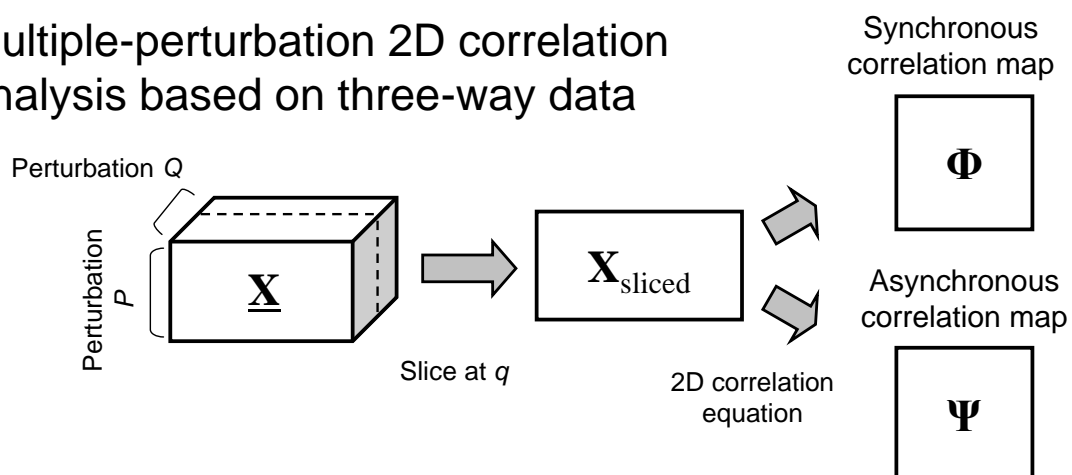


Figure 1. Schematic illustration of 2D correlation analysis for two-way and three-way data sets.

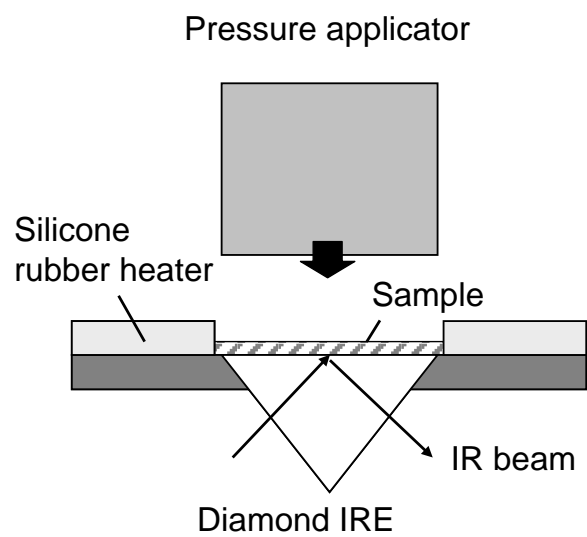


Figure 2. Schematic illustration of pressure- and temperature dependent ATR-IR measurement.



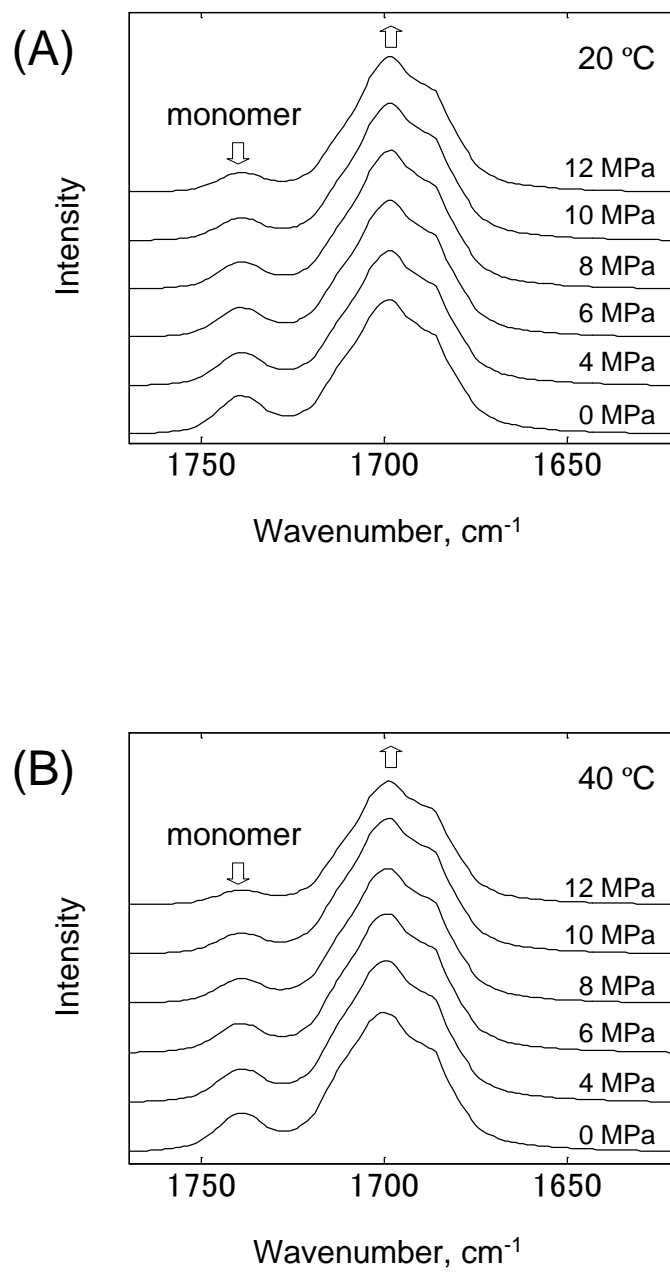


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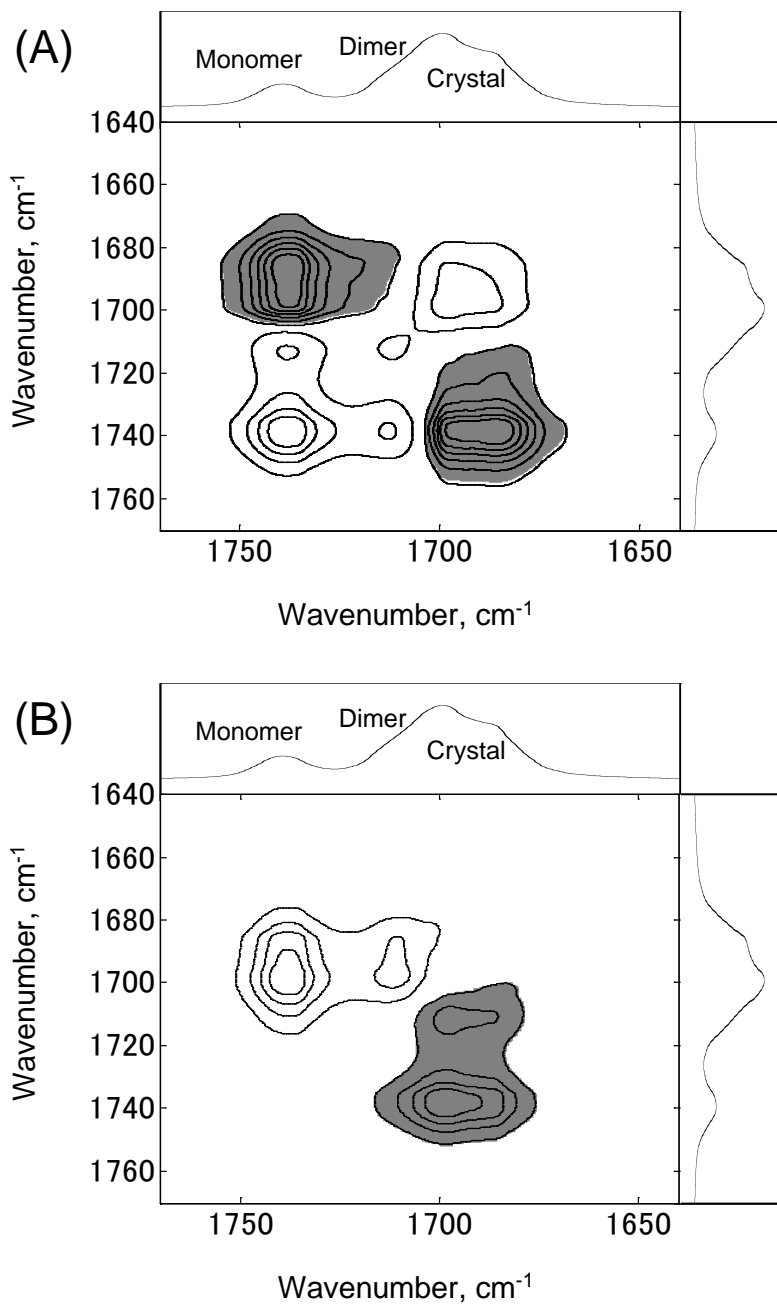


Figure 4. (A) Partial synchronous and (B) asynchronous correlation spectra calculated from pressure-dependent IR spectra of OA collected at 20 °C.

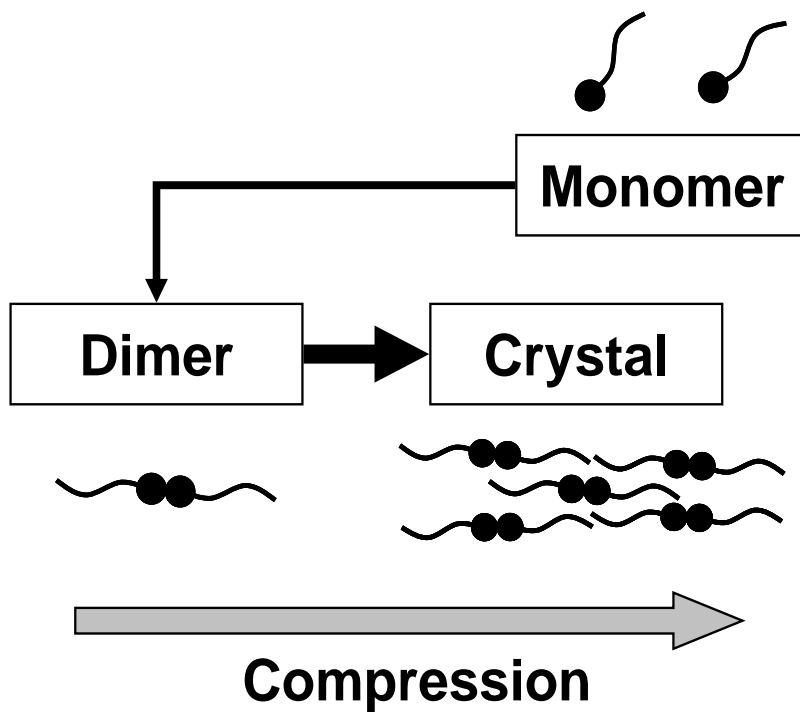


Figure 5. Schematic growth process of liquid crystals during compression.

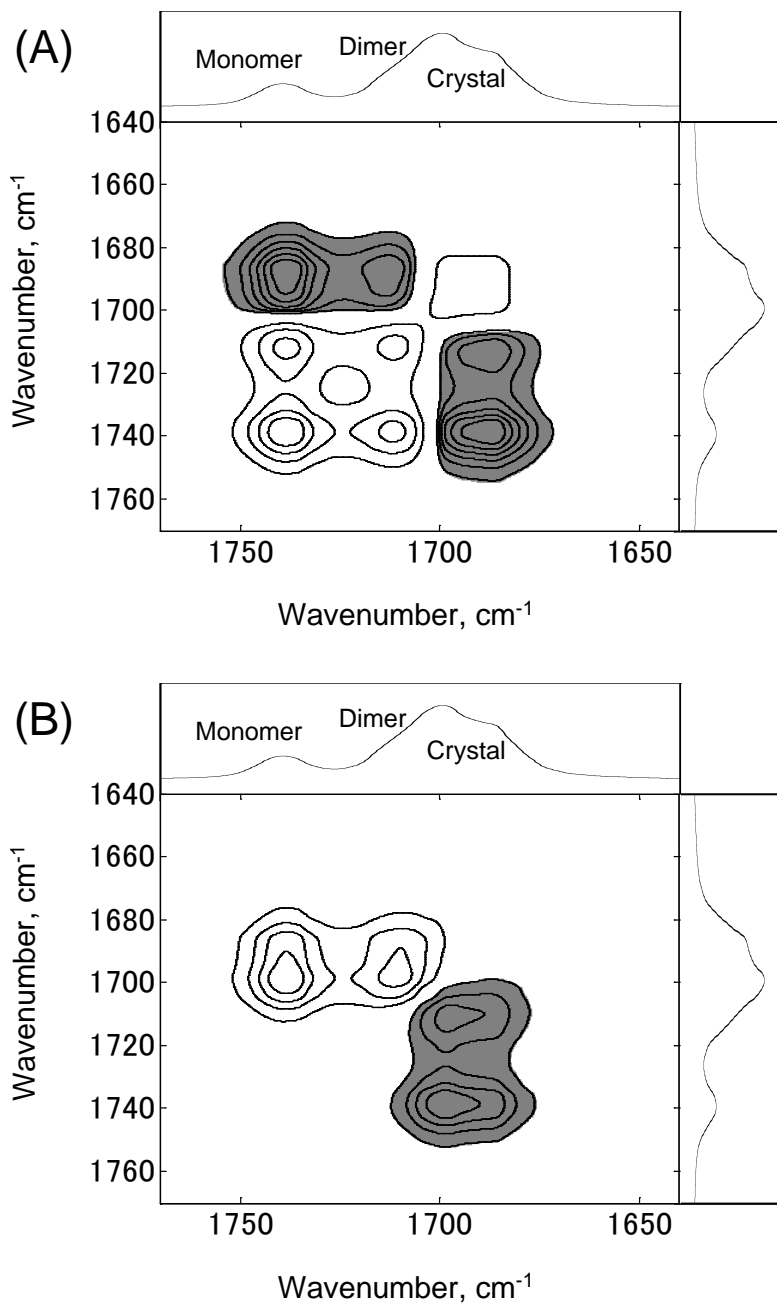


Figure 6. (A) Partial synchronous and (B) asynchronous correlation spectra calculated from pressure-dependent IR spectra of OA collected at 40 °C.

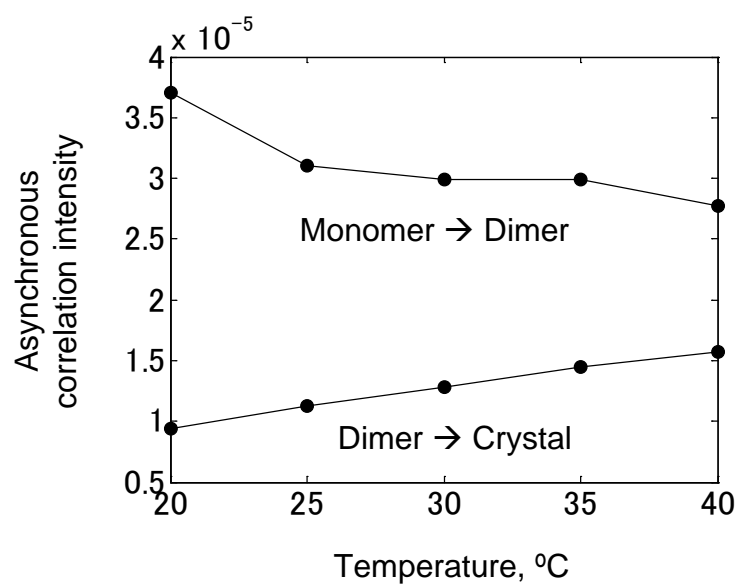


Figure 7. Partial asynchronous correlation intensities of the cross peaks at the coordinates (1740, 1714) and (1714, 1690).