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X-Ray diffraction and microcalorimetry study of the $\alpha \rightarrow \beta$ transformation of tripalmitin

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Abstract

The polymorphic $\alpha \rightarrow \beta$ transformation of tripalmitin was studied using two independent methods–thermodiffractometry and microcalorimetry–in the temperature range from 25.0 to 42.5°C. DSC was used as a preliminary tool to interpret the transformation process. In the DSC-scan the transition consisted of one endothermic and two exothermic peaks, one after the other, but in the isothermal microcalorimeter measurement the transition was a resultant of these three processes. The average reaction enthalpy was −45.6 J/g and −47.0 J/g by microcalorimetry and DSC, respectively. The concentration versus time curve of β-state during the $\alpha \rightarrow \beta$ transformation was fitted using the Avrami equation. The activation energy associated with the transformation was 410 kJ/mol as measured by thermodiffractometer and 455 kJ/mol by microcalorimeter. The uniformity of the results proved that these methods were reliable tools in the study of this thermally activated process.

Keywords: Polymorphism; Kinetics; Triglycerides; X-ray diffraction; Microcalorimetry

1. Introduction

Many pharmaceutical and food products are based on triglycerides. Fatty suppositories, ointments, margarines and chocolate are typical triglyceride-based products in our every day life. Triglycerides are esters of the trihydroxy alcohol glycerol. Triglycerides have variable chain properties because three independent hydrocarbon chains can have different chain lengths and saturated or unsaturated carbon bonds. It is well known that the changes in the physical properties and chain properties go

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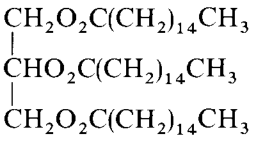
together. Therefore we chose only one triglyceride to this investigation. Tripalmitin was suitable because of its temperature behaviour.

Tripalmitin has the following molecular structure



$$\begin{array}{l} CH_2O_2C(CH_2)_{14}CH_3 \\ | \\ CHO_2C(CH_2)_{14}CH_3 \\ | \\ CH_2O_2C(CH_2)_{14}CH_3 \end{array}$$

Tripalmitin (glyceryl tripalmitate)

Triglycerides have three main polymorphic states, α, β' and β, which have different melting points. The melting points of the states for tripalmitin are 45, 55 and 65.5°C, respectively [1]. Faulty preservation causes structural changes to the triglycerides and changes in the physical properties. The common methods to stabilize the triglyceride, i.e. to prohibit the polymorphic transition, are to hydrogenate the unsaturated bonds and to add diglycerides.

The orientation of the monoacid triglyceride molecules in the β-state is shown in Fig. 1. The molecules are arranged in a tail to tail "tuning fork"-conformation [2]. The

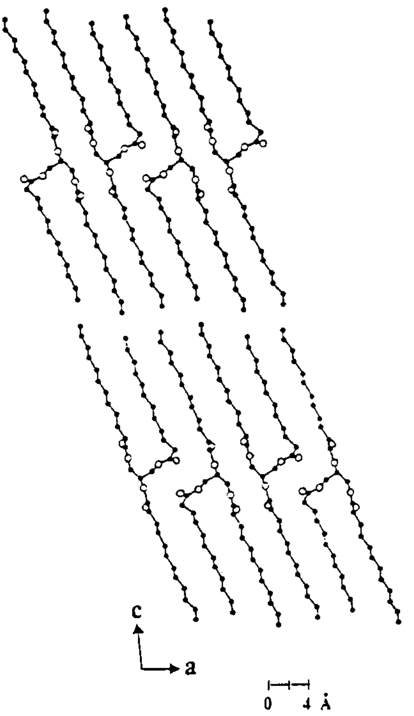


Fig. 1. The molecular arrangement of a monoacid triglyceride trilaurin in the β-state as seen along the short unit cell axis [2]. The lattice is formed of wide lamelles lying opposite to the paper. α- and β'-states are quite similar with this structure. Trilaurin differs from tripalmitin in the chain length only.

α-and β'-states retain this structure but the lattice parameters are different. The characteristic features of all states are the long interplanar spacing of approximately 40 Å and short spacing(s) of about 4 Å. These dimensions correspond to the longitudinal dimension of the molecule and the short distance between adjacent molecules, respectively.

The stability of the α-state decreases exponentially with increasing temperature. Triglycerides undergo a thermally activated transition $\alpha \rightarrow \beta' \rightarrow \beta$ from amorphous to crystalline state. The β'-state is an intermediate state with a limited temperature range of stability [3].

Previous authors have concluded that the transition is polymorphic in character if the initial and the final products have different crystallographic forms and melting points [1,4–10]. For example Coben et al. [11] and Laine et al. [12] found that in triglycerides the transition was not entirely polymorphic since the α-state had no distinct lattice parameters; there was no crystalline state before the transition. The difference between α-state and liquid state is mainly in the mobility of the molecules. The initial α-state was amorphous and the average distance between near neighbour was 4.14 Å which was the only short interplanar spacing obtained from X-ray diffraction measurements. The molecule has a high free energy and the transition towards a lower energy state is favourable. The transition rate depends on temperature. The transition is an ordering process rather than a polymorphic transition in its strict sense. The final state β is still amorphous as the reflections are quite broad.

In this work the kinetics and character of the $\alpha \rightarrow \beta$ transformation is studied using two independent methods, thermodiffractometry (TXRD) and microcalorimetry (MC). Differential scanning calorimetry (DSC) was used as a preliminary tool to interpret the transition process. The activation energy was calculated by TXRD and MC. The results were compared and the reliability of both methods was discussed. This study was an important predecessor to our next project, the stability of β'-state of margarines.

2. Theoretical background

Let c_β be the concentration of the β-state in a sample containing two polymorphic states. The value of c_β can be measured as a function of storage time for example with X-ray diffractometer [13]. The absorption of X-rays is constant through the transition because the absorption depends on the molecule, not on the degree of ordering. The concentration can be obtained from the diffraction intensity I of a characteristic reflection of the investigated β-state, i.e.

$$c_\beta(t) = \frac{I_\beta(t)}{I_{\beta,\max}}. \tag{1}$$

The peak intensity of a state depends linearly on the volume fraction of that state in the sample. $I_{\beta,\max}$ is the last value of $I_\beta(t)$ measured. Eq. (1) is not valid if there is any preferred orientation (texture) during crystal growth. Texture is a typical behaviour for materials which have been treated mechanically during crystallization. In this case it can be assumed that there is no texture.

The microcalorimeter measures heat flow as a function of time [14]. The heat flow is proportional to the conversion rate $\mathrm{d}n/\mathrm{d}t$ (n is moles) and to the change of the enthalpy in the reaction ΔH_r (in J/mol), i.e.

$$\frac{dq}{dt} = -\Delta H_r . \frac{dn}{dt}. \tag{2}$$

The integration of the signal from zero to infinity gives the reaction enthalpy ΔH_r. As ΔH_t is the value of the integration from zero to time t, plotting the reaction $\Delta H_t/\Delta H_r$ as a function of time one gets for the concentration of β-state

$$c_\beta(t) = \frac{\Delta H_t}{\Delta H_r}. \tag{3}$$

The most suitable function which fitted $I(t)/I_{max}$ versus t was the sigmoidal Avrami equation [15]

$$c(t) = 1 - e^{-kt^n} \tag{4}$$

where k is the reaction rate constant and n the constant which describes the mechanism of the transition. A sigmoidal shape was also obtained in previous papers [5]. In this study we were not interested in the reaction mechanism and the corresponding constants k and n. The fit was performed to calculate the half-time $t_{1/2}$ of the transition, only. The $t_{1/2}$-value is the time when $I(t)/I_{max} = 1/2$ in Eq. 1.

The transition rate k depends on temperature T according to the Arrhenius equation [15,16]

$$k_T = k_\infty e^{-(E_a/RT)} \tag{5}$$

where k_∞ is the asymptotic transition rate, E_a the activation energy, R the molar gas constant and T the temperature. The easier form to plot the Arrhenius equation is the linear representation ln k_T versus $1/T$, or

$$\ln k_T = \ln k_\infty - \left(\frac{E_a}{R}\right)\frac{1}{T} \tag{6}$$

The reaction rate constant k is determined as the inverse of time t. By determining the half time $t_{1/2}$, at several temperatures the activation energy E_a was calculated from the slope of the straight line, using the method of least squares. The half-time was chosen instead of k because the mathematical fitting according to Eq. (4) was incomplete and the most satisfactory measure at every temperature seemed to be the half-time.

The complete Arrhenius plot is useful when predicting the stability of α-state in colder preservation temperatures. The predictions are valid at temperatures below the melting point of α-state where the mechanism of the transition is similar as in the measurement temperatures.

3. Experimental

3.1. Sample preparation

The tripalmitin sample material (puriss., grade Fluga AG) was obtained from Orion Corporation Farmos, Turku, Finland. Samples were prepared from the liquid state using fast solidification to ensure that the sample solidifies completely to the α-state. The formation of the crystal state takes a rather long time because of the low mobility of the long hydrocarbon chains.

Samples were heated 15°C above the melting point 65.5°C for at least 10 min to melt all the crystals and large nuclei, i.e. to destroy the crystal memory. Samples were placed in the measuring vessel and melted. Solidification was performed quickly after heating by removing the sample on a cold (about −15°C) plate. The sample solidified within 15–20 s. The final sample was removed to the measurement position immediately after preparation.

3.2. *Thermodiffractometry*

The transition amorphous → crystalline, or $\alpha \rightarrow \beta$, was studied by X-ray powder diffractometer with the sample temperature controller. A Philips PW 1050 angle dispersive powder diffractometry, PW 1743 generator and PW 1964 scintillation counter were used with Ni-filtered CuK_α-radiation (0.15418 nm), voltage 45 kV, current 35 mA, fixed 1° divergence slit, receiving slit 0.2° and scatter slit 1°. The 2θ-angle was calibrated with copper. The scanning was performed in 0.02° steps using 1.0 s averaging time. The total scanning time should be short, especially at high temperatures, to ensure that the β-concentration is constant during the scan.

A thermal resistor and a Pt-100 thermometer was placed inside the sampleholder. The temperature of the sampleholder was controlled by microcomputer. The temperature scale from room temperature to 200°C was measured to ± 1°C. The temporary and continuous drifting of the temperature caused by "on/off"-operation of the thermal resistor smoothed out over long time measurements so that the long-time average temperature was accurate with the adjusted temperature and the error ± 1°C was an overestimate.

In order to minimize the temperature gradient between the surface and the bottom of the sample (sample thickness = 1 mm) the sample holder was surrounded by a sealed metal box (1 dm^3). The sampleholder (10 × 40 × 40 mm) was made of copper to ensure good heat conductivity. The small residual difference in temperature between the thin surface layer of the sample and the Pt-100 did not cause errors in the determination of activation energy because the slope in Eq. (6) did not change. The position of the straight line only changes. Residual differences were corrected in the calculations comparing the reaction rates obtained with microcalorimeter data at same temperatures. The temperature accuracy of the microcalorimeter was 0.01°C. The temperature scale of the thermodiffractometer was calibrated afterwards; the temperature was about 3.5°C too low. The difference was due to the temperature gradient.

The route of the radiation beam through the box was arranged through an elongated hole which was covered by a Mylar tape. The absorption and the diffraction of the tape had little or no effect on the measurements in the 2θ range from 1 to 30°. Data collection was carried out using Philips APD 1700 program.

The sample was held in place inside the diffractometer during the measurement. This minimized errors from non-homogeneous sample (ray hits the sample always at the same position) and temperature drifting. Three separate samples were used. Some samples were used at two different temperatures for convenience.

3.3. *Profile fitting of the diffractograms*

The peak heights in Eq. (1) were determined by a Profile Fitting-program. The measured diffractometer curve was fitted with a mathematical fitting curve which consisted of background scattering and separate reflections. Fig. 2 represents an average result of a profile fitting. Firstly, the background radiation on a peak-free area was determined at 2 points. Background was a straight line which increased as 2θ increased. Secondly, the reflections on the fitted area were determined. The positions, intensities and widths of the reflections were estimated beforehand. Finally was the profile fitting itself, the aim being to fit the FP-curve as close as possible to the measured RD-curve. The fitting was considered a success if the difference between RD- and FP-curve, i.e. FR-curve, was close to the background scattering. The growth of one peak was fairly followed as the reflections and background were well separated. The

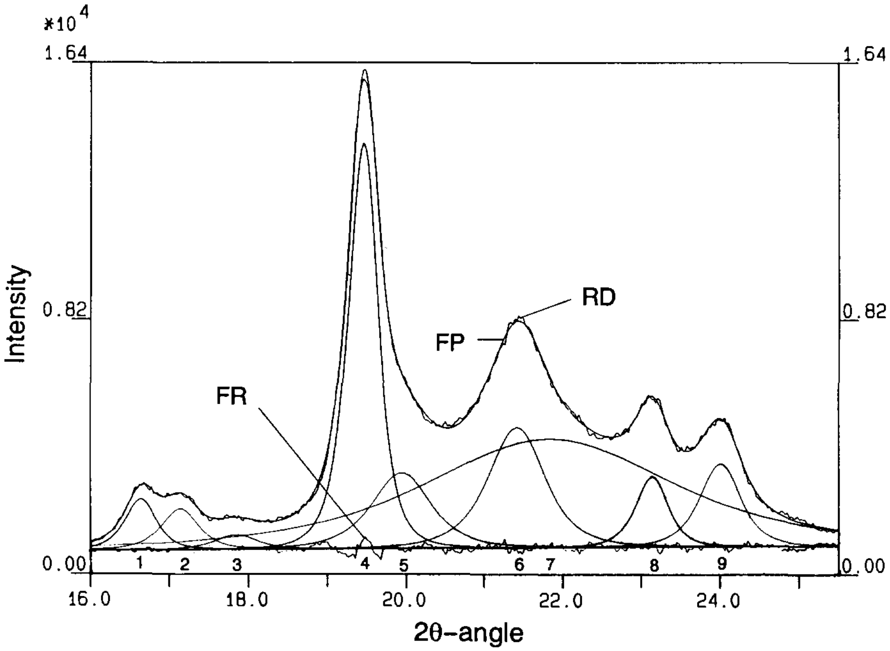


Fig. 2. The Profile Fitting-result of tripalmitin at 31.5°C when the storage time was 125.9 h. Nine separate reflections lie on the flat estimated background. Fitted profile (FP) fits well the measured rough diffractogram (RD). The residual part (FR) is the difference between FP and RD and it is oscillating around the background line.

main problem was keeping the peak positions and widths equal in successive diffractograms during a series of measurements.

3.4. *Differential scanning calorimetry*

A Perkin Elmer DSC model 7 with Thermal Analysis System Software was used. The temperature and energy calibration was performed with indium. About 1.5 mg samples were used. The sample pan was made of aluminium and the cover was perforated. The heating rate was 3 $\mathrm{K\,min^{-1}}$.

3.5. *Microcalorimetry*

A 2277 Thermal Activity Monitor, Thermometric AB, Sweden, microcalorimeter was used [17]. The samples (ca. 0.5 g) of tripalmitin were melted in the rubber sealed glass ampoule of the calorimeter and solidified as described earlier. After solidification the ampoule was placed in the calorimeter. The sample was placed for 20–25 min in the pre-equilibrium state after which it was lowered into the measurement position. The use of empty glass ampoule as a reference vessel was suitable because of the minor mass of the sample. The reference vessel was treated in the same way as sample vessel. The calibration of the instrument was carried out beforehand electrically with an inbuilt calibration resistor using empty measuring cylinders. Confirmation measurements were also made to ensure that there had been no faults in sample handling, e.g. fingerprints on the measuring ampoules, faults in weighting and leakage from the ampoules.

3.6. *Microcalorimeter data handling*

Before and after collecting the data points for the transition, the baseline was always measured. The baseline, which was assumed to be linear, was used to eliminate drift during the measurements.

The initial heat flow curves can be divided into two sections as in Fig. 3. The first large exothermic process at the very beginning of the measurement was probably due to mechanical and thermal relaxation of the cold-treated sample and ampoule. Nevertheless, there was no effect detected if the sample was cold-treated outside or inside the ampoule. This might give hints that the nucleation was a separate sequence before the growth of the crystals detected by diffractometer. The peak decreases very rapidly after which an autocatalytic curve was clearly present. The initial section was blocked out by a curve fitting procedure and the remaining autocatalytic curve was used in further analysis.

At 25 and 30°C the transition continued for hundreds of hours. The measurements were carried out in sections which were interpolated to get a connected curve. The samples were preserved in a precision oven between the actual measurement periods. The beginning and the end of the transition could not be recorded by calorimeter and extrapolation of the curve was required.

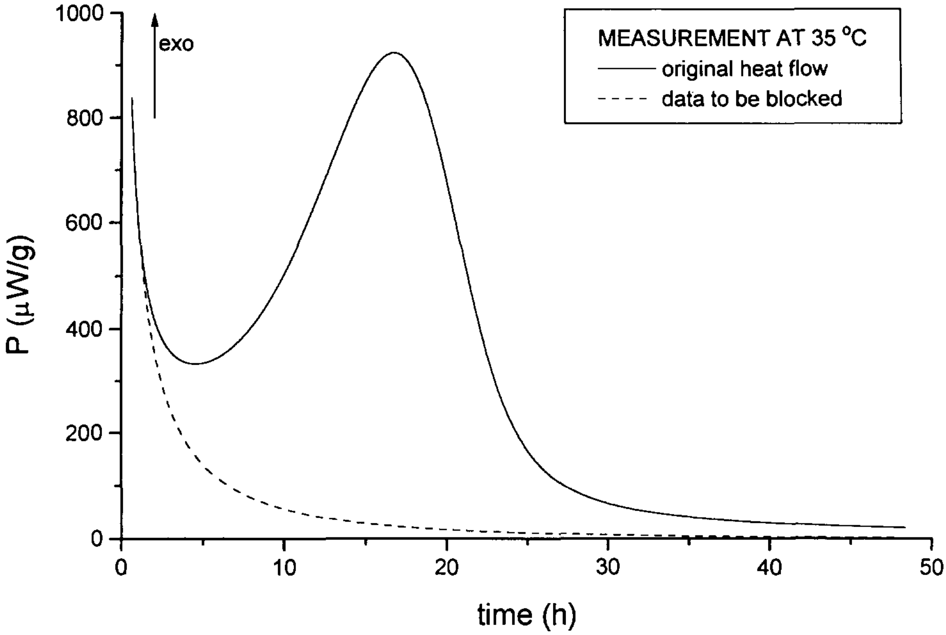


Fig. 3. In the beginning of the measured MC data (—) there was always an exothermal part (- - -) which was blocked out. The measurement was made at 35°C.

4. Results and discussion

4.1. DSC

DSC thermograms of the amorphous (α) and crystalline (β) tripalmitin are shown in Fig. 4. There was an initial endothermic transition at 44°C followed immediately by two exothermic transitions. The endothermic peak was interpreted as a separation process of molecules which consumes energy. Two following exothermic peaks were two separate ordering processes (β' and β). The resultant enthalpy of the three peaks was exothermic, $\Delta H_r = -47$J/g, as was expected for a crystallization process. The transition is monotropic, i.e. transition $\beta \rightarrow \alpha$ is not probable. The peak in Fig. 4 at 64°C is melting.

The three peaks between 44 and 53°C obtained for amorphous samples were absent for crystalline (β) samples. Crystalline material was prepared by heating above the melting point of the α-state at 50°C.

4.2. Thermodiffractometry

Thermodiffractometry measurements were performed at 6 temperatures between 31.5 and 42.5°C. The TXRD data was collected 6–12 times at each temperature. The

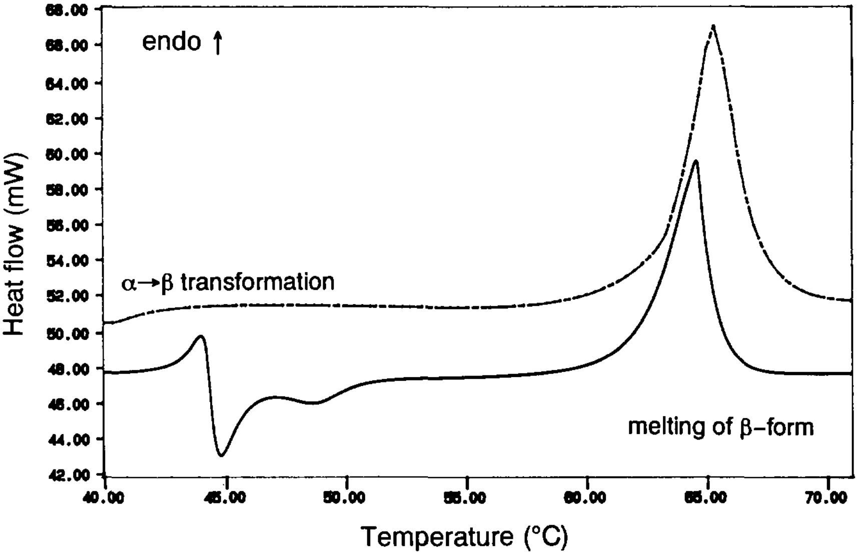


Fig. 4. DSC-thermogram of tripalmitin at α-state (lower curve) and at β-state (upper curve). The three peaks at left describe together the α→β transformation. The melting peak of β-state is at right. The transition enthalpy of the α→β transformation was −47.0 J/mol.

diffractograms of the transition at 31.5°C are shown in Fig. 5. The diffractogram is restricted to the 2θ-range 16.0–25.5°. The states observed, namely α-, β- and liquid, are shown in Fig. 6. The long spacing reflection is included. The β′-state was not observed. The assumption that the β-state is amorphous, at least partly, seems to be true since there is a wide amorphous reflection in both α- and β-states at $2\theta = 21.8°$ (see also Fig. 2).

The reflection studied was the strongest one of the β-state at $2\theta = 19.6°$. Each diffractogram was analyzed as described before and the peak height $I(t)$ at the moment t after the preparation of the sample was registered. I_{max} was the last value of $I(t)$ measured. A check was made that the last value measured had reached the end of the transition. The shape of the $I(t)/I_{\mathrm{max}}$ versus time -curve was sigmoidal as can be seen in Fig. 7. Sigmoidal shape was evident for all of the measurements. The transition rate of tripalmitin obeyed the Avrami equation which is used commonly for transition rate studies. The parameter n in Eq. (4) was between 2.1 and 2.9. Measurement results shown in Table 1 consist of temperature and the time $t_{1/2}$ when the peak height reached half the final value. Linear regression fitting according to the Eq. (6) is shown in Fig. 8. The prediction of the transition rate to lower temperatures seems to be possible as the Arrhenius plot works on the whole temperature range 31.5–42.5°C. The activation energy obtained from the slope of the straight line was (410 ± 40) kJ/mol. The error limits came from mathematical fitting error and include only partially the errors due to measurement inaccuracies.

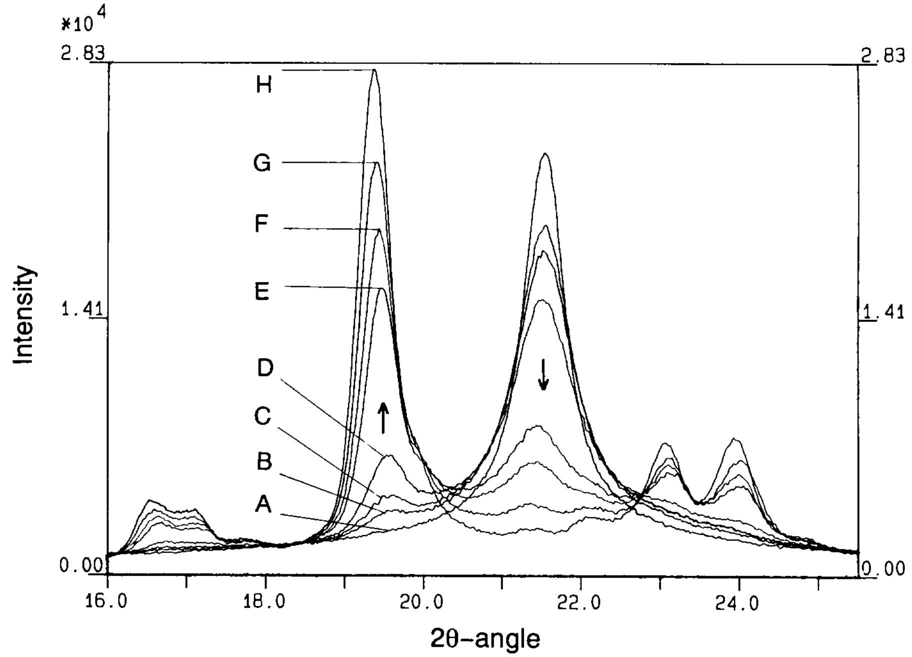


Fig. 5. The 2θ-range 16.0–25.5° presented as a function of the storage time at 31.5°C. The $\alpha \rightarrow \beta$ transformation was studied by following the increasing peak 4 in Fig. 2. The labels are A: 0 h, B: 41.6 h, C: 65.3 h, D: 93.3 h, E: 125.9 h, F: 137.3 h, G: 161.7 h and H: 233.6 h.

4.3. Microcalorimetry

Measurements were made at 25, 30, 33 and 35°C. Fig. 9 shows pretreated heatflow and calculated concentration curves at 35°C. The reaction rate at 25°C was too slow to get reliable results in appropriate time because the transition continued for several months. The results are shown in Table 2 and in Fig. 8. The $t_{1/2}$-values were determined from the concentration curve (Fig. 9). The reaction enthalpy averaged over three temperatures was -45.6 J/g which confirms that the integrating method described earlier in this paper was accurate. The activation energy was (455 ± 4) kJ/mol using the Eq. (6). The error limit comes from mathematical fitting error and it includes only partially the errors due to measurement inaccuracies.

5. Conclusions

The shape of the concentration–time curve was sigmoidal. The results obtained by thermodiffractometer, 410 kJ/mol and microcalorimeter 455, kJ/mol agreed well considering that the methods have totally different character. The microcalorimeter result was probably more correct than the one obtained by thermodiffractometer due

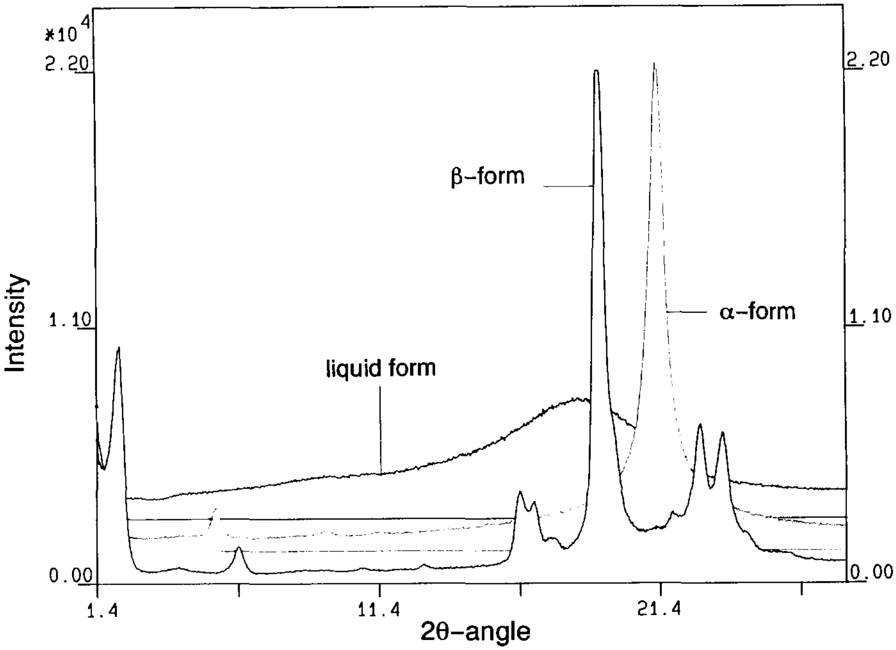


Fig. 6. Diffractograms of three observed states of tripalmitin. From behind: liquid, α-state and β-state.

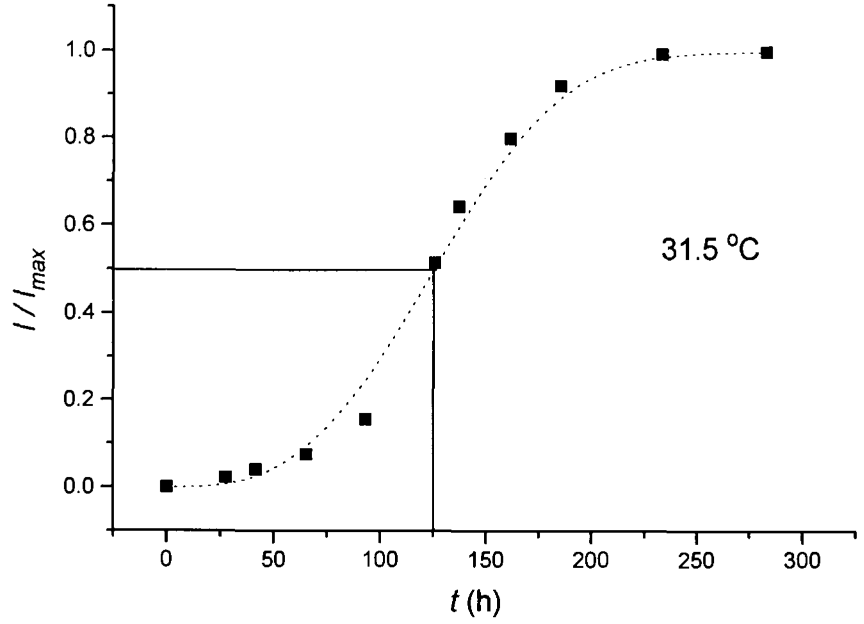


Fig. 7. The sigmoidal $I(t)/I_{max}$ curve fitted to the 10 measured points at 31.5°C with TXRD. $I(t)$ is the height of the reflection at the time t and I_{max} is the final height. The $t_{1/2}$-value is the time when $I(t)/I_{max} = 1/2$. The heights were determined from the profile fitting results as in Fig. 5.

Table 1
The half-times of the $\alpha \rightarrow \beta$ transformation obtained by TXRD

Temperature/°C	$t_{1/2}$/h
31.5	125.6
34.5	20.1
36.5	3.6
38.5	3.8
40.5	0.94
42.5	0.36

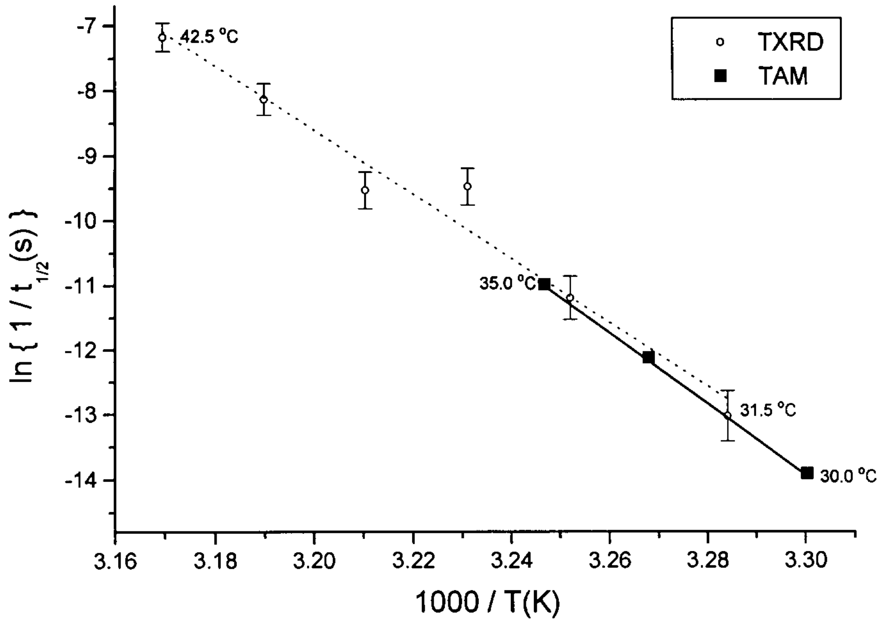


Fig. 8. The Arrhenius plot of the transition $\alpha \rightarrow \beta$ obtained with TXRD and MC. Some measurement points are labelled with measurement temperatures. The dashed line in this figure is fitted to thermodiffractometer points and the short line to the microcalorimeter points. The activation energy E_a is determined from the slope of the linear fitting curve.

to accurate temperature control. The large error limits of the thermodiffractometer result might be due to the sample differences.

The agreement of the transition enthalpies obtained with DSC (−47.0 J/g) and isothermal microcalorimetric technique (−45.6 J/g) was very good. The most obvious difference between these two measurement curves was that the isothermal microcalorimeter signal was the resultant of the three statistically simultaneous reaction

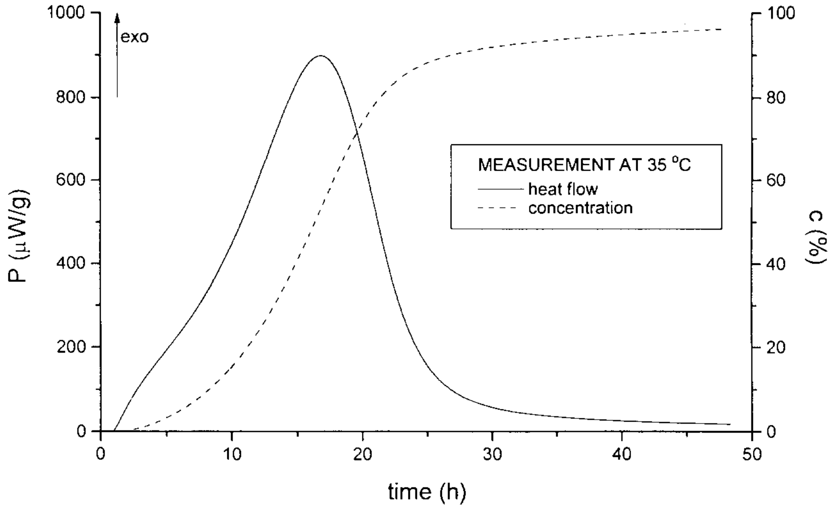


Fig. 9. The heat flow-signal (—) obtained from Fig. 3. The concentration curve (– – –) has the same sigmoidal nature as was observed in the TXRD results. The $t_{1/2}$-values were determined from this curve without separate fitting process.

Table 2

The half-times of the $\alpha \rightarrow \beta$ transformation obtained by MC

Temperature/°C	$t_{1/2}$/h	ΔH_r/J g^{-1}
25	–	–
30	305	45.5
33	50.9	45.5
35	16.3	45.7

processes as a function of time. In the non-isothermal DSC-curve the three reaction processes with different activation energies E_a appeared in sequence.

Acknowledgements

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