Physical Aging of Even Saturated Monoacid Triglycerides

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ABSTRACT

The polymorphism and kinetics of phase transformations of triglycerides have been investigated using a polarizing microscope equipped with a variable temperature gradient stage. Differential thermal analysis, X-ray, NMR, and IR data supplement the visual observations. The α-polymorphic transformation was observed to change with time in the cases of trilaurin, trimyristin, tripalmitin, tristearin, triarachidin, and tribehenin and can be described by the equation:

$$t = e^{-K\Delta T} - 1.$$

The greater stability of the α-polymorph chain triglycerides can be attributed to the larger energy of activation needed for the transition to occur. Estimates of the energy of activation (14-76 kcal/chain) imply that, not only does the transformation process involve simple Van der Waals CH_2-CH_2 interactions, but one must also consider chain-chain entanglement.

INTRODUCTION

Glycerides and triglycerides in particular have been studied for over a century to determine the various polymorphic forms which exist. However, few people have been concerned with the kinetics of transformation from one solid to another (or more simply aging) and the solidification of the melt to the various crystalline forms. Yet, these dynamic processes have great technological consequences, such as the mouthfeel properties and stability on everyday products. Triglycerides serve as a prime indicator for studying these systems, since they exist in almost all fatty materials and their polymorphic states are due to three principal cross sectional arrangements of long chains α, β', and β.

This paper presents evidence on the aging of pure monoacid triglycerides. A technique also is described using a polarizing microscope and a temperature gradient microscope stage which supplies supplementary information to thermal, x-ray, IR, and NMR methods. This apparatus also can be used as a simple device to determine the temperature stability of various fatty systems. More important, it eliminates many three dimensional heat transfer problems and provides a direct method of observation of the phenomena of physical aging of triglycerides.

BACKGROUND

Glycerides, in general, serve as the principal constituent of fat. Their presence is found in most living matter from plants to human beings. The functions of these lipids, especially in the case of shortenings, confectionary products, and biological membranes depend upon their crystalline structure, or, more appropriately, their polymorphic form.

One glyceride system which has been studied throughout the years for its polymorphic behavior is the triglyceride. However, in addition to the various crystalline forms which exist, it is also important to determine the degree of stability that each form exhibits. Some forms seem to be thermodynamically stable over a given temperature and pressure range while others are never thermodynamically stable but exist as a result of the kinetics of formation and transformation.

Obviously, this presents a problem, since a product could be prepared in one form with the desired properties, but, as a function of storage, temperature, and pressure (physical aging process), a transformation may take place from an unstable polymorph to one of greater stability. The end result is the deterioration of the product quality and its original properties. For this reason, it is of interest to understand the laws involved in the kinetics of transformations, or, more simply, aging.

The polymorphic behavior exhibited by triglycerides first was observed over a century ago when Heintz (1) observed two mp for tristearin. Duffy (2), in 1872, reported three polymorphs, and, in the following years, numerous scientists (3-7) disputed the various mp and forms of the simple triglyceride. It was really Malkin and coworkers (8) who demonstrated conclusively, using x-ray diffraction patterns, that the basis for the multiple melting was, indeed, polymorphism. However, numerous discussions ensued still debating the correct number of polymorphs. Malkin and Clarkson (9) claimed four polymorphs of tristearin existed, including a "glassy" form first observed by Ravich, et al. (10). At the same time, Lutton (11) and Bailey (12), conducting independent studies, accounted for only three forms. Today, it is accepted that saturated monoacid triglycerides exhibit with rare exception only three cross-sectional structures ($\alpha_L, \beta'_L, \beta_L$) which refer to the packing of the hydrocarbon chain. The subscript L refers to Lutton's convention. These forms can be observed using techniques, such as differential thermal analyses, x-ray single crystal studies, IR spectroscopy, and NMR.

TECHNIQUES FOR STUDYING AGING PROCESS

Microscopy

The type of stages used in previous investigations (7,13) can be considered isothermal microscope stages which evenly heat and cool the sample to the same temperature. Although they are suitable for nucleation and phase identification experiments, it is of little use for the investigation of the kinetics of solidification and aging.

When an isothermal stage is used, growth rates are often difficult to control, and temperature gradients must essentially be zero. Nevertheless, it is known that both the growth rate and temperature gradients in a sample are important factors in determining how a material solidifies. With this in mind, Hunt, et al., (14) at the Bell Telephone Laboratories, Murray Hill, N.J., developed a temperature gradient microscope stage for the study of pure systems and the effect of trace amounts of impurities upon the solidification process. This stage is pictorially described in Figure 1. The specimen cell consists of two thin glass slides (22 x 22 x 0.2 mm) sandwiching a thin film of the sample to be studied. One end of the cell rests on a cold plate, while the opposite edge serves as a heat reservoir or heat sink. The thin film of solution is essentially in a two dimensional heat transfer plane and a unidirectional temperature gradient. This arrangement eliminates many three dimensional heat transfer problems encountered in bulk samples. Most materials reach equilibrium in ca. 2 min. A controlled flow of dry air is flushed through the system to prevent frost formation on the cell and cold plate.

For the present work on aging of pure systems and complex systems, such as emulsions, a number of modifica-

tions were necessary. Since we are interested in both solidification and melting or freezing and thawing, the present stage is equipped with a reversible and variable D.C. motor (CONRAC Corp., Old Saybrook, Conn.) to move the cell at any rate between .01 mm/sec and 0.5 mm/sec. The motor driven shaft is connected to a Teflon frame which encompasses the specimen cell. Moving the cell in the direction of the cold plate will cause the liquid to freeze at the solid liquid interface. Moving the cell in the direction of the hot plate causes the interface to melt. When the solid/liquid interface has reached the equilibrium freezing or melting temperature, the interface position will remain stationary to the observer as the cell moves. This allows the study of the individual processes or as a combination of freezing and thawing cycles. Freeze-thaw cycles appear to be important when the stability of a system is being examined (13). In addition, both the hot plate and cold plate have been modified to control individually the plate temperature above the reservoir temperature. This allows for a wide choice of temperature gradients.

For the present work, it is important that the temperature profile be known at every point on the cell. The use of a fine thermocouple is impractical for accurate work, especially when the gradient is of the order of 10 C/mm. In addition, if the thermocouple is placed in the sample, it serves as a site for heterogeneous nucleation which may influence the experimental results. Rosano and coworkers (15,16) have developed an alternate and very accurate method of calibrating the stage for equilibrium conditions by using various salt solutions (NaCl, KCl, $BaCl_2$, $NaCO_3$) which form well defined eutectic compositions and freezing points, i.e. 21.6 C for NaCl 5.2 M). When equilibrium is attained, the distance from the tip of the cold plate to the eutectic-liquid interface is recorded for each solution. A plot of distance vs eutectic temperature then will yield the temperature profile of the cell. Above 0 C, pure solids which have well defined mp and do not exhibit polymorphism are used to give data points. These solids initially must be melted and spread on the glass slides to produce a thin film. They then are solidified by rapidly cooling them externally and placed on the gradient stage in the solid phase. At equilibrium, the solid above the equilibrium temperature on the gradient will melt leaving the well defined solid liquid interface. Once the cell has been calibrated for a given gradient, the temperature is known at every position of the cell at equilibrium. A cathetometer is used to measure the cell position within ±.01 mm. The unique feature of this microscope stage allows the observation of a growing interface to be observed at all times at a particular temperature and with a defined temperature gradient. The microscope used in this study is a Reichert "Zetopan" research microscope equipped with a polaroid camera.

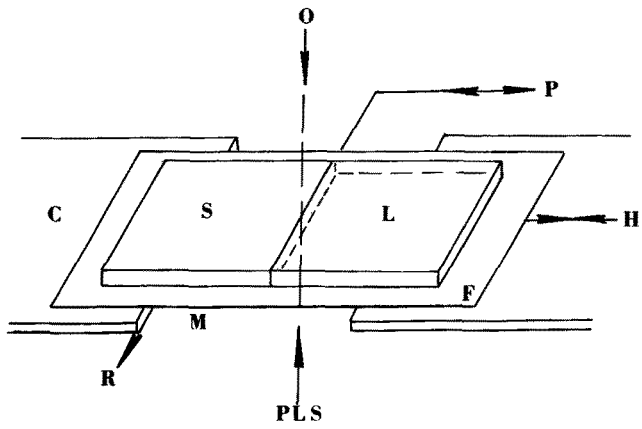

FIG. 1. Temperature gradient microscope stage. C = Cold reservoir, F = Teflon flame, H = Hot reservoir, L = Liquid phase, M = Microscope slides, O = Microscope objective, P = Pulley-cathetometer displacement apparatus, PLS = Polarized light source, R = Reference point, S = Solid phase, and T = Motor drive mechanism.

X-Ray Diffraction Patterns

X-ray studies still serve as the foundation for determining the different polymorphic forms. The short spacing dimensions for each form are those of Lutton (17) and are summarized in Table I. The system to be studied is placed in a fine capillary tube (.7 mm diameter, wall thickness 1/100 mm); General Rand Corp., Edison, N.J.) which is heated above the triglyceride mp and rapidly quenched to form the α-phase. The tube then is placed in a thermostated bath at the desired aging temperature for a given interval. After the aging interval, the sample temperature should be kept about 20 C below the α-transition temperature to

TABLE I

Polymorphic Forms of Saturated Mono Acid Triglycerides

	α	β′	β
Sub cell packing	Hexagonal $a = b \neq c$ $\alpha = \gamma = 90°$ $\gamma = 120$	Orthorhombic $a \neq b \neq c$ $\alpha = \beta = \gamma = 90°$	Triclinic $\alpha \neq \beta \neq \gamma$ $a \neq b \neq c$
X-ray short spacing (16)	4.15 s 2.40	4.2 vs 3.8 x 2.53 m 2.26 m	4.6 vs 3.84 s 3.68 s 5.24 m 2.85 m
IR spectrum (17)	Single band at 720 cm^{-1}	Doublet 719 at 727 cm^{-1}	Singlet band 717
NMR (18)			
Line width	6.8 gauss	---	13
Second moment	11.1	---	22
Microscope data (13)	Spherulite (−) Elongation	Spherulite (+) or (−) Elongation extinction	No spherulites Oblique extinction crystals
Mp (19) (± .5°C)			
Trilaurin	15	34	46.4
Trimyristin	32.8	45	58.0
Tripalmitin	45	56.6	66
Tristearin	54.7	63	73.5
Triarachidin	61.8	69	78
Tribehenin	68.2	74	82.5

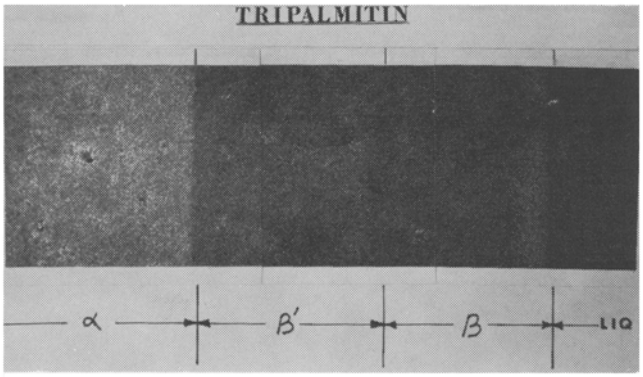

FIG. 2. Photomicrograph phase diagram of tripalmitin observed through cross polars. Magnification 80 x.

prevent further aging. The x-ray diffraction pattern is taken in a temperature controlled camera to determine if a phase change occurred.

Differential Thermal Analysis

The method of determining a particular phase using the Du Pont DTA 900 is most convenient, since the test could be conducted quite rapidly (heating rate 15 C/min), and sample preparation was minimal. The starting temperature for each of the samples is ca. 20 C below the α-transition.

IR Spectroscopy

Chapman (18) was one of the first scientists to use IR to classify the forms of the triglyceride. His results are summarized in Table I. The region of interest has to do with the CH_2-CH_2 rocking motion.

The sample is placed between two NaCl salt plates and melted using a temperature controlled IR cell. Rapid cooling is obtained by placing the cell in a refrigerated system. Precautions must be taken to prevent water from condensing on the cold plates and ultimately fogging them. Care also must be taken in cooling the plates, since rapid cooling will crack the plates. Once the triglyceride is in the α-form, the system is aged at the desired temperature while continuously scanning the IR spectrum. The tests were conducted on a Perkin Elmer 621 high resolution spectrophotometer.

This technique is used only to confirm the experiments conducted on the instruments mentioned above, since the aging temperature could not be controlled accurately due to the size of the I-R cell. A temperature gradient always existed.

NMR

Wide-line NMR can be used to define some of the polymorphic forms of glycerides. Chapman, et al., (19) give line widths for tristearin and tripalmitin. The α-form measures 7 gauss and the β- form 13 gauss. The line width for the β- prime form was not given for these systems.

In our study, a Jelco wide-line NMR (40 Hz) is used to study aging. A derivative curve is taken periodically and the line width measured to determine if aging had taken place. The samples are prepared originally in the α-forms by melting the triglyceride and then quenching the sample. Once again, the main problem with this technique is due to heat transfer within the sample, since the sample tube has a diameter of 15 mm. It must be realized that any bulk sample will, therefore, have a temperature gradient existing from the tube walls into the center of the sample. This temperature gradient may be minimized by placing a hollow glass rod evenly spaced in the tube to form an annulus. The sample is placed in the annulus and a thermocouple used to determine the temperature.

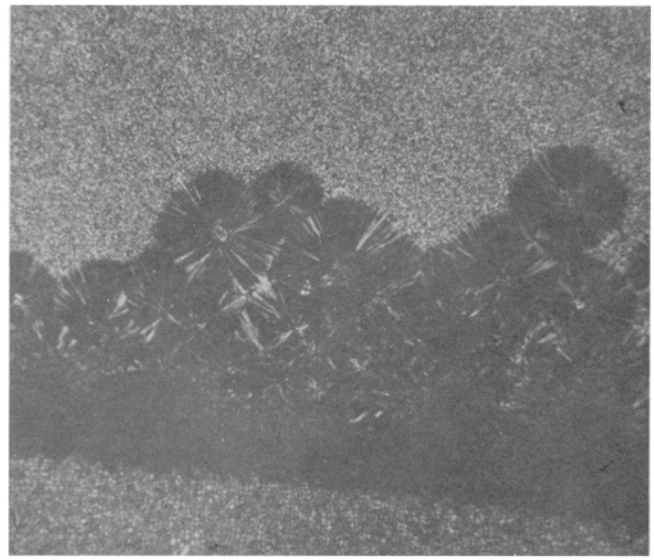

FIG. 3. Photomicrograph of the feathered β'-spherulites of tripalmitin using polarized light. Magnification 80 x.

Reagents

The monoacid (even) triglycerides were purchased from Applied Science Laboratories, State College, Pa., with the exception of triarachidin which was purchased from the Hormel Institute, Minneapolis, Minn. The purity was estimated by the manufacturers to be 99%. This was confirmed by us using gas liquid chromatography (GLC).

RESULTS

Microscope

A typical gradient used for study was 10 C/mm ranging from -20 → 80 C on the stage. The magnification was 80 x. Ca. 4 mg sample was placed between the microscope slides. This small amount produced a thin film when melted and then solidified to cover the entire specimen cell. The film was thin enough to allow optical identification of the crystalline material and also produce good reproducibility. It generally did not take longer than 2 min for any system to reach equilibrium. Less time (∿1 min) is required if the samples are placed on the stage in a solid film and allowed to melt.

Generation of Phase Diagram

A phase diagram of the monoacid triglycerides showing the various initial crystalline forms and the temperatures at which they form can be produced on the same microscope slide. The tripalmitin phase diagram is shown in Figure 2. The sample initially was melted on the slide and then pressed to form a thin liquid film free of air bubbles. The glass sandwich then was quenched rapidly to 0 C producing the α-phase spherulites. This was detected by observing the system through cross polars. Differential thermal analysis and x-rays were used to verify the α-phase by this method of solidification. Finally, the sample is placed on the microscope stage where the cold reservoir contains dry ice/acetone mixture, and the hot plate was maintained at +20 C. The hot reservoir then was increased to produce the desired temperature gradient mentioned above. As the gradient changed, the α-spherulites melt receding toward the cold plate in a uniform melting front leaving behind the liquid, β-phase, and β' phase in the appropriate temperature region. The melting front appeared to stop at the observed α-β'-transition temperature (45 C) quoted in the literature. If the sample is heated to melt all the crystals and immediately placed on the same temperature gradient,

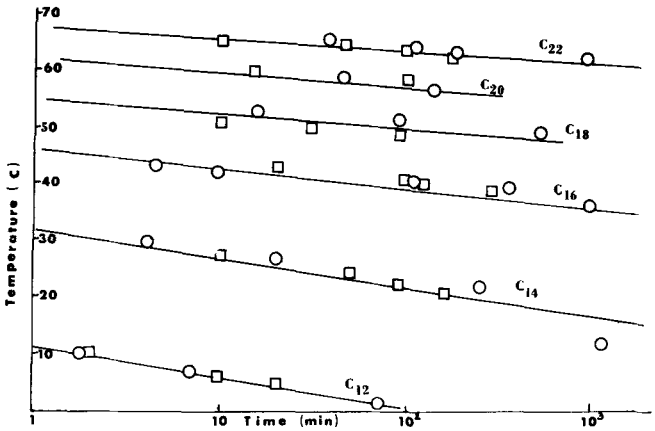

FIG. 4. Aging curves from the α-polymorphic form. ○ = Differential thermal analysis and □ = temperature gradient microscope stage (TGMS) determination.

α-spherulites form and propagate into the warmer region as a continuous freezing front. The interesting feature of this experiment is that the spherulite freezing front stops below the expected α-β′-equilibrium temperature. In the case of tripalmitin the α-β′-equilibrium temperature given in the literature and as seen in the first experiment is 45 C. In this experiment, the α-β′-front stopped at 38.5 C; a liquid section next is observed in the temperature region from 38.5-52 C but is followed by a second crystal region which is defined optically as a β-region. Immediately following this region is the liquid phase. The liquid section in the 38.5-52 C slowly solidifies (after ∿480 sections) in the form of large colored and feather-like spherulites. A photomicrograph of this solidification process for tripalmitin is given in Figure 3.

Aging

Figure 4 is an aging curve of the triglycerides. This was obtained in a manner similar to that described in above. The triglyceride to be studied was solidified initially in an α-form externally. It then was placed on the temperature gradient stage, and melting of α-front rapidly took place as the cell approached equilibrium. When the resolidification front reached the α-β′-transition temperature, the velocity of the front appeared to be zero. However, accurate measurements with the cathetometer over a period of time indicate movement of the α-β′-front beyond the equilibrium temperature. The aging curve can, therefore, be made by determining the temperature of the α-β′-front as a function of time.

Differential Thermal Analysis

Figure 4 describes the effect of temperature upon the time required for the triglyceride to transform from the α-form to either β′ or β. This method was most convenient for long range storage, since the sample could be stored in an externally controlled temperature bath and then tested at given intervals.

To determine if the system had aged, it is necessary to know the thermogram of the various triglycerides in the α-form. A typical series of aging curves is given in Figure 5 for trimyristin.

X-ray, IR and NMR techniques were used to reaffirm the aging curves obtained in Figure 6 and to determine which phase the sample had aged to, i.e. $\alpha \rightarrow \beta'$ or $\alpha \rightarrow \beta$.

DISCUSSION

The transition of a triglyceride from one solid to another can be described thermodynamically. At the transition point, the Gibbs free energy of the phases in equilibrium

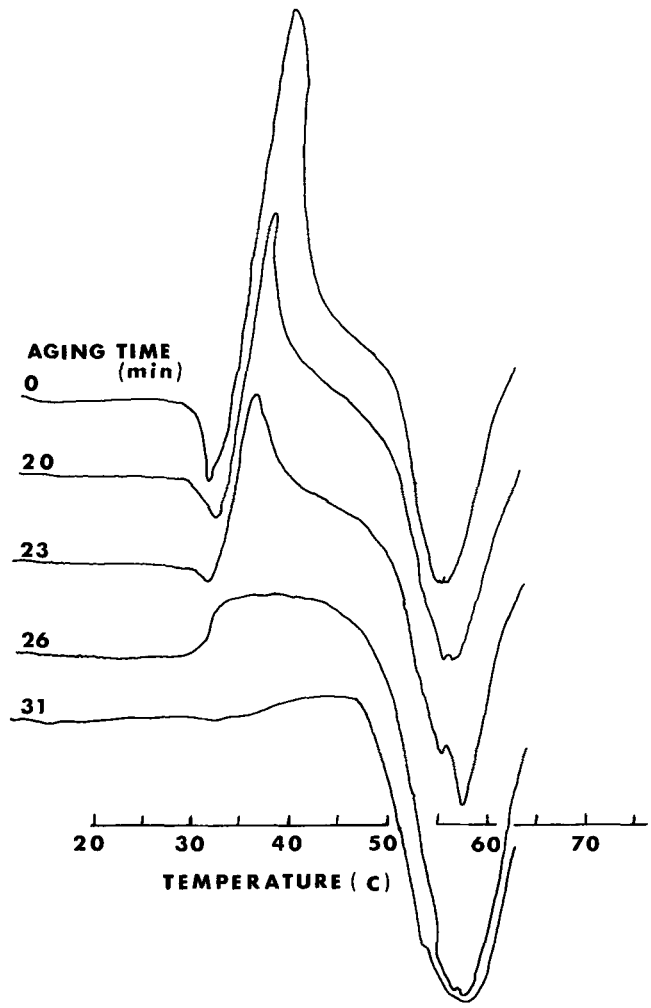

FIG. 5. Series of differential thermal analysis aging curves for trimyristin from α-polymorph. Aging temperature, -26 C.

are equal and the free energy curves at constant pressure intersect:

$$\left(\frac{\delta G}{\delta T}\right)_p = -S; \left(\frac{\delta G}{\delta p}\right)_T = v.$$

The change from low temperature to the high temperature form takes place with the absorption of latent heat, and its effect of pressure upon the transition temperature is determined by

$$\frac{dp}{dT} = \frac{\Delta S}{V_{II} - V_I}$$

The transitions which occur are either enantiotropic or monotropic. In the former case, each phase is stable within a region of temperature and pressure, while the other phase is unstable. In the latter case, one or more forms are unstable under all conditions. Most transformations which occur with triglycerides are considered either monotropic or a combination of monotropic and enantiotropic transitions. Since little work has been done on the kinetics of solidification and aging, this area is still wide open for discussion.

Our optical identification of the various phases and the phase diagram correlate quite well with the results of Quimby (20). The α-phase only contains "bright spherulites" that are not colored when viewed between cross polars with a halogen lamp. The isogyres are perpendicular to one another and quite sharp. This pattern is sometimes referred to as a "maltese cross."

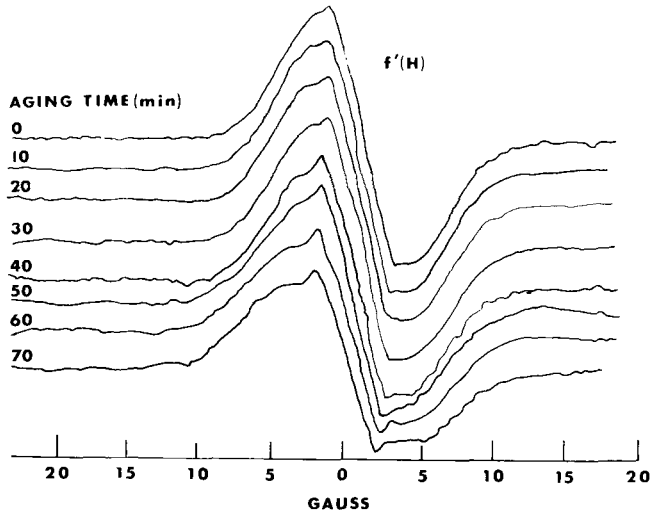

FIG. 6. Series of wide-line NMR aging curves for tripalmitin from α-polymorph. Aging temperature 40.5 C. f'(H) = derivative of hydrogen resonance.

TABLE II

Values of K

Amino acid	K(deg^{-1})	H^I (kcal/mole)
Trilaurin	.45	42
Trimyristin	.46	76
Tripalmitin	.672	152
Tristearin	.92	175
Triarachidin	.95	182
Tribehenin	2.30	228

This crystallization pattern is detected by the appearance of small birefrigent areas which grow steadily in size around the sites of primary nuclei. The extinction cross arms are oriented parallel to the pass directions of the polarizer and analyzer. There, orientation remains fixed when the crystals are rotated about a vertical axis on the microscope stage.

The most difficult distinction to make is that between the α-spherulite and the β′-spherulites. The β′ is distinguished by a colored and featherlike spherulite. The two isogyres are not as well defined as those in the α-phase. The β-phase is easiest to distinguish, since it lacks the spherulitic pattern with the "maltese cross" (Fig. 2).

The phase diagram is produced most easily by first forming α-spherulites over the entire slide and then introducing the temperature gradient. The reason for this becomes evident when experiments are conducted using the reversible motor to study interface solidification. The α-phase forms quite readily from the liquid once the temperature if below the observed α-β′-transition temperature. Currently, experiments are being conducted to relate the degree of super-cooling to the rate of freezing and the temperature gradient. These results will be discussed at a future date. β′- and β-phases, on the other hand, take a much longer time to solidify from the melt. However, the transformations from α→β′ and α→β and β′→β take place almost the instant the temperature reaches the equilibrium transformation temperature.

The aging phenomena described above appears to be quite significant, since it really questions the true meaning of the α-β′- and α-β-transition temperature from one phase to another as given in the literature.

The influence of temperature on the aging time is presented in Figure 4. The results obtained from differential thermal analysis studies and the temperature gradient microscope stage (TGMS) technique show good correlation, especially with aging times greater than 10 min. Differential

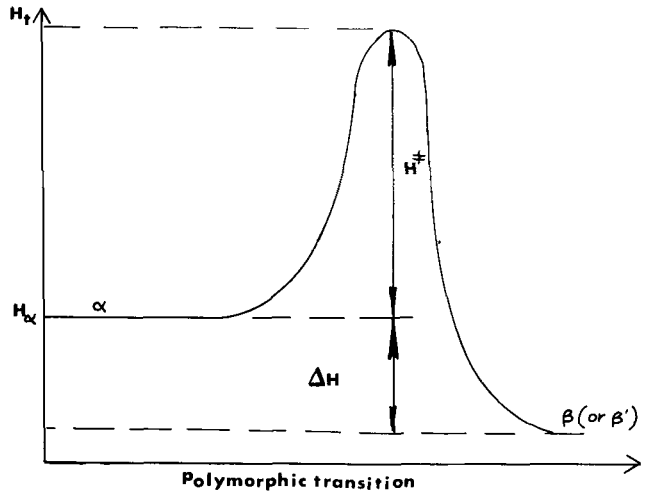

FIG. 7. Energy diagram for an α-polymorphic transition. H = enthalpy.

thermal analysis results tend to be somewhat scattered for aging times under 10 min which is mainly due to heat transfer in the sample and the time lost (∿5 min) in obtaining the thermogram.

These results indicate that the α-form is most stable for the higher chain triglycerides (C_{22}, C_{20}) when the temperature is kept at an equivalent number of degrees below the α-transition temperature. Curve fitting the data produces the general formula:

$$t = e^{K\Delta T_E} - 1$$

t = Aging time (min) to transform from α phase.

where $\Delta T_E = (T_{Equiv\ \alpha} - T)$

The values of K are given in Table II.

The fact that the stability of the α-form increases at a given ΔT with increasing chain length can be explained by the energy diagram in Figure 6. The α-form exists in a higher energy level than the more stable β-form. Charbonnet and Singleton (21) have confirmed this experimentally by determining heat of transitions from α→β for trimyristin (9.1 k cal/mole) tripalmitin (10.7 KaCl/mole) and tristeasin (12.2 kcal/mole) to be exothermic. However, ΔH of transition is not a measure of the stability of the system but H^I or the energy of activation is (Fig. 7). Classically, the rate can be expressed as follows:

$$\frac{\alpha \rightarrow \beta \text{ Transition}}{\text{Time}} = A\, e^{s^I/R_e}\, -H^I/pt.$$

For a given triglyceride let us assume that the entropy factor, $e^{S/R}$, is ca. constant, and the date in Figure 4 are used to determine the energy of activation. These results (Table II) indicate that the energy of activation is highest for the most stable α-phase (tribehenin). The magnitude of the energy of activation (10-228 kcal/mole) is reasonable, since it is assumed that to break CH_2-CH_2 interaction requires 250-300 cal/mole. For trilaurin (C_{12} chain) in the unsymmetric tuning fork orientation, we estimate the H^I necessary to break all Van der Waal's attraction is ∿20 kcal/mole. As the chains increase in carbon content, it is reasonable to assume that not only should the energy of activation increase due to the additional carbons but also because the chains become more entangled in the crystal structure (therefore, greater Van der Waal's interactions). The absolute entropy in the α- or β- state will be larger as the carbon content increases, but the entropy term (S^I) in the activated state will be lowered, since it is assumed that the chains are ordered and untangled in this high energy state.

A series of aging curves for trimyristin stored at 26 C are shown in Figure 5. The heating rate was programed at 15 C/min and the starting temperature was set at least 10 C below the α-transition temperature. It should be noted that the heating rate is an important factor in determining the thermogram for triglycerides. Although slower heating rates will yield sharper endo- or exotherms and can locate an equilibrium transformation temperature more accurately, the α- transition is not a thermodynamic equilibrium transition. Therefore, a high heating rate must be used to determine these metastable states. If a heating rate of 1 C/min was used, the trimyristin would automatically have aged in the differential thermal state regardless of the previous thermal history. This can be predicted from Figure 4 which indicates that the storage of trimyristin at 31 C (ΔT_E = 1) for ca. 1 min. will cause the α-phase to transform.

Physical aging, as noted earlier, also can be observed using wide-line NMR. Figure 5 is an example of tripalmitin aging at 40.5 C. The field strength was set at 10^4 gauss with a modulation frequency of 280 cps and a modulation width of 4 gauss. The α-form has a line width of 6.8 g as first observed by Chapman et al. (17). In a similar manner, as seen with the differential thermal analysis work (Figure 4), the triglyceride changes from the α-polymorph over a period of time to the more stable phase. The rate is dependent on ΔT_E. In this case, the line width continuously widens until the β-width is obtained. It is also interesting to notice the development of the narrow line component as the system ages.

The second moment of the proton resonance is primarily due to the long chain hydrocarbon. The C–H group of the glycerol and the terminal CH_3 group are assumed to make a rather small contribution to the wide-line spectra and will be mainly predominant in the narrow line. The smaller the second moment, or line width, (peak to peak width of the derivative curve) for the α form indicates that the hexagonal structure allows a considerable amount of molecular motion and would be less stable than the β-polymorph.

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