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Slow Recrystallization of Tripalmitoylglycerol from MCT Oil Observed by ^{2}H NMR

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The crystallization and recrystallization of fats have a significant impact on the properties and quality of many food products. While crystallization has been the subject of a number of studies using pure triacylglycerols (TAG), recrystallization in similarly pure systems is rarely studied. In this work, perdeuterated tripalmitoylglycerol (^{2}H-PPP) was dissolved in medium chain triacylglycerol oil (MCT) to yield a saturated solution. The solution was heated to cause partial melting of the solid and dissolution of the molten fraction of ^{2}H-PPP in MCT and was then cooled to the original temperature to induce recrystallization from the supersaturated solution. ^{2}H NMR was used to monitor the disappearance of ^{2}H-PPP from the solution and showed that recrystallization occurred in two steps. The first step was rapid, taking place over a few minutes, and accounted for more than two-thirds of the total recrystallization. The second step was much slower, taking place over a remarkably long timescale of hours to days. It is proposed that dissolution occurs from all parts of the crystals, leaving an etched and pitted surface. The first step of crystallization is the infilling of these pits, while the second step is the continued growth on the smoothed crystal faces.

KEYWORDS: Fats; triglyceride; triacylglycerol; recrystallization

INTRODUCTION

The interactions between triacylglycerols (TAG) in the liquid and solid phases of fats give rise to many phenomena and many different behaviors in the area of lipids. Thus, the formation of bloom in chocolate, migration from a confectionery center, development of graininess in margarines, and the behavior of fats during fractionation processing are all dependent on the movement of TAG molecules between solid and liquid phases. Little work has been carried out in this area to date, although Haghshenas et al. (*1*) quantified the rate of exchange between the solid and the liquid phases of tripalmitoylglycerol (PPP) in a medium chain triacylglycerol (MCT) solvent. This involved the addition of radiolabeled (^{14}C), crystalline PPP to a saturated solution of PPP in MCT oil, with exchange of PPP molecules between the solid and the solution phases being determined by radiodetection. This method was not ideal, due to temperature fluctuations, thus Löfborg et al. (*2*) used deuterium-labeled PPP to determine the rate of exchange using nuclear magnetic resonance (NMR). In addition, partial acylglycerols have been shown to inhibit the exchange of molecules between the solid and the liquid phases (*3*).

At a time when there is a drive towards reducing or eliminating trans fatty acids from the diet (*4*), food manufacturers are looking towards other sources of structuring fat. Thus, in the current oils and fats industry, there is a desire to reduce or remove trans fats from edible fats. Partial hydrogenation, forming trans fats, has been used to provide structuring to previously liquid oils. In the absence of trans fats, it is necessary to turn to saturated fats. There are few commercially available oils containing high levels of saturates, and of those that do, fractions with higher saturate levels must be produced via fractionation. In light of this, Timms recently referred to fractionation as "the fat modification process for the 21st century" (*5*), since it provides a natural means to control fat properties. Fractionation, as applied to fat, involves the fractional crystallization of a solid phase (stearin) and separation of this from the liquid phase (olein). One oil often fractionated is palm oil. In the fractionation of palm oil, the solid phases formed comprise two principal TAGs, namely, PPP and 1,3-dipamitoyl-

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2-oleoylglycerol (POP). The way in which the stearin crystallizes, and the consequent ease of separation from the liquid olein, depends on several factors including the fractionation temperature, stirring rate, and composition of the crystallizing solid.

The crystallization and recrystallization of fats and oils are complex, and studies aimed at understanding it take two approaches. The first approach examines the effect on crystallization of, for example, changing the composition of natural oils by blending (see, for example, ref (*6*)), and is essentially an empirical approach. The second approach uses pure TAG models to determine fundamental parameters (see, for example, ref (*7*)), a more theoretical approach. The work described here extends the previous studies by Löfborg et al. (*2*) (which looked at the exchange of PPP molecules between the solid and the liquid phases) to investigate the recrystallization of PPP in a similar model system, using NMR as before.

MATERIALS AND METHODS

TAG Crystals. Perdeuterated PPP (^{2}H-PPP, $C_{51}D_{98}O_6$, Larodan, Malmö, Sweden) was crystallized from hexane (PA quality) and carefully vacuum dried. As determined by differential scanning calorimetry (DSC) (DSC 821; Mettler Toledo), the PPP crystals were in the stable polymorphic form (β) with a mp of 64.5 °C, similar to that previously published for PPP (*8*). The melting enthalpy obtained was 179 kJ/mol, which is slightly higher than the previously published value by Timms (*9*) of 171 kJ/mol for PPP but in close agreement with the value by Van Miltenburg and Ten Grotenhuis (*10*) of 177 kJ/mol. Light microscopy showed a typical crystal size of 20–60 μm.

TAG Oil. Medium-chain TAG oil (MCT; Karlshamns AB, Karlshamn, Sweden) was used as a solvent for PPP. The oil contained 96.6% TAG, 3.2% diacylglycerol, <0.1% monoacylglycerol, and <0.1% free fatty acid. The main fatty acids in the MCT oil were caprylic acid (octanoic acid, 61.8%) and capric acid (decanoic acid, 37.6%).

Preparation of the Sample. An excess of ^{2}H-PPP crystals (0.201 g) was mixed with MCT oil (10 mL), giving a total weight of 9.733 g of a saturated solution of ^{2}H-PPP in MCT over residual crystals of ^{2}H-PPP. After thoroughly dispersing the crystals throughout the volume, approximately 300 mg of the dispersion was transferred into a 10 mm NMR sample tube by pipette. By repeated visual inspection, it was found that spinning samples in the NMR probe at a 25 Hz spinning rate prevented sedimentation of the crystals in the dispersion. This phenomenon is similar to the suppression of thermal convection by sample spinning (*11*). Hence, all experiments were performed under such spinning conditions.

NMR Experiments. As reported previously (*2*), because of the anisotropy of spin interactions, NMR spectra are usually broad in solid powders. This also applies to ^{2}H nuclei in C–^{2}H bonds in the solid state, whose dominant spin interaction is the so-called quadrupole coupling that leads to line broadening of the order of 10^5 Hz (*12*). However, in the liquid state, molecular motions are fast and isotropic, leading to an averaging of anisotropic spin interactions and resulting in much narrower (about 1–10 Hz) NMR lines. Thus, by using high resolution NMR, the signal from the solid phase is effectively cancelled, while the ^{2}H nuclei residing in the liquid phase are readily detected. The actual experiments were performed with 10 kHz spectral width; therefore, the integral intensity of the recorded ^{2}H NMR spectra measured the amount of ^{2}H nuclei (and hence ^{2}H-PPP) in the liquid phase.

Following preparation, the sample was transferred to an NMR instrument (Bruker DMX 500, 76.8 MHz resonance frequency for ^{2}H). The temperature within the NMR probe was set to about 28 °C, and the sample, initially at room temperature, was allowed to equilibrate for a long time (not essential, see later). After this, the temperature was increased by 5 °C (taking about 2 min for the whole sample volume to be within ±0.1 °C of the set temperature as measured by an in situ thermocouple during a separate run), and the amount of ^{2}H-PPP in solution was determined as described above. After 1 h, the temperature within the probe was decreased to the original temperature (28 °C), and the disappearance of ^{2}H-PPP from the liquid phase, as a consequence of recrystallization, was determined as a function of time by measuring every 30–40 s. The signal was typically obtained by averaging 64 scans. The longitudinal relaxation time T_1 of ^{2}H-PPP in MCT was measured to approximately 30 ms, at 28 °C, and the recycling time was set to more than five times this value to ensure recording artifact-free intensities. Note that T_1, set by the strongly temperature-dependent and potentially very complex (*13*) rotational molecular dynamics of the PPP molecules, varies strongly by temperature [T_1 = 300 ms was measured previously at 40 °C (*2*)]. The measured signal intensity was normalized to the initial intensity prior to cooling (which was set to 1).

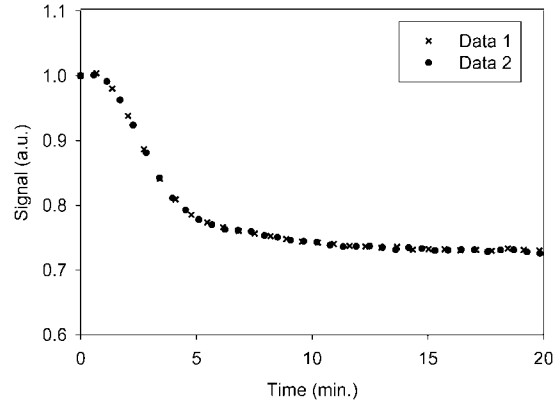

Figure 1. Comparison of two experiments, illustrating reproducibility of data at early times.

RESULTS AND DISCUSSION

On heating from 28 to 33 °C, the amount of dissolved ^{2}H-PPP quickly (within 3 min) reached a reproducible level that corresponded to the saturation concentration at the higher temperature. Thus, the initial heating from room temperature to 28 °C did not require a lengthy period to reach equilibrium. Because temperature equilibration takes about 2 min (see above), this ~3 min period is certainly longer than the intrinsic time constant for dissolution.

When the temperature was reduced to the original 28 °C, the signal intensity from the ^{2}H decreased as the ^{2}H-PPP recrystallized. **Figure 1** shows two data sets from early on in the crystallization, illustrating the good reproducibility of the drop in the signal.

In **Figure 2**, we display the integral intensity of the recorded ^{2}H NMR spectra starting at the instant of setting the temperature back to 28 °C. Clearly, within a few minutes of decreasing the temperature back to its original value of 28 °C, more than two-thirds of the ^{2}H-PPP that had previously dissolved on heating was deposited into the solid phase. Note that the first few points after reducing the temperature are also influenced by the 2 min temperature response time of the NMR probe. Thereafter, there was a clear and distinct crystallization process during which time the amount of dissolved ^{2}H-PPP very slowly approached its original equilibrium value.

Increasing the temperature to 33 °C and keeping the sample there for 10 min to 1 h always resulted in the same behavior upon lowering the temperature, irrespective of whether or not equilibrium had been re-established following recrystallization after the previous cooling step.

When interpreting the results, the following points must be kept in mind. First, experiments performed in solutions that contained ^{2}H-PPP under saturation level at 28 °C resulted in constant intensities for the same temperature course. Hence, the slow kinetics is not a result of some experimental artifact.

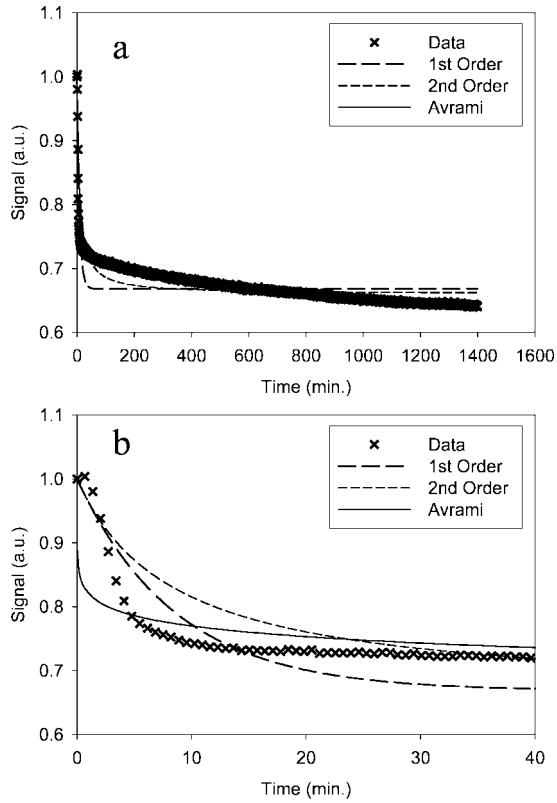

Figure 2. Comparison of single kinetic models fitted to the decrease in liquid signal, showing (**a**) all data and (**b**) early data.

Second, the solution always contained crystals of β-^{2}H-PPP. Hence, crystallization from the supersaturated solution can commence immediately without any induction period. Moreover, growth on pre-existing crystals that were in β-form produces the same polymorph.

First-order (eq 1) and second-order (eq 2) models, as well as the Avrami model (eq 3), were fitted to the fall in signal intensity of the ^{2}H-PPP in the liquid phase:

$$\alpha_t = \alpha_\infty + (\alpha_0 - \alpha_\infty)\exp(-kt) \quad (1)$$

$$\alpha_t = \alpha_\infty + \frac{1}{\left[1/(\alpha_0 - \alpha_\infty) + kt\right]} \quad (2)$$

$$\alpha_t = \alpha_\infty + (\alpha_0 - \alpha_\infty)\exp(-kt^n) \quad (3)$$

where t = time, α_τ = signal intensity at time t, α_0 = signal intensity at time 0 (normally set to 1), α_∞ = signal intensity at infinite time (equilibrium), k = rate constant, and n = Avrami exponent.

None of the equations fit well (**Figure 2a**), although the Avrami model was the best (**Table 1**). However, even this model failed to fit well since the data showed a two-step process, as described above. The first step took on the order of 10 min, while the second step took several hours. All models had difficulty fitting the data at the point where the first stage gave way to the second (**Figure 2b**).

For the data fits, the signal intensity could be fixed to the initial intensity (i.e., α_0 set to 1). However, to account for the temperature falling over the first few points, α_0 can be fitted (i.e., a notional starting point) while disregarding the first three points. This was the approach taken here. Additionally, to account for two recrystallization stages, equations can be combined (double first order, double second order, double Avrami, and a combination of first order for the first step and Avrami for the second, or vice versa) and fitted to the data.

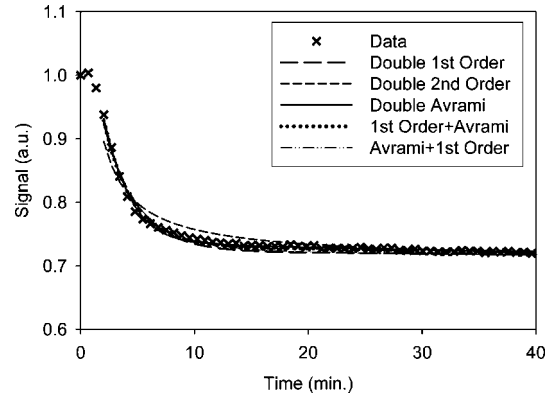

Figure 3. Comparison of double kinetic models fitted to the decrease in liquid signal, showing early data only; at later times, all lines fit the points closely.

Table 1. Comparison of Quality of Fit of Various Models To PPP Recrystallization[a]

model	no. of parameters	residual sum of squares (sum of squared errors)
1st order	3	0.10991089
2nd order	3	0.10077926
Avrami	4	0.06508941
double 1st order	5	0.00884638
double 2nd order	5	0.0105827
double Avrami	7	0.00466901
1st order + Avrami	6	0.00464576
Avrami + 1st order	6	0.00794754

[a] Note: number of points = 2045.

Table 1 lists the details of the fitting. The doubled models fit the data much better, although all models still had difficulty at shorter times (**Figure 3**). Nevertheless, the best-fitting models utilized the Avrami equation for the second stage.

The second stage had an Avrami exponent very close to 0.5. This suggests rodlike crystal geometry, athermal nucleation, and rate determined by diffusion. In this case, nucleation sites already exist on the crystal surface (thus, athermal nucleation) and crystallization can be considered to be a process of diffusion of molecules to the crystal surface followed by some surface reaction that incorporates the molecule into the crystal (which may involve molecular diffusion on the surface). The parameter values derived from the equation fitting suggest that the recrystallization would not be complete even within a month (**Figure 4**), although this is an extrapolation far from the data points!

On the other hand, the Avrami exponent for the first stage is close to 2. Three situations can give rise to this value: (i) rodlike crystals, thermal nucleation, and rate determined by nucleation; (ii) disclike crystals, athermal nucleation, and rate determined by nucleation; and disclike crystals, thermal nucleation, and rate determined by diffusion.

It is not possible, from the data here, to establish which of these situations applies or even whether the Avrami model is appropriate (note from **Table 1** that the fit of a first-order model to the first stage is slightly better). However, the fitting of any model to the first, very rapid stage of crystallization is unreliable since so few (~25) points are available and those within the first couple of minutes are measured during the cooling phase from 33 to 28 °C.

Why should recrystallization take place in two stages in this system? Consider the starting crystals. As the mixture is heated

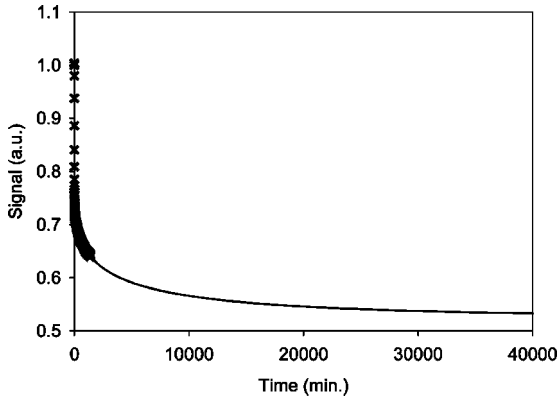

Figure 4. Extrapolation of the double Avrami model (line) fitted to the decrease in liquid signal (points).

up, the crystals will partially dissolve. As observed in other systems (*14*), pits may be etched on the surface of the crystals at random point defects or points where line defects break the surface. Thus, dissolution is proposed by some to be a process of pit etching and layer stripping. The crystal surface, therefore, would be highly roughened following partial dissolution. If the sample was to be cooled, recrystallization would occur. It is well-known that rough crystal surfaces lead to very much higher growth rates than smooth crystal faces (*14*). It may be that the initial rapid crystallization rate is a process of filling and smoothing over of these pitted crystal faces. In such a case, the presence of etched pits in the crystal surface might favor an athermal nucleation mechanism (i.e., zero activation energy).

The crystallization rate in the second stage might be much slower due to the smoother crystal surfaces remaining after this initial, rapid crystallization. Should dissolution take place from all crystal faces, these will be left roughened. This might give rise to rapid initial crystallization on all faces. As the surface becomes smoother, it may be that the crystallization occurs preferentially on certain faces. This could explain the differences found in the Avrami exponent, that is, perhaps two-dimensional growth initially, followed by one-dimensional growth later. Further work is necessary to determine the validity of this hypothesis.

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