Activation Energy of Crystallization for Trihydroxystearin, Stearic Acid, and 12-Hydroxystearic Acid under Nonisothermal Cooling Conditions

Ricky Sze Ho Lam† and Michael A. Rogers*‡

†Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N5A8
‡Department of Food Science, Rutgers University, The State University of New Jersey, New Brunswick, New Jersey 08901-8554, United States

ABSTRACT: The nucleation activation energy under nonisothermal cooling conditions was determined for 12-hydroxystearic acid (12HSA) (1-D crystals), stearic acid (2-D crystals), and trihydroxystearin (3-D crystals). The relative nucleation rates of trihydroxystearin and stearic acid were inversely proportional to the supercooling-time trajectory parameter (β), while 12HSA was linearly proportional to β. The differences in the proportionality to β are attributed to microscopic versus macroscopic phase separation. This suggests that both stearic acid and trihydroxystearin follow a probability density function for the number of molecules which crystallize as a function of supercooling (i.e., the greater the cooling rate, the greater the number of molecules which are incorporated into the crystal lattice). On the other hand, 12HSA molecules all crystallize when supercooled. The activation energies for stearic acid, 12HSA, trihydroxystearin, and triglycerides were 1.52, 5.40, 7.87, and 24.80 kJ/mol, respectively. The activation energy is partly affected by the polarity of the crystallizing molecules relative to the solvent. As the polarity of the crystallizing molecules increases, the activation energy decreases. However, this was not always observed because the activation energy for stearic acid was less than that of 12HSA. Therefore, the activation energy is not only a function of the molecular polarity but also due to a specific interaction between the nucleating molecules. The specific interaction affects the ability of the polar regions of the molecule to phase separate from the apolar solvent. As 12HSA and stearic acid dimerize, the carboxylic acid regions of the molecule are shielded from the solvent, but 12HSA cannot effectively shield the hydroxyl groups from the crystalline surface, resulting in a higher interfacial tension and, thus, higher activation energy.

INTRODUCTION

Often, crystallization is thought to occur in three-dimensional (3-D) space, whereby spherulitic aggregates result and minimize the surface area to volume ratio and interfacial tension.1 However, recent advances in crystal physics have thrust low-dimensionality crystals (i.e., two-dimensional (2-D) platelets and one-dimensional (1-D) fibers) into the forefront of numerous fields, including pharmacology,2–4 medicine,5–7 and nanomaterials.8–10 Currently, there is a limited understanding of how these molecules nucleate and form supramolecular aggregates which no longer minimize the surface area of the crystal.11

It has been well established that changes to the crystallization conditions greatly affect the mechanical properties, the flow behavior, and the supramolecular structure of materials regardless of the dimensionality of crystal growth.12,13 For example, increasing the cooling rate increases the number of nuclei, decreases the crystal size, and modifies the polymorphism and crystal imperfection.14,15 The thermodynamic conditions during nucleation and crystal growth dictate the structures formed by 12HSA, stearic acid, and trihydroxystearin crystals.15 At high cooling rates, highly branched networks are formed due to crystallographic mismatches, while, at low cooling rates, large aggregates with few branching points are formed.15 In industrial applications, crystallization often occurs under nonisothermal crystallization conditions where the temperature changes as crystallization progresses.16,17 Understanding how nucleation and crystallization occurs under nonisothermal cooling conditions is of paramount importance.

Three systems, 12HSA, stearic acid, and trihydroxystearin, were chosen to be modeled to determine the activation energy of nucleation due to their molecular similarities and different dimensionalities of crystal growth. Upon crystallization, 12HSA forms 1-D nanofibers,18–23 stearic acid forms 2-D platelets,24–26 and trihydroxystearin forms 3-D spherulitic crystals.15,27 Traditionally, nucleation is studied under isothermal cooling conditions due to the ease of experimental design and application of theoretical models. The activation energy for nucleation may be determined under nonisothermal cooling conditions using a supercooling-time trajectory parameter (β).16,17 β describes the amount of supercooling experienced by a system during crystallization.16,17 Polarized light microscopy (PLM) is utilized to visualize the number of nucleation sites formed as a function of time.16,17 The number of nucleation sites is used to determine the dependence of

Received: May 1, 2011
Revised: June 2, 2011
Published: June 03, 2011
\( \beta \) on nucleation rate \((J)\) and may be used to calculate the activation energy \((E_a)\) of a system.\(^{16,17} \) The supercooling-time—trajectory parameter, \( \beta \), takes into account both the time and the temperature or the amount of supercooling experienced by the material during crystallization.\(^{16,17} \)Effectively, \( \beta \) is represented by the area under the temperature—time curve experienced by the material cooled at a specific rate:\(^{16,17} \)

\[
\beta = \frac{1}{2} \Delta T_c t_c
\]  

(1)

where \( \Delta T_c \) is the supercooling experienced by the system (i.e., the temperature difference between the melting temperature and the temperature at which the first crystal appears) and \( t_c \) is the time it takes to go from the melting temperature to the appearance of the first nucleus. Equation 1 may be rewritten if the system is cooled at a specific cooling rate \((\psi = \Delta T_c/t_c)\):\(^{16,17} \)

\[
\beta = \frac{1}{2} \left( \frac{\Delta T_c}{\psi} \right)^2
\]  

(2)

For triacylglycerides (TAGs) an exponential scaling relationship is empirically observed between \( \beta \) and the nucleation rate \((J)\):\(^{17} \)

\[
J_{\text{max}} = e^{-k\sqrt{\beta}}
\]  

(3)

Combining eqs 2 and 3 reveals the relationship between the nucleation rate and the cooling rate:\(^{16} \)

\[
J_{\text{max}} = e^{-k(\Delta T_c/\sqrt{\psi})}
\]  

(4)

Equation 4 may be rewritten to include the activation energy parameter.\(^{16} \) Previously, it has been reasoned that the activation energy is the heat removed from the system between the melting temperature and the appearance of the first nuclei.\(^{16} \) This heat removed \((Q_m)\) is the specific heat \((C_p)\) of the system, which is \( Q_m = C_p \Delta T \).\(^{16} \) Here, \( C_p \approx 2.0 \text{ J g}^{-1} \text{ K}^{-1} \) for lipid based molecules.\(^{16} \) Therefore, it is possible to substitute \( Q_m/C_p \) for \( \Delta T \) in eq 4 and to obtain the following relationship:\(^{16} \)

\[
J_{\text{max}} = e^{-k(Q_m/C_p\sqrt{\psi})} = e^{-(Q_m/Z\sqrt{\psi})} = e^{-(X/\sqrt{\psi})}
\]  

(5)

\( Q_m \) represents the activation energy for nucleation per unit mass \((J/g)\) and \( Z \left( \text{J g}^{-1} \text{ K}^{-1/2} \text{s}^{1/2} \right) \) represents

\[
Z = \frac{\sqrt{2}C_p}{k}
\]  

(6)

the terms \( Z \) and \( X \) can be determined by the exponential fits of the relative nucleation rate \((J/J_{\text{max}})\) versus \( \beta \) or \( \psi \). It is possible to determine the activation energy for nucleation by calculating \( Q_m = ZX(J/g) \). This model has been adapted to account for molecules requiring negligible supercooling before nucleation, such as 12HSA, by applying a Taylor expansion to the probability density function.\(^{21} \) The probability density function (pdf) makes it possible to explain nucleation kinetics in a statistical manner by applying the kinetic theory of gases.\(^{21} \) In order to demonstrate the argument logically, the kinetic theory of gases is reviewed.\(^{21} \)

The rate of reaction \((v)\) is determined by the number of molecules with enough energy to overcome the energy barrier for the reaction \((N^*\)).\(^{21} \) Therefore \( v = k [N^*] \), where \( k \) is the rate constant for the reaction and \( N^* \) corresponds to the concentration of molecules in the activated state.\(^{21} \) In gases, \( N^* \) are the molecules with sufficient energy and proper orientation which are capable of undergoing the reaction.\(^{21} \) In nucleation, \( N^* \) are the molecules in the metastable state prior to the nucleation event.\(^{21} \) The proportion of molecules that may nucleate is given by \((N^*) = p(x)(N_T)\), where \( N_T \) is the total concentration of molecules in a system and \( p(x) \) is the pdf that describes the frequency distribution of the particular event.\(^{21} \) The pdf approach was first developed when the relative nucleation rate \((J/J_{\text{max}})\) was found to be exponentially dependent on \( \sqrt{\beta} \).\(^{21} \) Therefore, if the rate constant for nucleation \((k)\) is greater than zero, the exponential pdf, \( p(\sqrt{\beta}) \), is of the form\(^{21} \)

\[
p(\sqrt{\beta}; k) = \begin{cases} 
ke^{-\sqrt{k\beta}}; & \sqrt{\beta} \geq 0 \\
0; & \sqrt{\beta} < 0 
\end{cases}
\]  

(7)

This pdf applies to the randomly distributed variable belonging to the set \( \sqrt{\beta} \in [0; \infty) \).\(^{21} \) Therefore, this pdf is appropriate to describe nucleation kinetics nonisothermally.\(^{21} \)

The lower activation energy for 12HSA compared to triglycerides is due to microscopic phase separation in 12HSA compared to the macroscopic separation in TAGs.\(^{11,21} \) This is partially due to an increased polarity of 12HSA, which facilitates the formation of a nucleus by allowing 12HSA to dimerize with relative ease.\(^{11} \) Therefore, smaller critical nuclei are required when going from a crystal embryo to a stable nucleus.\(^{11} \) The nucleation behavior is dependent on the solvent employed.\(^{11} \) Not only does the solvent polarity play a crucial role in the activation energy but so does the ability of the solvent to interact with the crystallization molecules.\(^{21} \) Therefore, solvent—gelator interactions greatly affect the nucleation behavior and structural features of the crystals formed.

The aim of this study is to examine the role of molecular structure on the activation energy of nucleation. It is hypothesized that as the polarity of the crystallizing molecule increases, the activation energy will decrease. The increased chemical potential may facilitate phase separation and generation of crystal embryos. It is predicted that, under nonisothermal crystallization, triglycerides will have a higher activation than trihydroxystearin while stearic acid will have a higher activation energy than 12HSA.
MATERIALS AND METHODS

Stearic acid (Sigma Aldrich, Oakville, ON) and trihydroxystearin (Nu-Chek Prep, Elysian, MN, USA) were purchased and used as received. 2.5% (w/w) samples were added to heavy mineral oil (Sigma Aldrich, Oakville, ON, CA) and melted at 90 °C for 30 min to erase crystal memory. Cooling rates between 1 and 10 °C/min were applied at 1 °C/min increments, and the following cooling rates were also utilized, 0.5, 1.5, 2.5, 15, and 20 °C/min, to cool the samples to 30 °C.

Polarized Light Microscopy. A Nikon Eclipse E400 light microscope equipped with a Nikon DS-FiL color camera and a long working distance 10× lens and condenser (Nikon Instruments Inc., Melville, NY, USA) were used to acquire polarized light micrographs (PLMs) and bright-field micrographs. A temperature controlled stage (LTIS120 and PE94 temperature controller (Linkam, Surrey, U.K.) was used to control the cooling rates. The image resolution was 2560 by 1920 pixels. Images were analyzed using Adobe Photoshop Extended CS4.0 (Adobe Systems Incorporate, San Jose, CA, USA) to account for the number of crystals on each micrograph. Sample preparation consisted of applying a drop of the melted sample onto a glass microscopy slide. A coverslip was not applied to avoid crystallization in a confined environment. The samples were imaged as the sample was cooled.

Differential Scanning Calorimetry. A Q2000 differential scanning calorimeter (DSC) (TA Instruments, New Castle, Delaware, USA) was used to determine the enthalpy of the phase transition and the melting point. 8–10 mg of sample was hermetically sealed in aluminum pans, and thermograms were collected as the sample was first heated to 90 °C and isothermally held for 30 min to erase the crystal memory. The samples were then cooled at 3 °C/min to a final temperature of 30 °C to capture the exothermic phase transition. TA analysis software (TA Instruments, New Castle, Delaware, USA) was applied to integrate the transition associated with crystallization to determine the enthalpy. The onset of crystallization was determined by the observable inflection point on the collected thermograms.

Figure 2. Bright-field micrographs of 12HSA (A–C) and polarized light micrographs of stearic acid (D–F) and trihydroxystearin (G–I) in mineral oil cooled at 10 °C/min with their respective crystallization temperatures.

Figure 3. Number of nuclei on the corresponding light micrographs as the sample is nonisothermally cooled for 12HSA (A and B), stearic acid (C and D), and trihydroxystearin (E and F) in mineral oil, where time zero is the maximum cooling temperature. Symbols: ●, 0.5 K/min; ○, 1 K/min; ▲, 1.5 K/min; △, 2 K/min; ▽, 2.5 K/min; ◇, 3 K/min; ▼, 4 K/min; △, 5 K/min; ■, 6 K/min; □, 7 K/min; ◆, 8 K/min; ◇, 9 K/min; ◆, 10 K/min; ♦, 15 K/min; ★, 20 K/min.
RESULTS AND DISCUSSION

The crystallization behavior of a material is sensitive to the cooling rate. Rapid cooling increases the degree of supercooling (i.e., the crystallization temperature ($T_c$)) and increases the thermodynamic driving force. PLMs and bright-field micrographs were utilized to visualize the crystal morphology, number of crystals, and crystal size (Figures 1 and 2). Trihydroxystearin crystallized as 3-D spheres (Figure 1G and 2G), stearic acid crystallized as 2-D platelets (Figure 1D and 2D–F), and 12HSA formed 1-D crystals (Figure 1A–C and Figure 2A–C). Rapid cooling rates resulted in more nuclei to act as crystal growth sites (Figure 3) and smaller crystals (Figure 2). The crystallization process may be monitored by counting the number of nuclei present over time on the micrographs and plotting the number of nuclei versus time (Figure 3). The first derivative of the number of nuclei versus time ($\frac{\partial n}{\partial t}$) was taken to produce the rate of nucleation versus time (Figure 4). The rate of nucleation versus time (Figure 4) allows the maximum rate for nucleation to be determined for each cooling rate (Figure 3).

From Figure 4, a theoretical maximum nucleation rate ($J_{max}$) may be determined by fitting the nucleation rate ($J$) with its corresponding supercooling-time trajectory parameter ($\beta$) to an exponential decay function. $J_{max}$ corresponds to the intercept when $\beta$ is zero. By utilizing the $J_{max}$, it can be normalized and plotted as a function of both $\beta$ and $\varphi^{0.5}$ (Figure S). The data collected for stearic acid (Figure SC and D) and trihydroxystearin (Figure SE and F) was found to be inversely proportionate to $\beta$ and $\varphi^{0.5}$. This inverse relationship was previously observed for triglycerides. Previous data for 12HSA in the same solvent (Figure SA and B) indicates that two distinct regions exist with differing sensitivities to the cooling rate. There are two distinct regions for 12HSA (i.e., above and below cooling rates of $5-7^\circ\text{C}/\text{min}$). Below $5-7^\circ\text{C}/\text{min}$, no observable dependence between cooling rate and nucleation rate was observed. Therefore, the nucleation rate below $5-7^\circ\text{C}/\text{min}$ is a function of the mass transfer of crystallizing molecules to the nucleus. Above $5-7^\circ\text{C}/\text{min}$, the rate of nucleation is dependent on the cooling rate, indicating that the rate of crystallization is driven by a time-dependent thermodynamic driving force and not mass transfer. Utilizing the scaling relationships between $J_{max}$ vs $\beta$ and $J_{max}$ vs $\varphi^{0.5}$, it is possible to determine the activation energy for these systems using a statistical approach.

The polarity of these compounds in descending order would be 12HSA, stearic acid, trihydroxystearin, and TAGs. Thus, our original hypothesis would suggest that the activation energy from lowest to highest should be in the same order. The activation energies for stearic acid and trihydroxystearin were calculated to be 2.12 and 7.87 kJ/mol. The activation energies for triglycerides and 12HSA were previously reported as 24.8 and 5.40 kJ/mol. In general, the activation energy was affected by the polarity of the crystallizing molecule; however, the one exception was the lower activation energy for stearic acid compared to 12HSA. This result is counterintuitive to our original hypothesis, where 12HSA was predicted to have the lowest activation energy. As the polarity of the crystallizing molecules increased compared to that of the apolar solvent, the driving force for phase separation increased, facilitating the formation of crystal embryos.
It is speculated that the difference in polarity between triglyceride and the mineral oil would be minimal; thus, the chemical potential between the two compounds is small and, hence, the activation energy would be the largest. Therefore, a large thermodynamic force (i.e., supercooling) is required before nucleation may occur. Trihydroxystearin is slightly more polar compared to triglycerides, and thus, a higher chemical potential is observed, resulting in a lower activation energy. Stearic acid and 12HSA have the smallest activation due to their increased polarity compared to that of trihydroxystearin. Yet, differences in polarity do not explain the fact that stearic acid has a lower activation energy compared to that of 12HSA. The observed differences in activation energy between stearic acid and 12HSA could also be affected by factors which may limit adsorption of molecules to the crystal interface, such as surface diffusion, step adsorption, and incorporation into kink sites.

Stearic acid and 12HSA are both 18 carbon fatty acids; however, 12HSA has a hydroxyl group at position 12. The polar carboxylic acid head groups on 12HSA and stearic acid facilitate phase separation. Therefore, as stearic acid and 12HSA cool, the molecules form dimers, effectively shielding the polar carboxylic acid group away from the apolar solvent interface (Figure 6).

Figure 5. Dependence of the relative nucleation rate \((J/J_{\text{max}})\) versus the supercooling-time exposure \((\beta)\) for 2.5% 12HSA (A), 2.5% stearic acid (C), and 2.5% trihydroxystearin (E) in mineral oil, and the dependence of the relative nucleation rate \((J/J_{\text{max}})\) versus the cooling rate for 2.5% 12HSA (B), 2.5% stearic acid (D), and 2.5% trihydroxystearin (F) in mineral oil.

Figure 6. Molecular dimers of 12-hydroxystearic acid (A) and stearic acid (B).
When carboxylic acid groups are hidden from the crystal—solvent interface, the interfacial tension at the forming interface is reduced. For 12HSA, the formation of the dimer does not minimize the contact between the polar hydroxyl groups and solvent, which results in a higher interfacial tension compared to the case of stearic acid (Figure 6A). The increased polarity of 12HSA should translate to a lower activation energy nucleation, in part, such that a more polar compound results in higher interfacial tension and thus higher activation energy.

Formation of a crystal embryo is facilitated by enthalpic forces driven by the formation of noncovalent bonds between molecules that result in a release of heat. This driving force is countered by entropic forces attributed to the demixing encountered during nucleation. The enthalpy of the phase change was measured using DSC (Figure 7), which allowed for the entropy to be calculated (Table 1). Stearic acid, 12HSA, and trihydroxystearin had enthalpies of 774.4 ± 51.29, 974.4 ± 28.54, and 216.1 ± 29.78 J mol⁻¹, respectively. These three values were calculated to be statistically different with a 95% confidence interval. The trend in the enthalpic forces did not correlate with the trend observed in the activation energy. Trihydroxystearin has the lowest enthalpy per mole because the noncovalent interactions formed during nucleation are predominately van der Waals interactions and there is minimal hydrogen bonding associated with the hydroxyl groups. Stearic acid forms van der Waals interactions as well as hydrogen bonds between the carboxylic acid head groups. Finally, 12HSA has the highest enthalpy because in addition to the noncovalent interactions formed by stearic acid, 12HSA has additional hydrogen bonding associated with the hydroxyl groups.

From the enthalpies obtained, the entropy for demixing was calculated. When the sample reaches its crystallization temperature, the Gibbs energy is zero; therefore, it is possible to calculate the demixing entropy associated with the system:

$$\Delta G = \Delta H - T\Delta S$$  

(eq8)

where $\Delta G$ is the Gibbs energy, $\Delta H$ is the enthalpy for the phase transition, $T$ is the crystallization temperature in kelvin, and $\Delta S$ is the entropy. The entropy of demixing is associated with the phase separation in the formation of a crystal embryo. This value indicates the loss of entropy, which is thermodynamically unfavorable. The demixing entropies for stearic acid, 12HSA, and trihydroxystearin were calculated to be 2.50 ± 0.16, 2.86 ± 0.08, and 0.68 ± 0.09 J mol⁻¹ K⁻¹, respectively. Using a 95% confidence interval, stearic acid and 12HSA were calculated to not be statistically different while both were statistically different than trihydroxystearin. The entropy associated with the phase transition follows a similar trend as the activation energy associated with nucleation. This trend suggests that the phase separation process and the associated crystal-interfacial tension are central to the formation of a stable nucleus. This suggests that the ability of the molecules to arrange in such a fashion to minimize the polar groups from the interface between the apolar solvent is central in the formation of a stable nucleus, which supports the concept of a specific interaction.

### CONCLUSION

The nature of the activation energy for nucleation under nonisothermal cooling conditions was determined for 12HSA, stearic acid, trihydroxystearin, and triglycerides. It was found that the relative nucleation rate of trihydroxystearin and stearic acid were inversely proportional to the supercooling-time trajectory parameter. The activation energies for stearic acid, 12HSA, trihydroxystearin, and triglycerides were 2.12, 5.40, 21.78, and 24.8 kJ/mol, respectively.

The relative polarity influences the activation energy for nucleation, in part, such that a more polar compound results in a lower activation energy. The difference in molecular polarity explains the differences in the activation energies between triglycerides and trihydroxystearin compared to stearic acid and 12HSA. However, the difference in activation energy between stearic acid and 12HSA cannot be attributed solely to the polarity, and a specific interaction by the nucleating molecules must be accounted for. The specific interaction is the molecular arrangement within the crystal and the ability of the crystal to facilitate the depletion of polar groups from the apolar solvent at the crystal—solvent interface. The importance for the specific interaction may be observed by examining the entropy of demixing for these compounds, which is 2.50 ± 0.16 J mol⁻¹ K⁻¹, 2.86 ± 0.08 J mol⁻¹ K⁻¹, and 0.68 ± 0.09 J mol⁻¹ K⁻¹ for stearic acid, 12HSA, and trihydroxystearin, respectively. Hence, the absolute entropy values correspond well to the activation energies for these molecules.

### AUTHOR INFORMATION

**Corresponding Author**

*E-mail: rogers@aesop.rutgers.edu.*
REFERENCES

(15) Lam, R. S. H.; Rogers, M. A. CrystEngComm 2010.