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Two-Dimensional Correlation Analysis of Temperature-Dependent FT-IR Spectra of Oleic Acid

ABSTRACT In the present study, variable temperature FT-IR spectroscopic investigations were used to characterize the spectral changes in oleic acid during heating oleic acid in the temperature range from −30°C to 22°C. In order to extract more information about the spectral variations taking place during the phase transition process, 2D correlation spectroscopy (2DCOS) was employed for the stretching (C=O) and rocking (CH2) band of oleic acid. However, the interpretation of these spectral variations in the FT-IR spectra is not straightforward, because the absorption bands are heavily overlapped and change due to two processes: recrystallization of the γ-phase and melting of the oleic acid. Furthermore, the solid phase transition from the γ- to the α-phase was also observed between −4°C and −2°C. Thus, for a more detailed 2DCOS analysis, we have split up the spectral data set in the subsets recorded between −30°C to −16°C, −16°C to 10°C, and 10°C to 22°C. In the corresponding synchronous and asynchronous 2D correlation plots, absorption bands that are characteristic of the crystalline and amorphous regions of oleic acid were separated.

KEYWORDS 2DCOS, generalized 2D correlation spectroscopy, oleic acid, phase transition, variable-temperature FT-IR spectroscopy

INTRODUCTION

Oleic acid is a monounsaturated omega-9 fatty acid found in various animals and vegetable sources. It is the major unsaturated fatty acid of stratum corneum (SC), the outermost layer of human skin. SC lipid bilayers consist mainly of three fractions: namely, ceramides, free fatty acids, and cholesterol and its derivatives. It is known that the degree of unsaturation of fatty acid chains in membrane lipids has a profound effect on membrane fluidity. The saturated form of this acid is stearic acid. Oleic acid occurs naturally in greater quantities than any other fatty acid. It is present as glycerides in most fats and oils. High concentrations of oleic acid can lower the cholesterol level in the blood. Oleic acid is one of the major components of oils (olive, castor, and peanut oil) used in pharmacy and medicine. Furthermore, oleic acid is widely used in pharmaceutics as an ingredient.
of semisolid formulations (e.g., ointments, lotions, emulsions, and gels). However, ingestion of large amounts of oleic acid would produce unpleasant gastrointestinal disorders. Oleic acid crystallizes in two polymorphic phases, \( \alpha \) (mp 13.3°C) and \( \beta \) (mp 16.6°C), depending on the crystallization condition. The \( \alpha \)-phase is obtained by rapid cooling and the \( \beta \)-phase by extremely slow crystallization. Another polymorphic named the \( \gamma \)-phase is also obtained by cooling the \( \alpha \)-phase below \(-4^\circ\text{C}\). Previous FT-IR and Raman spectroscopic investigations of oleic acid\(^{[2–4]}\) suggested that the phase transition between \( \gamma \) and \( \alpha \) is of an order–disorder type and, in the \( \alpha \)-phase, the segment of the alkyl chain on the methyl side of the double bond undergoes extensive conformational disorder, while the segment on the carboxyl side of the double bond maintains an all-trans conformation. These changes are clearly reflected in the position as well as intensity of the \text{C}=\text{O} \) and \text{CH}_2 \) modes. Hence, these modes are used for analysis.

Literature survey reveals that to the best of our knowledge, to date no one has applied the present technique to study the structural variations taking place during the heating process of oleic acid. A lot of study has been done for the phase transition of oleic acid, but all those studies are based 1D analysis. Through present measurements, not only are we able to monitor the crystallinity and conformational changes in heating/cooling cycles, but we are also able to assign the state of order of specific absorption bands in the investigated spectra. FT-IR spectroscopy in combination with variable temperature measurements is a powerful analytical technique to analyze crystallinity or conformational changes of solids (e.g., polymers).\(^{[5–7]}\) Nevertheless, FT-IR spectra are often complicated, but in such cases, 2D correlation spectroscopy (2DCOS) is a very powerful tool to overcome this weakness and allow one to investigate overlapping absorption bands.\(^{[6–10]}\)

Previously FT-IR and Raman spectroscopic investigations have been applied to oleic acid.\(^{[3,11–16]}\) From these measurements, it has already been reported that the solid state phase transition between \( \gamma \)- and \( \alpha \)-modification is observed at \(-2.2^\circ\text{C}\).\(^{[5]}\) In continuation to our earlier work on spectroscopic studies of oleic acid,\(^{[17]}\) our present study focuses on the differences in the structural and phase transition changes of oleic acid during heating beyond the melting point. In order to separate the overlapping \( \nu(\text{C}=\text{O}) \) and \( \rho(\text{CH}_2) \) absorptions of the crystalline and amorphous regions, we applied generalized 2DCOS.

**EXPERIMENTAL**

**Sample Preparation and Instrumentation**

Oleic acid has been sandwiched between two NaCl disks and this sandwich was mounted in a variable-temperature cell with a temperature controller unit of Hotset RR 210 (Luedenscheid, Germany). The sample was then cooled down from room temperature to \(-30^\circ\text{C}\). From this temperature the sample was heated in increments of 2°C over the temperature range \(-30^\circ\text{C}\) to \(22^\circ\text{C}\). After each increment the cell was maintained at that temperature for 3 min to equilibrate the sample and the FT-IR spectrum of oleic acid was measured on a Bruker IFS 88 FT-IR spectrometer (Ettlingen, Germany) equipped with a mercury cadmium telluride (MCT) detector. The FT-IR spectra were measured with a spectral resolution of 4 cm\(^{-1}\) and 16 scans.

**Generalized Two-Dimensional Correlation Spectroscopy (2DCOS)**

Generalized 2D correlation spectroscopy, which was first proposed by Noda, has a characteristic pair of synchronous \( \Phi(v_1, v_2) \) and asynchronous \( \Psi(v_1, v_2) \) 2D correlation spectra in which the two spectral variables \( v_1 \) and \( v_2 \) are wavenumbers. Detailed information for the evaluation of synchronous and asynchronous 2D correlation spectra can be obtained by applying Noda’s rule.\(^{[8–10]}\)

The 2D IR technique was originally employed to analyze data obtained from infrared rheo-optical studies of polymers. These studies were designed to probe molecular level responses of samples undergoing flow, deformation, and subsequent relaxations.\(^{[18–21]}\) The 2D IR spectroscopy based on such strain-induced dynamic IR measurements has been successfully used to probe the segmental dynamics of macromolecules.\(^{[18]}\) Some notable features of the two-dimensional approach are the simplification of complex spectra consisting of many overlapped peaks; enhancement of spectral resolution by...
spreading peaks over the second dimension; and identification of various inter- and intramolecular interactions through selective correlation of IR peaks. The appearance of positive and negative cross-peaks located at the off-diagonal positions of a 2D spectrum indicates various forms of correlation features among IR bands. Correlation among bands that belong to, for example, the same chemical group, or groups interacting strongly, can be effectively investigated by 2D spectra.

For the 2DCOS analysis the FT-IR datasets were extracted and only the region of the $\nu$(C=O) and $\rho$(CH$_2$) absorption band in the wavenumber region 1740–1660 cm$^{-1}$ and 650–750 cm$^{-1}$ was used. Furthermore, all FT-IR spectra were baseline corrected prior to the 2DCOS analysis. The calculations of 2D correlation spectra were performed by using the 2Dshige software (http://sci-tech.ksc.kwansei.ac.jp/~ozaki/2D-shige.htm).

RESULTS AND DISCUSSION

Temperature-Dependent Changes in the $\nu$(C=O) Band Region

Figure 1 shows the FT-IR spectra as a function of temperature in the 1740–1660 cm$^{-1}$ region. The band located at 1688 cm$^{-1}$ is intense at −30°C and its intensity decreases with increasing temperature. This particular band is responsible for the stretching of the C=O group in both the $\alpha$- and $\gamma$-form of oleic acid. This band is characteristic for the crystalline form of the oleic acid. Beyond the melting, a band at 1711 cm$^{-1}$ appears, which is specific for the amorphous regions and reaches its highest intensity at 22°C.

To analyze the changes in crystallinity of the $\gamma$- and $\alpha$-phases of oleic acid during the temperature variation, a crystallinity index was derived from the peak area intensity in the 1688–1710 cm$^{-1}$ wavenumber range. The low-wavenumber crystalline wing of the $\nu$(C=O) absorption was integrated from 1689 to 1662 cm$^{-1}$ and the amorphous wing from 1740 to 1709 cm$^{-1}$ applying a baseline from 1740 to 1662 cm$^{-1}$. The crystallinity index for the heating process of oleic acid in the temperature region from −30°C to 22°C is shown in Figure 2. In the temperature range −30°C to −16°C, the crystallinity of the $\gamma$-phase of oleic acid increases due to recrystallization. Between −16°C and −10°C, the crystallinity decreases only slightly, but from −6°C to 0°C a sharp step is observed and can be identified as a solid-phase transition between the $\gamma$- and the $\alpha$-phase. Upon further heating the melting process of oleic acid is accompanied by a drastic decrease of the crystallinity index between 10°C and 16°C.

Generalized Two-Dimensional Correlation Spectroscopy

2DCOS analysis was employed to confirm the observed changes during recrystallization of the $\gamma$-phase, the phase transition from $\gamma$ to $\alpha$, and finally the melting of oleic acid and possibly to detect further changes from the synchronous and
asynchronous spectra. For this purpose the experimental temperature range from \(-30^\circ\text{C}\) to \(22^\circ\text{C}\) has been split up into the subranges \(-30^\circ\text{C}\) to \(-16^\circ\text{C}\), \(-16^\circ\text{C}\) to \(10^\circ\text{C}\), and \(6^\circ\text{C}\) to \(22^\circ\text{C}\). Figures 3, 4, and 6 show the synchronous (A) and asynchronous (B) 2D correlation spectra based on the spectral changes in the wavenumber region from \(1740\) to \(1660\) cm\(^{-1}\). In the 2D correlation spectra positive peaks appear white and negative peaks are gray-shaded.

In the synchronous 2D COS spectrum of the temperature range \(-30^\circ\text{C}\) to \(-16^\circ\text{C}\) (Fig. 3A), two autopeaks are developed at around \((1697, 1687)\) cm\(^{-1}\) and \((1697, 1697)\) cm\(^{-1}\) together with two negative cross-peaks at \((1697, 1687)\) cm\(^{-1}\), indicating the spectral variations of the amorphous and crystalline components during the recrystallization of the \(\gamma\)-phase of oleic acid. In the corresponding asynchronous 2D COS spectrum (Fig. 3B), two pairs of cross-peaks are observed at \((1697, 1687)\) cm\(^{-1}\) and \((1716, 1687)\) cm\(^{-1}\). It should be noted that the asynchronous 2D correlation spectrum resolves two absorption bands at \(1716\) cm\(^{-1}\) and \(1697\) cm\(^{-1}\) arising from the amorphous regions. Thus, the band at \(1716\) cm\(^{-1}\) is assigned to the \(\text{C}=\text{O}\) stretching mode of low-order amorphous regions (less hydrogen bonded), whereas the band at \(1697\) cm\(^{-1}\) is characteristic for amorphous regions with some structural order (more hydrogen bonding). According to the rule of Noda,\(^{[8–10]}\) the cross-peak at \((1697, 1687)\) cm\(^{-1}\) changes sign and indicates that the amorphous regions with some structural order (1697 cm\(^{-1}\)) change prior to the crystalline regions (1687 cm\(^{-1}\)). Based on the cross-peak at \((1716, 1687)\) cm\(^{-1}\), the crystalline regions (1687 cm\(^{-1}\)) are formed before the low-order amorphous regions recrystallize.

Figure 4A shows the synchronous 2D correlation spectrum in the temperature range \(-16^\circ\text{C}\) to \(10^\circ\text{C}\).

Similarly to the recrystallization process, only two autopeaks at about 1685 and 1697 cm\(^{-1}\) together with negative cross-peaks at (1697, 1685) cm\(^{-1}\) are observed. This is characteristic of the opposite intensity variation of the \(\nu(\text{C}=\text{O})\) bands of the crystalline and amorphous states of oleic acid. The asynchronous 2D correlation spectrum (Fig. 4B) shows one negative cross-peak at \((1697, 1685)\) cm\(^{-1}\), which reflects a correlation between the ordered amorphous band (1697 cm\(^{-1}\)) and the crystalline absorption band (1685 cm\(^{-1}\)). Thus, this cross-peak changes sign\(^{[8–10]}\) and indicates that the band of the ordered amorphous regions changes before the band of the crystalline regions. In contrast to the recrystallization process, in this temperature range the intensity of the characteristic crystalline absorption band decreases, whereas the intensity of the absorption band of the amorphous regions increases. Furthermore, the solid-phase transition from the \(\gamma\)- to the \(\alpha\)-modification takes place between \(-4^\circ\text{C}\) and \(-2^\circ\text{C}\). For this reason, the intensity changes of the \(\rho(\text{CH}_2)\) rocking vibration were monitored in the wavenumber region \(744\)–\(678\) cm\(^{-1}\). Figure 5 shows the temperature-dependent FT-IR spectra in the \(\gamma\)-phase recrystallization range \((-30^\circ\text{C}\) and \(-16^\circ\text{C}\), Fig. 5A), in the \(\gamma\)- to \(\alpha\)-phase transition range \((-6^\circ\text{C}\) to \(0^\circ\text{C}\), Fig. 5B), and for the melting process \((0^\circ\text{C}\) and \(16^\circ\text{C}\), Fig. 5C) for the \(\rho(\text{CH}_2)\) rocking vibration region. From the intensity changes observed in these figures, the absorption bands can be assigned to the different phases outlined in Table 1.

For the melting process of oleic acid in the temperature range from \(6^\circ\text{C}\) to \(22^\circ\text{C}\), the synchronous 2D correlation spectrum (Fig. 6A) displays two autopeaks at about (1689, 1689) cm\(^{-1}\) and (1710, 1710) cm\(^{-1}\) together with corresponding negative cross-peaks at (1710, 1689) cm\(^{-1}\).
This cross-peak indicates the opposite spectral changes of the crystalline (1689 cm\(^{-1}\)) and amorphous (1710 cm\(^{-1}\)) regions of the \(\nu(C=O)\) stretching mode. The asynchronous 2D correlation spectrum (Fig. 6B) shows two cross-peaks at (1689, 1679) cm\(^{-1}\) and (1710, 1689) cm\(^{-1}\). With the cross-peak (1689, 1679) cm\(^{-1}\), the asynchronous 2D correlation spectrum resolves the two highly overlapped bands at about 1689 and 1679 cm\(^{-1}\), which arise from the crystalline regions. Additionally, this cross-peak indicates that the absorption band at 1689 cm\(^{-1}\) changes prior to the band at 1679 cm\(^{-1}\). Thus, the band at 1689 cm\(^{-1}\) may be assigned to the \(\nu(C=O)\) stretching mode of the less ordered crystalline regions of oleic acid, whereas the band at 1679 cm\(^{-1}\) is characteristic of the same vibrational mode in highly ordered crystalline regions. Because \(\Phi\) (1710, 1689) is <0, the asynchronous cross-peak \(\Psi\) (1710, 1689) >0 changes sign and becomes negative.\[^{8-10}\] This indicates that the less ordered crystalline regions (1689 cm\(^{-1}\)) change before the less ordered amorphous domains (1710 cm\(^{-1}\)).

### Temperature-Dependent Changes in the (CH\(_2\)) Band Region

In the synchronous 2DCOS spectrum in the temperature range 6°C to 22°C (Fig. 7A), positive peaks

**TABLE 1** Assignments of FT-IR Bands for the \(\nu(CH_2)\) Vibration Region in the Temperature Range −30°C to 22°C of Oleic Acid

<table>
<thead>
<tr>
<th>Phase</th>
<th>(\gamma)</th>
<th>(\alpha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavenumber</td>
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<td>cryst</td>
</tr>
<tr>
<td>688</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>701</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>725</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>736</td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

**FIGURE 5** FT-IR spectral variations for the recrystallization of \(\gamma\)-phase (A), for the \(\gamma\rightarrow\alpha\)-phase transition (B), and the melting process of \(\alpha\)-phase (C) in the wavenumber region 744–688 cm\(^{-1}\) of oleic acid.

**FIGURE 6** (A) Synchronous and (B) asynchronous 2D correlation spectra of the \(\nu(C=O)\) asynchronous 2D correlation band for the melting process (6°C to 22°C) of oleic acid.

**FIGURE 7** (A) Synchronous and (B) asynchronous 2D correlation spectra of the \(\nu(CH)\) stretching band for the melting process (6°C to 22°C) of oleic acid.
are developed at around (680, 680) cm\(^{-1}\) and (700, 710) cm\(^{-1}\) with additional peaks. In this 2DCOS spectrum, no negative peaks are observed. It is a very complicated 2DCOS spectrum as compared to the C=O stretching vibration 2DCOS spectrum at the same temperature. In the corresponding asynchronous 2DCOS spectrum (Fig. 7B), negative cross-peaks are observed at (680, 700) cm\(^{-1}\) and (680, 725) cm\(^{-1}\); corresponding positive autopeaks are observed at (680, 700) cm\(^{-1}\) and (740, 720) cm\(^{-1}\). These additional bands are characteristic of the rocking mode of CH\(_2\) indicating the spectral variations of the amorphous and crystalline components during the recrystallization of the phase of oleic acid. The intensity of the background underlying these bands is relatively low so that, as indicated above, we may conclude that the disorder is in \(\alpha\)-phase.

In the synchronous 2DCOS spectrum of the temperature range \(-30^\circ\text{C}\) to \(-16^\circ\text{C}\) (Fig. 8A), autopeaks (positive) are developed at around (687, 687) cm\(^{-1}\) and negative cross-peaks at (697, 687) cm\(^{-1}\). These observations indicated the spectral variations of the amorphous and crystalline components during the recrystallization of the phase of oleic acid.

In the corresponding asynchronous 2DCOS spectrum (Fig. 8B), cross-peaks are observed at (680, 687) cm\(^{-1}\) and (687, 720) cm\(^{-1}\). It should be noted that the asynchronous 2D correlation spectrum resolves four absorption bands at (700, 720) cm\(^{-1}\), (700, 720) cm\(^{-1}\), (700, 680) cm\(^{-1}\), and (720, 740) cm\(^{-1}\).

**CONCLUSIONS**

The structural changes of oleic acid in the temperature range \(-30^\circ\text{C}\) to \(22^\circ\text{C}\) were studied by variable-temperature FT-IR spectroscopy and subsequently analyzed by 2DCOS analysis. Through our analysis we found that 2DCOS results are exposing hitherto unseen features and will lead us to new levels of quantitative analysis of vibrational spectra and their relation to structural dynamics in condensed phases.

The \(\nu(\text{C}=\text{O})\) absorption band region and partly the \(\nu(\text{CH}_2)\) absorption region were employed to study the structural changes as a function of temperature.

In the temperature range from \(-30^\circ\text{C}\) to \(-16^\circ\text{C}\), oleic acid occurs in the \(\gamma\)-phase and recrystallizes during heating to a perfectly ordered structure. The solid-state transition between the \(\gamma\)- and \(\alpha\)-modification was observed between \(-6^\circ\text{C}\) and

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**FIGURE 8** (A) Synchronous and (B) asynchronous 2D correlation spectra of the \(\nu(\text{CH})\) stretching band for the melting process (\(-30^\circ\text{C}\) to \(-16^\circ\text{C}\)) of oleic acid.

**FIGURE 9** (A) Synchronous and (B) asynchronous 2D correlation spectra of the \(\nu(\text{CH})\) stretching band for the melting process (\(-16^\circ\text{C}\) to \(10^\circ\text{C}\)) of oleic acid.
0°C and is characterized by the disappearance of the 701 cm⁻¹ absorption band at −2°C. Melting of oleic acid was observed in the temperature range 6°C to 22°C, with the major changes taking place between 10°C and 16°C. Furthermore, the existence of two ν(C=O) bands for the crystalline and amorphous regions were identified and the sequence of their intensity changes was determined. The two highly overlapped crystalline ν(C=O) absorption bands located at 1679 cm⁻¹ (strongly hydrogen bonded) and 1689 cm⁻¹ (less hydrogen bonded) can be assigned to well-ordered and less ordered crystalline regions, respectively. Furthermore, ν(C=O) bands of the amorphous regions with some structural order and without order could be resolved at 1697 and 1714 cm⁻¹, respectively. Finally, a detailed band assignment has been derived for the ρ(CH₃) vibration region. We found that the intense rocking bands at 688 cm⁻¹ and 701 cm⁻¹ are due to the crystalline α- and γ-form of oleic acid, respectively.

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