Triglyceride Nanocrystal Aggregation into Polycrystalline Colloidal Networks: Ultra-Small Angle X-Ray Scattering, Models and Computer Simulation.

Fernanda Peyronel¹, David A. Pink^{2,1}, Alejandro G. Marangoni^{*1}

¹ Food Science Department, University of Guelph, Guelph, ON, Canada

²Physics Department, St. Francis Xavier University, Antigonish, NS, Canada

ABSTRACT

Triacylglycerols (TAGs) are the majority molecules present in edible fats and oils. Many of the functional characteristics of fat products depend on the colloidal fat crystal network present. Identifying the hierarchies of these colloidal networks and how they spontaneously self-assemble is important to understand their functionality and the oil binding capacity, and new insights into the nano- to meso-scale structure in these colloidal fat networks have been reported in recent years. Ultra small angle X-ray scattering (USAXS) is a technique new to the study of edible oil structures and, when combined with modelling and computer simulation, has enabled significant advances to be made in understanding the nano- to micro-scale crystalline structures of edible oils. In the four years since crystalline nanoplatelets (CNPs) were characterized, models have been made of these highly anisotropic nanoscale structures in which they were treated as the primary unit. In those models, CNPs were represented as close-packed rigid layers of spheres, so chosen because the van der Waals sphere-sphere interaction is known. The intent of the models was to predict the hierarchy of colloidal fat networks that would self-assemble from the components in edible oils. Initially, CNP aggregation was modeled under the assumption that all CNPs are present before aggregation begins and that their solubility in the liquid oil is very low. The models successfully predicted the fractal dimensions subsequently measured using USAXS. This brief review reports on some of the latest models and simulations together with the results of USAXS experiments carried out on binary lipid systems, such as SSS in OOO, as well as certain complex systems that contain many different TAG molecules. The excellent agreement between the two approaches has established that USAXS is a powerful tool in the elucidation of the nano- to meso-length scales in fats and oils.

1-INTRODUCTION

Edible fats are a class of colloidal gels in which an oleogel crystal network is formed as the triacylglyceride (TAG) molecules crystallize from the melt. Crystalline nano-platelets (CNPs) [1–6] have been found to be the basic components of the complex macro-colloidal structure of these edible fats. These CNPs aggregate to form

hierarchical structures where it is likely that low melting TAGs remain trapped in liquid state within the solid matrix. It is well known, that the macroscopic structure of the colloidal network can be changed by altering the processing conditions like undercooling temperature, cooling rates and shear [7–11]. It is of fundamental importance to understand what are the implications of having different CNPs sizes and morphologies as well as to understand how these CNPs self-assemble via nucleation and growth, and which structures they form. In these colloidal systems of solid fats particles in oil, it is likely that the aggregation of CNPs into bigger aggregates is ultimately responsible for many of the macroscopic properties, such as oil binding capacity and viscoelasticity.

A novel technique to visualize nano-crystals was developed in 2010 by Acevedo and Marangoni [2]. CNPs were successfully imaged for fully hydrogenated canola oil in high oleic sunflower oil. Rigorous sample preparation [2] which included shearing the system at 30,000 rpm followed by the use of cryo-transmission electron microscopy (TEM) revealed the existence of highly anisotropic crystalline nanoplatelets (CNPs) composed of TAG bilayers. The CNPs observed for that system possess lateral dimensions of $\sim 10^2$ - 10^3 nm and thicknesses of ~ 20 -80 nm. Those authors also showed that CNP sizes were affected by the ratio of high-melting to low-melting TAG species, as well as the degree of supersaturation, cooling rate and shear rate during the crystallization [1]. Following those first observations, other authors have shown the existence of the CNPs in lipid systems like: cocoa butter (CB) [12][4][3], fully hydrogenated soybean oil (FHSO) in soybean oil (SO) blends [13][14][15], FHCO in canola oil (CO) blends [16], tristearin(SSS) in triolein (OOO) [5] and cocoa butter substitutes [6]. The question as to whether the sample preparation for the cryo-TEM observation changed the size of the CNPs was answered by Peyronel et al. [5] when *in-situ* experiments were carried out using ultra small angle x-ray scattering (USAXS). These authors demonstrated that the SSS CNPs sizes obtained using USAXS were essentially the same as the ones observed using the Acevedo and Marangoni visualization technique.

The USAXS technique is well suited to study opaque systems and an important advantage over other technique is that it requires little sample preparation. Thus shearing is not a requirement so that its effect can be studied separately. The absolute X-ray scattering intensity is reported as a function of the magnitude of the scattering wave vector, q , which is related to the length scale, L , of the system via $L = 2\pi/q$. This technique can cover length scales from ~ 100 nm to ~ 15 μ m, well suited to study the nano- to meso-scale structures of edible fat colloidal networks. It is, however, important that the interpretation of the USAXS data be complemented with results from other experiments or by predictions from computer simulations.

The development of models which predict or identify aggregating structures can be an important aspect to the interpretation of the data. Pink and collaborators [17,18] modelled lipid CNPs as a rigid aggregate of spheres chosen so that the rigid structures representing the CNPs mimicked those observed by Acevedo and Marangoni [2]. Spheres belonging to different CNPs interacted via van der Waals sphere-sphere interaction. It was assumed that the CNPs were essentially insoluble in the liquid oils so that all model CNPs were present at the start of the simulation. Surfaces of the spheres comprising CNPs were modified as desired to represent the different scenarios that are believed to exist according to the TAGs present in the liquid-solid mixtures. These considerations are described in more detail below. Predictions for the USAXS measurements were made and later confirmed by the experiments.

This article begins with an outline of the models, their computer simulation and the predictions arising. A brief description of the system used to collect and analyze the data is presented which is followed by an account of the experimental USAXS results obtained for different lipid systems.

2-MODELS, THEORY AND COMPUTER SIMULATION

Here we are primarily concerned with describing modelling aggregation of structures which have been formed from triacylglycerol (TAG) molecules. The spatial scales range from tens of nanometers to hundreds of microns. However, molecular scale studies will be described when relevant. Here we briefly touch on the variety of techniques related to modelling aggregation. Aggregation is a wide-ranging field and involves the formation of clusters in a colloidal suspension. During this process, particles dispersed in the liquid phase stick to each other and spontaneously form particle clusters, flocs, or aggregates. The process can be driven by external controlled means such as, for example, enzymatic activity as in the case of rennet-driven aggregation of casein micelles, or via the addition of polymers to induce flocculation. The process can also be driven by the van der Waals attraction between the components of the colloidal suspension such as particles in aerosols or solid structures in oils. These processes have been modelled in essentially two ways: (i) analytically via the Smoluchovsky equation, the Langevin equation and analogous dynamical equations and (ii) models which rely upon computer simulation to elucidate the dynamics and/or static structures. Of importance to applications to solid structures in edible oils, especially at high solid concentration, is tortuosity. A number of definitions of tortuosity in 2-dimensions have been given which involve local or average curvatures, or has been defined in terms of an effective diffusion coefficient. In what follows, we focus our attention upon modelling the hierarchy of solid networks which come about via solid aggregation in edible oils.

At low solid fat content, the starting point is the existence of highly-anisotropic crystalline nanoplatelets (CNPs) first characterized by Acevedo and Marangoni [2] and the question to be addressed is: to what aggregation structures do such CNPs give rise? The models assume that a majority solid has condensed as the sample is cooled from a sufficiently high temperature to about room temperature leaving either a 1-component liquid oil or one that contains minority components. One consideration will be whether there is essentially only one solid component or whether the minority components condense together with the majority solid component, and how the majority and minority components are structurally related. Another question is whether crystal morphology plays a role in aggregation. For a given system, different solid morphologies are effectively determined by the cooling rates which give rise to the solids. If these procedures all result in essentially similar, highly-anisotropic 2-dimensional CNPs as reported by Acevedo and Marangoni, then the only physical difference between CNPs which possess different morphologies would be the slightly different van der Waals interactions due to the small differences in mass density. Mass fractal aggregates can be defined by the relationship between the mass, $M(R_g)$ and the radius of gyration, R_g , $M(R_g) = KR_g^D$. The small differences in the van der Waals interaction might affect the pre-factor, K , but not the fractal dimension, D , and so would have no effect upon the structure of aggregates. Since the models described here start with an assumption of highly-anisotropic CNP existence, then they ignore questions of crystal morphology.

We shall restrict ourselves to CNPs which have very low solubilities at the temperature of the liquid oils in which they are immersed. This important restriction is reflected in the assumption that all CNPs have been formed before significant aggregation begins and that, once they are formed, the CNPs are unchanging structures. CNPs have been modelled using coarse grained models [19,20]. In order to help understand and guide scattering experiments geared towards structural characterization [21,22] of these systems, static structure functions, $S(q)$, for the different structures that emerged from simulations, were computed as functions of wave vector magnitude q and used to predict the results of small and ultra-small angle X-ray scattering (SAXS and USAXS).

Figure 1. Models of CNPs and TAGwoods. **A:** {10, 6, 1} CNP. **B:** A {5, 1, 1} structure. **C:** Interaction between two spheres belonging to different structures. **D:** Two coated spheres. **E:** Coated CNP. **F:** Interaction energy (equation 1) between two spheres as a function of their radius, R , and centre-centre distance, r . **G:** A TAGwood formed from CNPs (upper) and its representation by a {4, 1, 1} structure (lower). Reproduced with permission from Pink, et al. J. Applied Phys., 2013, 114, 234901. Copyright 2013, AIP Publishing LLC.

(a) 2-component oil. Tristearin solids in triolein liquid oil. When an oil comprising a mix of liquid tristearin (SSS) and triolein (OOO) is cooled to room temperature, SSS condenses into solids. These are irregularly-shaped highly-anisotropic crystalline structures 100-1000 nm on a side and 50-200 nm thick. To model such structures we have to be able to calculate the van der Waals interaction between two such rigid objects an arbitrary distance apart with arbitrary relative orientation. This was achieved by representing a CNP, as shown in Figure 1A, as a rigid array of monodisperse spheres, each of radius R . Such model CNPs are described by a set of integers, $\{l, m, n\}$ where the first two specify the number of spheres in the rows of the hexagonal lattice and the third integer gives the number of layers of such lattices. Figure 1A shows a {10, 6, 1} model CNP. The advantage of this model is that one knows the analytical form of the van der Waals sphere-sphere interaction [23] and, if one makes the assumption that the total interaction between two such model CNPs is the sum of all pairwise sphere-sphere interactions with one sphere in each model CNP, then one can trivially calculate the CNP-CNP interaction for arbitrary separation and relative orientation. This is illustrated in Figure 1C where the interaction, $V_d(R, r)$, is shown. Figure 1F shows the form of the interaction.

We write the interaction between two identical homogeneous spheres, each of radius R , a centre-to-centre distance, r , apart as

$$V(r) = V_d(R, r) \quad r > 2(R + \Delta) \quad (1)$$

$$V(r) = V_B \quad r \leq 2(R + \Delta) \quad (2)$$

where Δ is the thickness of a region into which another sphere, belonging to a different CNP, cannot penetrate, and $V_B < 0$ is a binding energy. The distance, 2Δ , is approximately the nearest distance to which a pair of parallel TAGs can approach and is approximately ≥ 0.2 nm. One choice for V_B is $V_B = V_d(R, 2R + 2\Delta)$ while other choices can explore aspects of very tight binding. The interaction free energy, $V_d(R, r)$, is given by Parsegian [23,24] (Table S.3, p155) as

$$V_d(R, r) = \frac{-A}{6} \left[2R^2 \left(\frac{1}{r^2 - 4R^2} + \frac{1}{r^2} \right) + \ln \left(\frac{r^2 - 4R^2}{r^2} \right) \right] \quad (3)$$

where A is the Hamaker coefficient. Here $A \approx 10^{-20}$ J since the two spheres are immersed in OOO oil. In air or vacuum, $A \approx 5 \times 10^{-20}$ J [25]. Metropolis Monte Carlo simulation techniques [26,27] were used to obtain steady state structures. At each Monte Carlo step an attempt was made to move each object in a random sequence. Attempted moves involved spatial translation of the centre of mass and rotation by a random angle around a randomly chosen axis passing through the centre of mass. Such objects could be either CNPs or their aggregates. The range of movement of an aggregate was assumed to be proportional to $M_A^{-1/2}$ where M_A is the mass of the aggregate and was taken to be proportional to the number of spheres in that cluster. All simulations were run at a temperature of 30°C.

The disadvantage of this model is that, in common with many simple models, the interaction is not simply the sum of 2-body interactions [28].

Structure functions, $S(q)$, were computed in the usual way [29]

$$S(\vec{q}) = \frac{1}{N} |\sum_j \exp(i\vec{q} \cdot \vec{r}_j)|^2 \quad (4)$$

$$S(q) = \langle S(\vec{q}) \rangle_{|\vec{q}|=q} \quad (5)$$

where $\vec{r}_j$ is a vector to the centre of the sphere labeled j , ($1 \leq j \leq N$) and N is the total number of spheres. If the system possesses a fractal structure over a sufficient range of q , then it has been found that $S(q) \sim q^{-D}$ [21,30] where D ($D \geq 1$) is the fractal dimension of that structure.

In all realizations of this model, the sphere radius was $R = 0.5u$, where u is an arbitrary unit of length, and the simulations were carried out in cubic boxes of dimensions $L \times L \times L$ with periodic boundary conditions. The value of L was either $50u$, $100u$ or $150u$. In some cases, notably in following the relaxation from DLCA aggregates to RLCA aggregation (below), the largest values of L were required in order to unambiguously identify values of D . All CNPs were represented by $\{10, 10, 1\}$ rigid arrays of spheres and their number density, ϕ , took on values $\phi = 0.06, 0.13, 0.22$. All simulations, including those below, were initially relaxed for up to 10^5 Monte Carlo steps while production runs involved up to 5×10^6 steps with the largest being necessary in following the relaxation of structures from DLCA to RLCA (below). The values of Δ ranged over $\Delta = 0.1u$ for uncoated CNPs to $\Delta = 2u$ for coated CNPs (below).

It was predicted that CNPs would aggregate in to (1-dimensional) TAGwoods ($D = 1$), an example of which is shown in Figure 1G (upper). In order to explore their aggregation, TAGwoods were represented as rigid $\{L, 1, 1\}$ spheres, shown in Figure 1G (lower) with L taking on values $L = 2, 5, 10, 20$. TAGwood number densities $\phi = 0.0005, 0.001, 0.06, 0.13$ were used. TAGwoods were found to aggregate, via diffusion limited cluster-cluster aggregation (DLCA), into clusters with fractal dimension of 1.7-1.8, which increased to 2.0-2.1 as the system relaxed. The latter is characteristic of reaction limited cluster-cluster aggregation (RLCA) [22,29]. These clusters were found to be isotropically distributed in space with a fractal dimension of 3. The hierarchy of CNP aggregation was predicted to be as shown in Figure 2 and was confirmed by USAXS [5]. It was also predicted that, for short-time observations, which can be realized by fast cooling followed immediately by measuring $I(q)$, A and B would be observed with a random distribution ($D = 3$) of TAGwoods. For long-time observations, A-D will be observed.

Figure 2. Aggregation of uncoated CNPs ($\Delta = 0.1u$). **A:** CNPs ($D = 2$). **B:** TAGwoods ($D = 1$). **C:** DLCA $\rightarrow$ RLCA clusters ($D = 1.7=2.1$). **D:** uniformly-distributed TAGwood clusters ($D = 3$). The horizontal black line indicates the same length (approximately 1000 nm) on the different length scales. For short-time observations (fast cooling followed immediately by X-ray scattering) A and B will be observed with a random distribution ($D = 3$) of TAGwoods. For long-time observations, A-D will be observed. Reproduced with permission from Pink, et al. J. Applied Phys., 2013, 114, 234901. Copyright 2013, AIP Publishing LLC.

(b) Complex oils. Tristearin solids in complex oils. Models of CNPs in complex oils were also made and studied for which Δ ranged from $\Delta = 0.5u$ to $\Delta = 2u$. Here, the complex oils contained a component, typically SSS, which would condense from the melt at a high temperature, as in the case described above. In addition to SSS, however, the oil contained minority components combined with the majority liquid OOO component. Some of these minority components might condense at room temperature as continuous coatings or as localized nano-scale crystallites onto the surfaces of SSS CNPs and examples of this are shown in Figure 3. The question of whether minority components of the solvent oil can exhibit nanoscale coatings onto the surfaces of a CNP was addressed by [31] and we outline this below.

Figure 3. Coated CNPs and a side-coated TAGwood. **A:** uniformly coated CNP. **B:** CNP coated only on the (100) and (010) surfaces. **C:** CNP uniformly coated with crystallites. **D:** A side-coated TAGwood composed of CNPs coated only on their (100) and (010) surfaces.

Modelling coated CNPs led to a range of predictions and interpretations depending upon whether the coatings were isotropically distributed over the surface of a CNP or were preferentially attached to the sides, the (100) and the (010) surfaces, and whether the coating was "thin" or "thick". With a thick coating, the resulting aggregates could be quite different from the case of pure SSS solids in pure OOO oil as shown in Figure 1. It was found, for example, that with as little as $\sim 1\%$ of the solvent oil as minority components, a thick coating could be attained. This observation enabled one to understand why such a seemingly low concentration of minority solvent oil components could have a significant effect upon the physical properties of an edible oil. The reason that a minority component might prefer to be attached to the (100) and (010) surfaces but not to

the large (001) surface is because, on the former, the hydrocarbon chains are parallel to these surfaces with a high density of CH₂ groups. On the (001) surface, the chains are perpendicular to the surface with a lower density of CH₃ chains leading to weaker binding. Figure 4 shows the predictions for thin isotropic coatings (Figure 4A) and for thick isotropic coatings (Figure 4B).

Figure 4. SSS solids in a complex oil. Schematic log-log plot of approximate USAXS intensities, *I*, for two cases of isotropic coating of CNPs. The values of log *q* are approximate. Predicted parameters, *P* and *s* of the Unified Fit and Guinier-Porod analyses. **A:** Thin isotropic coating of oils or crystallites. Regions identified by exponents, *P*₁, *P*₂, *P*₃ and a shape-integer, *s*. **B:** Thick isotropic coating of nanoscale phase separated oils or crystallites. Regions identified by exponents, *P*₁, *P*₂ and a shape-integer, *s*. The total scattering intensity, *b*, is a sum of two curves with no non-zero slope connecting them because the coating on the CNPs is too thick to permit aggregation.

Table I shows the parameter values for *P* and *s* of the Unified Fit and Guinier-Porod analyses, respectively, predicted by the models described here. The coating can be isotropic via a uniform coating of liquid-like TAGs or a uniform coating of solid crystallites (Figure 3 A, C).

Table I. Parameters, *P* and *s*, predicted for Unified Fit and Guinier-Porod data analyses

P, s	Liquid-Liquid Uniform	Liquid-Liquid 001 & 010	Solid-Liquid Uniform
------	-----------------------	-------------------------	----------------------

THIN			
P₁	4	4	4
P₂	~1.8 to 2.1	~1.0 to 2.1 long relaxation time?	~1.8 to 2.1
S	1 + some 2	1	1 + some 2
P₃	3	3	3
THICK			
P₁	>4 (Diffuse)	4	<4 (Rough)
S	2	1	2
P₂	3	3	3

One example of the effect of anisotropic coating is shown in Figure 3D where a side-coated TAGwood has been formed from side-coated CNPs (Figure 3B). Because of the coating the lateral attractive interaction between TAGwoods is weak though the interaction between ends can remain strong. The result of this is to create end-linked TAGwoods snakes which can aggregate via their uncoated ends. The complexity of the aggregation is determined by the branching ratio: the numbers of TAGwoods which can bind together at their uncoated ends. If this number is unity i.e. only one TAGwood can bind to an end of a second TAGwood, then only 1-dimensional snakes can form and the intertwining appears to be negligible regarding structures which might contribute to oil binding capacity. If, however, this number averages between 1 and 2, then "branched snakes" can result with a corresponding increase in aggregation via intertwining. These are similar to swollen randomly branched polymers e.g. [32] which exhibit a fractal dimension of 2. An example of such an aggregate, projected onto a 2-dimensional plane, is shown in Figure 5. If this effect is realized then, computer simulations of our models shows that an additional slope yielding a P-value of $P = 2$ will be observed in the X-ray scattering intensity.

Figure 5. End-linked TAGwoods where between 1 and 2 TAGwoods can be linked to an end of a third TAGwood. The 3-dimensional structure is projected onto a plane. The fractal dimension of the TAGwoods is 1 and that of the 3-dimensional aggregate of TAGwoods is 2, similar to that of a swollen randomly branched polymer.

(c) Recent models of TAGs using atomic scale molecular dynamics. The possibility that coating of CNP surfaces can take place on a nanoscale in oils which contain minority components was studied in [31]. They considered a flat surface representing an SSS crystal in contact with two liquid oils, comprising approximately 90% OOO with either 10% elaidin-diolein or 10% olein-dielaidein. They used atomic scale molecular dynamics [33,34] at room temperature in the NVT ensemble, and compared the free energies of (i) random mixes of the two samples and (ii) samples in which the minority components were assumed to be in contact with the SSS surface with the OOO removed from contact. They found that the free energy of the sample with olein-dielaidein in contact with the SSS surface lay significantly lower than the random mix of these two oils but that the free energies of the samples with elaidin-diolein on the SSS surface or randomly mixed with OOO were equal within the confidence limits. They concluded that the sample with two chains containing *trans* double bonds could coat the SSS surface. Atomic scale models studied by molecular dynamics have been used to study condensation in single-component TAGs with all-trans chains. A recent simulation [35] investigated the stability of OOO between a pair of parallel surfaces representing two SSS crystalline nanoplatelets. The intent was to understand “oil binding capacity”, an important concept regarding the ability of fats particles to retain oil. They found that OOO would be expelled from between the SSS nanoplatelets so that pure SSS would display essentially zero oil binding capacity for OOO. However, if the surfaces of the nanoplatelets were made “soft” through, for example, a coating of a minority fluid component as assumed in the models described above, then OOO was retained between the nanoplatelets. This provided some impetus for the work described above [31] in which phase separation on a nanoscale was shown to exist. Measurements not unrelated to these simulations, used a surface force apparatus with two mica surfaces, to measure adsorption of (anhydrous) OOO oil in contact with modified mica surfaces. Studies of OOO oil between mica surfaces coated with phospholipids via self-assembly [36,37] [32, 33] found that this changed the mica surface from hydrophilic to hydrophobic resulting in OOO exhibiting no order at the surface. The results of MacDougall et al [31] are in accord with that.

Simulations such as these involving edible oils have been growing in importance [38]. Brasiello et al. [39] developed coarse-grained models to study TAG liquid-solid phase transitions and applied it to tridecanoin. The models represent 3 atoms (CH_3 , CH_2 , CH , N , O) by single coarse-grained atoms and three models were

developed which differentiate, more or less, between the groups. The intent was to discover to what extent one can reduce the complexity of such models and successfully predict phase transition temperatures and density changes at phase transitions. Only the T4 model was able to do so. Such models might lend themselves to supporting the assumptions of coating onto CNP surfaces assumed to come about in the models described above. Earlier work on TAGs was in [40] where a NERD force field and Gaussian quantum mechanical computations for the polar groups of the glycerol core, were used to compute thermodynamic quantities which were found to be in good agreement with those measured. Hall et al. [41] modelled tripalmitin(PPP) and OOO in an attempted to observe the liquid-to-crystalline phase transition in the former. Although the results are encouraging and suggest the structure of the crystalline phase, they do not compute the X-ray scattering to show they have achieved a sufficient approximation to crystallinity. Hsu & Violi [42] were concerned with the thermal stability of various TAGs when used as high-strength lubricant films. They defined stability as "the ability of TAGs to stay in a disordered phase during the cooling process" and found that this was related to the order-disorder transition temperature.

3- X-RAY MEASUREMENTS

USAXS is an X-Ray technique that enables to explore opaque colloidal systems to characterize, among other things, the size of scatterers and their fractal structures. The USAXS range is shown besides the wide angle X-ray scattering, WAXS and the small angle X-ray scattering, SAXS in Figure 6, where a sample USAXS scattering curve is shown in the inset. The range of q vectors in the USAXS region corresponds to spatial scales ranging from about ~ 100 nm to $15\mu\text{m}$. Since USAXS covers decades, the intensity is typically displayed in a log scale, and the same is done with the q scattering vector.

Figure 6. Intensity as a function of the magnitude of the scattering wave vector q showing the regions covered by USAXS, SAXS and WAXS. The insert shows an X-ray scattering pattern in the USAXS region for a triacylglycerol mixture of 20% SSS in OOO.

(a) USAXS equipment

This section covers a brief description of the equipment use at the Advanced Photon Source (APS), Argonne National Laboratory. The X-ray scattering experiments were performed using a Bonse-Hart camera [43,44]. For the edible fat experiments, the 1D-collimated USAXS mode was used [45] and is shown schematically in Figure 7. The Bonse-Hart instrument uses two sets of crystals pairs; the collimating pair, located in the beam path before the sample, and the analyzer pair, located after the sample and before the photodiode detector (Figure 7).

Figure 7. Schematic of the USAXS (adapted from [46]) experiment at APS for 1-D collimation used on beam-line 15 ID at the Advanced Photon Source.

The USAXS geometry utilizes multiple crystal reflections to provide ultra high resolution only in the vertical direction. This allows for the study of microstructure sizes from a few nm to 10-20 μm . The 1-D geometry arrangement introduces smearing along the horizontal axis, which means that the data requires a numerical desmearing procedure [47] to recover the differential scattering cross section. The length scale, L , of the material probed by a given value of q can be computed from $L = 2\pi/q$. The Bonse-Hart instrument permits the collection of a sufficient number of data points in only a few minutes to cover the range $100 \text{ nm} < L < 10\text{-}30 \text{ }\mu\text{m}$.

At the APS, the instrument control and data acquisition are performed using the Spec software for diffraction experiments. Each data point is a single measurement for either 0.5, 1, or 2 seconds, as one goes from lower to higher q . *Indra*, an IgorPro-based software [48] is used for data reduction, removal of multiple scattering, and to obtain the absolute intensity [45].

(b) Software and Interpretation of the Fitting Parameters

The *Irena* IgorPro-based software [48] is used for data interpretation using both the Unified Fit [49,50] and the Guinier-Porod models [51]. Both models employ a non-linear regression analysis to find the best parameters. The analysis proceeds by identifying a number of “levels” - regions of q - space characterized by the values of two parameters: (i) a parameter P related to the slope of the curve and a fractal dimension, D , and (ii) a parameter R_g , which identifies the average radius of gyration of the scatterers giving rise to a structure with the fractal dimension D [49,50]. In practice, one can find from 2 to 5 or 6 levels [52,53].

In fat and oils systems, Unified Fit is used to find a maximum of 3 slopes and two radii of gyration for level 1 and level 2. The Guinier-Porod model is used to analyze the shape of the scatterers[16].

For an ideal two-phase system with a well defined interface, Porod's law predicts [54] that as q goes to infinity, $I(q)$ decreases as q^{-4} for large q , where the value of -4 for the slope represents the 2-dimensional surface of a smooth object. When $\ln I(q)$ is linear over a sufficiently large range of q , the material possesses a fractal structure [29] and the exponent P is interpreted as follows [53,55] : If $3 \leq |P| < 4$ then $6 - |P|$ is the surface fractal dimension, D_s ($2 < D_s \leq 3$). When $1 \leq |P| < 3$, then the value of $|P|$ is a mass fractal dimension, $D_m = |P|$. However, if $|P| > 4$, all that is known is that the surfaces of the aggregating particles are diffuse.

(c) Experiments

This section summarises the work of 5 papers. Results for two binary lipid system are shown first (Experiment 1 and 2), followed by the results of two pure solid lipids (Experiment 3) and finishes with the results for three complex lipid systems (Experiment 4).

Experiment 1

This first experiment involved SSS in OOO, studied for concentrations of 5, 10, 15 and 20% w/w of SSS [56]. This is one of the simplest lipid solid-liquid systems because at room temperature all the SSS is in its solid state.

The parameters reported from the fittings of the data were: (1) the values of s from the Guinier-Porod model, (2) slopes from the Unified Fit model and (3) radii of gyration from the Unified Fit model. Radii of gyration was used to either compute the diameter of a sphere ($s = 2$) or the length and diameter of a cylinder ($s = 1$). The data and the best fit obtained for 20% SSS in OOO at a slow cooling rate of crystallization of 1 deg/min, and after storage of 22 days at room temperature ($\sim 22^\circ\text{C}$), are shown in Figure 8.

Figure 8. Absolute USAXS intensity as a function of scattering vector for a sample containing 20% SSS in OOO. The grey line shows the fitting using the Unified Fit model while solid black lines show slopes obtained. d_1 and d_2 are dimensions of structures obtained from radii of gyration. Inset shows a cryo-TEM image of the material. See text for more details.

At the smallest length scale, or in the q -region $2 \times 10^{-3} < q < 2 \times 10^{-2}$ the morphology of the surface of the CNPs is obtained from the slope, while the size of the CNPs is obtained from the radius of gyration obtained from the curvature of the line. The fundamental structures were shown to range in length between ~ 110 nm and ~ 430 nm. These numbers are in close agreement with the values observed for the CNPs using cryo-TEM, (inset of Figure 8) for which a length between 60 nm to 450 nm was obtained. $P_1 = 4$ indicates a smooth surface, which gives a surface fractal dimension of $D_{s1} = 2$, in agreement with the (001) surface of CNPs observed under cryo-TEM.

Results from the Guinier-Porod model gave a value of $s = 1$ for the region, $4 \times 10^{-4} < q < 2 \times 10^{-3} \text{ \AA}^{-1}$ indicating the presence of cylinders. This was in accord with predictions of the model (above) that the CNPs would aggregate into cylinders, which were named TAGwoods. The TAGwoods are formed by the stacking of CNPs via their (001) faces. This was especially noticeable when the sample was cooled rapidly (Figure 9) which was manifested in a value of $P_2 = 1$ and predicted by the computer simulations (Figure 4B-b and Table 1). The simulations also predicted that the length of TAGwoods, shown as L in Figure 8 got smaller as the fraction of solids increased [18]. This was observed in the samples containing 5, 10 and 15% SSS in OOO but not shown here [57]. When the system was given more time to both aggregate and to relax, by using a slow cooling rate,

the TAGwoods aggregated and formed structures with a mass fractal value in accord with DLCA/ RLCA, $1.8 < D_{m2} < 2.1$ [29,58,59] as shown in the intermediate q region of Figure 8. Similar fractal dimensions were obtained for other concentrations (5, 10 and 15 SSS in OOO) [57]. Both the presence of TAGwoods and the fractal structures obtained from their aggregation were in accord with the predictions of modelling and computer simulations [5,18].

The smallest q region, $q < 4 \times 10^{-4} \text{ \AA}^{-1}$, gave $P_3 = 3$ for the case of slow cooling (Figure 8). These values correspond to a mass fractal dimension $D_{m3}=3$, which indicates that on the average, the aggregates are uniformly distributed in space. This result was predicted by the computer simulations on models of TAGwoods aggregation [5,18]. However, when the system was analyzed under a fast cooling rate (30 deg/min), a value of $P_3 = 3.8$ was obtained. This indicates a surface fractal dimension of $D_{s3}=2.2$, for which no radius of gyration was measured as the dimensions were larger than the smallest q value observed.

Experiment 2

In order to learn how the cooling rate affected the CNPs' formation and subsequent aggregation, 20% SSS in OOO and 20% PPP in OOO were studied under two different cooling rates: (1) a fast cooling rate of 30 deg/min and (2) a slow cooling rate of 0.5 deg/min (Figure 9). PPP was studied and compared with SSS in order to see if the 16 carbons present in each of the 3 fatty acids (FA) in the PPP molecule caused a difference when compared with the 18 FA in the SSS molecule. Not surprisingly, the results were the same. Both samples showed $P_1=4$, but the radius of gyration for the slow cooled case $\sim 430 \text{ \AA}$, was almost twice as large as the fast cooling case, $\sim 240 \text{ \AA}$. These results indicate that the CNPs created via a fast cooling rate are smaller than the ones obtained via a slow cooling rate regardless whether SSS or PPP was used.

Figure 9 Absolute USAXS intensity as a function of scattering vector q for two samples cooled from the melt to 20°C at 30 deg/min (o) and at 0.5 deg/min (+). (A) 20% SSS in OOO (B) 20% PPP in OOO. $P_1 = 4$ for all four cases, while $P_2 = 1$ for the fast cooled samples and $1.8 < P_2 < 2.1$ for the slow cooled ones. $P_3 = 3$ for the SSS slow cooled sample while $P_3 = 3.3$ for the PPP slow cooled sample. $P_3 = 3.8$ for both samples cooled at a fast cooling rate.

Both PPP and SSS results showed that the P_2 value depends on the cooling rate. The $P_2 = 1$ result for the fast cooling case indicated the presence of TAGwoods, while the $1.8 < P_2 < 2.1$ result for the slow cooling case indicated that aggregation took place via DLCA or RLCA [29]. The interpretation given to the existence of TAGwoods and the DLCA aggregation was predicted by the modelling and the computer simulations (above).

For the smallest q range P_3 was either 3 (SSS) or 3.3 (PPP) for the slow cooling case or, 3.8 for the fast cooling case for both SSS and PPP.

Peyronel et al. [57] also showed that the slow cooled SSS sample re-arranged over time to give a slope $P_2 = 2.0$ that extended beyond the smallest q value detected by the instrument. This suggests that the clusters made of TAGwoods that were originally uniformly distributed in space had merged into bigger aggregates, keeping in line with DLCA as the slope $P_2=2.0$ was unchanged.

Experiment 3

The study of two pure systems was carried out by performing experiments on a single solid lipid: pure SSS and pure PPP [56]. This experiment was needed to understand the structures that come about when there is no liquid oil present in between the solid colloidal network. X-Ray scattering is due to a difference between the electron densities of at least two of the components that make the sample. Since the purity of both SSS and PPP was better than 99 %, in order to see scattering, voids must be present in the solid network. From a simulation point of view, no solidification theory was explicitly considered [56]. The system was viewed as forming grains by following the grain-growth kinetics of normal coarse-grained matter, in accord with the model dynamics of Smith[60] and Burke [61,62] and described by Humphreys and Hatherly [63]. The simulations took into account that the solids were created based upon the existence of solid seeds. The growth kinetics took into account that the grain boundaries moved in accordance with forces derived from their curvatures, which were determined by the average radius of curvature. The structure function was computed and compared with the results from the experiments. Two levels of structure were detected using the Unified Fit model as shown in Figure 10.

Figure 10 Absolute scattering intensity at room temperature ($\sim 24^\circ\text{C}$) as a function of q in a log-log graph for 100% SSS (+) and 100% PPP ($\blacktriangle$) prepared from the melt and after 22 days of storage at room temperature. $P_1 = 3.9$ for SSS while $P_1 = 3.8$ for PPP. $P_2 = 3$ for both cases.

The average volume of the nano-voids was characterized by a radius of gyration R_{g1} of about 370 ± 20 nm for SSS and 437 ± 28 nm for PPP [56]. The P_1 values of 3.9 and 3.8 for SSS and PPP respectively gave a surface fractal dimension $D_s = 6 - |P|$ of 2.1 and 2.2. This surface fractal dimension characterizes the surface of the grain boundaries or the walls of the nano-voids. The structural information obtained from the radius of gyration was used to give an estimation of the average size of the surface that makes up the grain walls. The dimensions of the grain boundaries must necessarily, on average, correspond to those of the grains that make up the solid. Assuming that the shape of the “holes” is rectangular, the sides were computed as to have lengths between 650 nm and 1070 nm. These numbers confirm that the nano-voids’ sizes are in accordance with the values obtained for CNPs sizes using USXAS when less than 20% solids were present [5].

The second slope observed for $q < 4 \times 10^{-4} \text{ \AA}^{-1}$ using the Unified Fit model gave $P_2 \sim 3.0$. This value indicates a uniform distribution of the nano-voids through out the material, a result predicted by the computer simulations. The simulations predicted a broad localized peaks at $q \approx 0.32 u^{-1}$, where u indicates the units used to define the simulation space that gave the size of the grain boundaries which matched the observed 650 nm and 1070 nm using USAXS.

The models, computer simulations, computer predictions and the experiments carried out on the pure solids materials had shown that it is necessary to complement these two techniques in order to correctly interpret the meaning of the slopes.

Experiment 4

This section presents the results of experiments carried out on three different mixtures made with a combination of solid and liquid lipids.

Three different lipid systems were studied: (1) partial hydrogenated canola oil (HPCO) in OOO (2) SSS with shea butter and high oleic sunflower oil (HOSO) (3) SSS and OOO with cotton seed oil [57]. The data and the fittings using the Unified Fit model for these three systems are shown in Figure 11. Values of the radius of gyration, R_{g1} for all three cases were in the range of ~ 230 Å to ~ 800 Å while the R_{g2} were in the range between ~ 2000 Å to ~ 7000 Å. These values are average values that by no means indicated a fixed size of either a CNP or the aggregates made with these CNPs.

Figure 11 Absolute scattering intensity at room temperature ($\sim 24^{\circ}\text{C}$) as a function of q in a log-log graph showing the scattering of (A) PHCO in OOO, 48 hours after preparation (B) 20 % SSS with 16 % Shea and 64 % HOSO, 48 hours after preparation (C) 15 % SSS with 51 % Cotton Seed Oil and 34 % OOO, 6 days after preparation.

Experiment 4 - Discussion of P_1 results

To interpret the P_1 results, it was necessary to look not only at the computer simulation predictions (Table 1) but also at the TAGs making up the mixtures analyzed.

Case (A): Figure 11A - HPCO in OOO. The results of $P_1=3.8$ was interpreted as crystallites formed on the surfaces of the CNPs, making it appear rough, hence a value of P_1 smaller than 4 (Table 1). The solids present in PHCO are a mixture of SSS, SSP, PPS and SS_C-20:0 [57]. It is believed that the SSS, which is present in the highest percentage, will form the CNPs and the other solids will attach to its surface. The value of $P_1=3.8$ is in accord with the computer simulation for the case in which solid-liquid nano-phase separation takes place due to minority materials attaching to the surfaces of the solid CNPs [64].

Case (B): Figure 11B. The case of SSS with shea butter and HOSO falls into the case of CNPs coated with a soft deposition of TAGs, as it correlates with the predictions of the computer simulations [64] for a "soft" or diffuse thick layer regime (Table 1). It is then speculated that this soft layer was made up by the TAGs containing 2 unsaturated hydrocarbon chains, as there is 15% present in the mixture [57].

Case (C): Figure 11C. The P_1 value for the case of SSS with OOO and cotton seed oil was found to be higher than 4, which suggested that the CNPs surfaces are at least partially diffuse (Table 1). Based on the computer simulations, a diffuse surface on the CNPs is indicative of either (1) that there is an isotropic coating on all the CNP faces or (2), that the coating is anisotropically distributed only on the 100 and 010 surfaces of the CNP. In order to decide which case is favourable, the TAGs composition must be looked at. The system study in case c contained 15 % TAGs with 3 saturated hydrocarbon chains, 6.3 % of TAGs with 1 unsaturated hydrocarbon chain, 25.3 % TAGs with 2 unsaturated hydrocarbon chains and 49.5 % TAGs with 3 unsaturated hydrocarbon chains. It was assumed that the TAGs with 1 unsaturated hydrocarbon chain bind to the CNP surface first, followed by TAGs with 2 unsaturated fatty acids, thus resulting in a "solid TAG" density gradient from the solid surface into the solvent (OOO).

Experiment 4- Discussion of P_2 results

The values obtained for the parameter s from the Guinier-Porod model was 1.5 for case (A), 1.2 to 1.0 for case (B) and 1.0 for (C). Case (A) suggests that the system contains a mixture of TAGwoods and CNPs, or that the TAGwoods are in the process of becoming “super-platelets” by stacking side by side. This explanation would be in accord with the idea of having CNPs dotted with smaller crystallites that would either prevent a perfect side by side aggregation or an aggregation all together. For cases (B) and (C) the most plausible interpretation is that coated TAGwoods are forming 1-dimensional strings comprising TAGwoods connected end-to-end. The coated TAGwoods would appear since the CNPs were coated themselves as discussed previously for the P_1 value. The results for the case (B) in Figure 11 were the same ones obtained for samples stored for 8 months [57] at 5°C. These two observations at different storage time seem to suggest that once the coated TAGwoods are formed, they remain in that state without any farther aggregation.

Experiment 4 - Discussion of P_3 results

The slope observed for the q -region $< 5 \times 10^{-4} \text{ \AA}$ showed a $P_3 = 4.1$ for the three cases (Figure 11 A, B, C). This value of P indicates a diffuse 2-dimensional surface on the larger scale. It was proposed that the long TAGwood attached end to end, forming strings that intertwined dynamically so as to look like a diffuse surface. No further computer simulations results were available at the time of the writing of this manuscript.

4-CONCLUSIONS

This article shows the importance of complementing USAXS results with computer modelling and simulations. The simulations were carried out by first creating the primary units to then allow them to aggregate by using only van der Waals interactions. Predictions of the intensity of the X-ray scattering were made and excellent agreement with the experiments was obtained. The interpretation of the USAXS experiments via the computer simulations suggest that the processing condition and the TAGs present in the material dictate the morphology of the CNPS and the patterns that emerge from the CNPs aggregation.

The following general conclusions can be drawn:

1- Models of SSS CNPs in OOO (liquid) oil interacting via van der Waals forces, predicted that the hierarchy of aggregation after long time relaxation to steady states was : (i) highly anisotropic CNPs ($D_s = 2$) formed 1-dimensional TAGwoods ($D_m = 1$). (ii) TAGwoods aggregated via DLCA/RLCA to form structures with $D_m = 1.7$ to 2.1. (iii) these structures filled space uniformly there by exhibiting a fractal dimension, $D_m = 3$.

2- For fast cooling experiments, the models predicted that only TAGwood formation ($D_m = 1$) would be observed, and not TAGwood aggregation, for which there would have been insufficient time to take place. Accordingly, the TAGwoods will be uniformly distributed in space with a mass fractal dimension $D_m = 3$.

3- For SSS CNPs in complex oils, a multitude of aggregation structures can be realized depending upon whether and how nanoscale phase separation takes place on the surfaces of SSS CNPs. Some predictions have been made for the cases of isotropic uniform phase separation and non-isotropic phase separation on (100) and (010) surfaces. These effects will be observed for as little as ~1 % of the solvent oil being in the form of nanoscale phase separable moieties. This enables us to understand how small quantities of such components can have significant effects via their nanoscale phase separation at CNP surfaces.

4- Using the Unified Fit model, Level-1 analysis showed that the scatterers were 2-dimensional objects with either a smooth, a rough, or a diffuse surface. These 2-D objects had an average radius of gyration, R_{g1} , between 200 Å to 1500 Å.

5- Level-2 Unified Fit analysis displayed values of P_2 between 1 and 2, while the Guinier-Porod model gave values $1 \leq s \leq 1.5$. A value of $s = 1$ indicated the presence of 1-D objects, the TAGwoods. A value of $s = 1.5$ indicates either a mixture of un-aggregated CNPs and deformed TAGwoods or incomplete side-by-side TAGwood aggregates. Based on the predictions from computer simulations and confirmed by the experiments, the TAGwoods aggregated into fractal structures with a mass fractal dimension $1.8 \leq D_{m2} \leq 2.0$ only when they are formed by CNPs with clean surfaces and after the system is given enough time to relax. These structures are characteristic of diffusion or reaction limited cluster-cluster aggregation (DLCA/RLCA). When the TAGwoods have thickly coated sides, then there seems to be no aggregation, even after 8 months of storage with a P_2 value that remains at 1.

6- Level-3 Unified Fit analyses showed that the aggregates: (i) could be evenly distributed in space, (ii) could exhibit a rough surface or (iii) could exhibit a diffuse surface. The evenly distributed aggregates of TAGwoods appeared when CNPs had clean surfaces and the system had time to relax.

7- Fast versus slow cooling in SSS/OOO mixtures: (i) CNPs were bigger in sizes for slow cooling samples according to their radii of gyration, (ii) slow cooling samples showed TAGwoods that aggregate as in DLCA/RLCA. (iii) The TAGwood aggregates were uniformly distributed in space up to 22 days after sample preparing. (iv) 2 years after sample preparation, the TAGwood aggregates had merged into even larger ones with dimensions not possible to be seen by the current set up of the Bonse-Hart instrument at APS.

8- From the nanoscale phase separation simulations, the results suggested a mechanism whereby many-component oils undergo phase separation on a nanoscale so as to create a transition oil region between the surface of the CNP and the bulk oil phase. The TAG molecules containing the most saturated hydrocarbon

chains will attach first to the CNP surface, followed by those containing less saturated hydrocarbon chains in such a way as to create a “soft” interface between the solid and the liquid surrounding it.

9- Nanoscale voids (nanovoids) were observed in pure SSS and PPP. The observations revealed that the nanovoid wall sizes were in agreement with the observed CNP’s sizes for SSS and PPP.

USAXS is a well-established technique that has found a new use in advancing the studies to characterize the nano- to micro-scale structures in edible oils. The latest challenge for the fat industry has been to replace unhealthy fats by new ones that can sustain the same functionality. However the functionality has been hampered by oils that leak out of the colloidal fat networks. USAXS has proven to be a powerful technique that enables the characterization of lipid system’s structure at a length scale where the oil gets trapped. With the help of models and predictions using computer simulations, an interpretation of the 3-D structures that emerge from the aggregation of the CNPs is now possible. This understanding is essential in the quest to keep the oil trapped in the colloidal fat network. The results shown here will help the industry make judicious decisions about the correct lipid systems to be used for a desired functionality. As we continue to characterize new fats, a library of CNPs sizes and mesoscale structures will emerge and a replacement of one fat for an alternative will be made possible.

ACKNOWLEDGEMENTS

It is a pleasure for DAP to thank Bonnie Quinn, Shajahan Razul and Charles Hanna for their collaborations.

Thanks also to NSERC for grants and to ACEnet for ample computing time and technical advice. ChemMatCARS

Sector 15 is principally supported by the National Science Foundation/Department of Energy under grant

number NSF/CHE-0822838. Use of the Advanced Photon Source was supported by the U. S. Department of

Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-06CH11357.

- [1] Acevedo NC, Marangoni AG. Toward Nanoscale Engineering of Triacylglycerol Crystal Networks. *Cryst Growth Des* 2010;10:3334–9.

- [2] Acevedo NC, Marangoni AG. Characterization of the Nanoscale in Triacylglycerol Crystal Networks. *Cryst Growth Des* 2010;10:3327–33.
- [3] Maleky F, Marangoni AG. Nanoscale effects on oil migration through triacylglycerol polycrystalline colloidal networks. *Soft Matter* 2011;7:6012–24.
- [4] Maleky F, Smith AK, Marangoni A. Laminar Shear Effects on Crystalline Alignments and Nanostructure of a Triacylglycerol Crystal Network. *Cryst Growth Des* 2011;11:2335–45.
- [5] Peyronel F, Ilavsky J, Mazzanti G, Marangoni AG, Pink DA. Edible Oil Structures at Low and Intermediate Concentrations: II. Ultra-Small Angle X-Ray Scattering of in situ Tristearin Solids in Triolein. *J Appl Phys* 2013;114:234902.
- [6] Peyronel F, Marangoni A. In search of confectionary fat blends stable to heat: Hydrogenated palm kernel oil stearin with sorbitan monostearate. *Food Res Int* 2014;55:93–102.
- [7] Maleky F, Acevedo NC, Marangoni AG. Cooling rate and dilution affect the nanostructure and microstructure differently in model fats. *Eur J LIPID Sci Technol* 2012;114:748–59.
- [8] Maleky F. Oil Migration Through Fats - Quantification and Its Relationship to Structure. In: Marangoni AG, editor. *Struct. Anal. Edible Fats*, Urbana, IL: AOCS Press; 2012, p. 207–30.
- [9] Herrera ML, Hartel RW. Effect of Processing Conditions on Crystallization Kinetics of a Model Milk Fat System. *J AOCS*, 2000;77:1177–87.
- [10] Toro-Vasquez JF, Dibildox-Alvarado E, Herrera-Coronado V, Charó-Alonso M. Triacylglyceride Crystallization in Vegetable Oils: Application of Models, Measurements, and Limitations. In: Widlak N, Hartel R, Narine S, editors. *Cryst. Solidif. Prop. Lipids*. 1st ed., Champaign, IL, USA: AOCS Press; 2001.
- [11] Martini S, Cardona J a. R, Ye Y, Tan CY, Candal RJ, Herrera ML. Crystallization Behavior of High-Oleic High-Stearic Sunflower Oil Stearins Under Dynamic and Static Conditions. *J Am Oil Chem Soc* 2013;90:1773–86.
- [12] Maleky F, Marangoni A. Thermal and Mechanical Properties of Cocoa Butter Crystallized under an External Laminar Shear Field. *Cryst Growth Des* 2011;11:2429–37.
- [13] Acevedo NC, Block JM, Marangoni AG. Critical laminar shear-temperature effects on the nano- and mesoscale structure of a model fat and its relationship to oil binding and rheological properties. *Faraday Discuss* 2012;158:171.
- [14] Acevedo NC, Block JM, Marangoni AG. Unsaturated Emulsifier-Mediated Modification of the Mechanical Strength and Oil Binding Capacity of a Model Edible Fat Crystallized under Shear. *Langmuir* 2012;28:16207–17.
- [15] Acevedo NC, Marangoni AG. Functionalization of Non-interesterified Mixtures of Fully Hydrogenated Fats Using Shear Processing. *Food Bioprocess Technol* 2014; 7: 575-587.
- [16] Maleky F, Acevedo NC, Marangoni AG. Cooling rate and dilution affect the nanostructure and microstructure differently in model fats. *Eur J Lipid Sci Technol* 2012;114:748–59.

- [17] Pink DA. Intercrystalline Interactions. In: Marangoni AG, editor. Struct. Anal. Edible Fats, AOCS; 2012.
- [18] Pink DA, Quinn B, Peyronel F, Marangoni AG. Edible Oil Structures at Low and Intermediate Concentrations: I. Modelling, Computer Simulation and Predictions for X-ray Scattering. J Appl Phys 2013;114:234901.
- [19] Muller M, Katsov K, Schick M. Biological and synthetic membranes: What can be learned from a coarse-grained description? Phys Rep 2006;434:113–76.
- [20] Brini E, Algaer EA, Ganguly P, Li C, Rodriguez-Ropero F, van der Vegt NFA. Systematic coarse-graining methods for soft matter simulations – a review. Soft Matter 2013;9:2108.
- [21] Zhang F, Ilavsky J. Ultra-Small-Angle X-ray Scattering of Polymers. Polym Rev 2010;50:59–90.
- [22] Jullien R. From Guinier to fractals. J Phys I Fr 1992;2:759–70.
- [23] Parsegian VA. Van der Waals forces : a handbook for biologists, chemists, engineers, and physicists. Cambridge ; New York: Cambridge University Press; 2006.
- [24] Hamaker HC. The London-Van der Waals attractions between spherical particles. Physica 1937;4:1058.
- [25] Hunter RJ. Foundations of Colloid Science. vol. 2nd. Oxford, UK: Oxford University Press; 2000.
- [26] Binder K, Heermann D. Monte Carlo Simulation in Statistical Physics: An Introduction. vol. 3rd. Springer; 1997.
- [27] Landau DP, Binder K. A Guide to Monte Carlo Simulations in Statistical Physics. vol. 2nd. Cambridge, UK.: Cambridge University Press; 2000.
- [28] Dzyaloshinskii IE, Lifshitz EM, Pitaevskii LP. General Theory of van der Waals' Forces. Sov Phys Uspekhi 1961;73:153.
- [29] Viscek T. Fractal Growth Phenomena. vol. 2nd. Singapore: World Scientific;; 1999.
- [30] Matsushita M. Experimental observations of aggregations. In: Avnir D, editor. Fractal Approach to Heterog. Chem. Surfaces, Colloids, Polym., Chichester: Wiley and Sons; 1989.
- [31] MacDougall CJ, Shajahan Razul M, Papp-Szabo E, Peyronel F, Hanna CB, Marangoni AG, et al. Nanoscale characteristics of triacylglycerol oils: phase separation and binding energies of two-component oils to crystalline nanoplatelets. Faraday Disc 2012;158:425–33.
- [32] Janssen H-K, Stenull O. Scaling Exponents for a Monkey on a Tree: Fractal Dimensions of Randomly Branched Polymers. PhysRevE 2012;85 051126.
- [33] Hess B, Kutzner C, van der Spoel D, Lindahl E. GROMACS 4 : Algorithms for Highly Efficient , Load-Balanced , and Scalable Molecular Simulation. J Chem Theory Comput 2008;4:435–47.
- [34] Berendsen HJC, van der Spoel D, van Drunen R. GROMACS: A Message-Passing Parallel Molecular Dynamics Implementation. Comput Phys Commun 1995;91:43–56.

- [35] Razul MSG, MacDougall CJ, Hanna CB, Marangoni AG, Peyronel F, Papp-Szabo E, et al. Nanoscale Characteristics of Molecular Fluids in Confined Spaces: Triacylglycerols Oils. *Food Funct* 2014.
- [36] Dedinaite A, Claesson PM, Campbell B, Mays H. Interactions between Modified Mica Surfaces in Triglyceride Media. *Langmuir* 1998;14:5546–54.
- [37] Dedinaite A, Campbell B. Interactions between Mica Surfaces across Triglyceride Solution Containing Phospholipid and Polyglycerol Polyricinoleate. *Langmuir* 2000;16:2248–53.
- [38] Brasiello A, Russo L, Siettos C, Milano G, Crescitelli S. Multi-Scale Modelling And Coarse-Grained Analysis Of Triglycerides Dynamics. *Comput Aided Chem Eng* 2010;28:625–30.
- [39] Brasiello A, Crescitelli S, Milano G. Development of a coarse-grained model for simulations of tridecanoin liquid–solid phase transitions. *Phys Chem Chem Phys* 2011;13:16618–28.
- [40] Sum AK, Biddy MJ, Pablo JJ De, Tupy MJ. Predictive Molecular Model for the Thermodynamic and Transport Properties of Triacylglycerols. *J Phys Chem B* 2003;107:14443–51.
- [41] Hall A, Repakova J, Vattulainen I. Modeling of the Triglyceride-Rich Core in Lipoprotein Particles. *J Chem Phys B* 2008;112:13772–82.
- [42] Hsu W, Violi A. Order - Disorder Phase Transformation of Triacylglycerols : Effect of the Structure of the Aliphatic Chains. *J Chem Phys B* 2009;113:887–93.
- [43] Ilavsky J, Allen AJ, Levine LE, Zhang F, Jemian PR, Long GG. High-energy ultra-small-angle X-ray scattering instrument at the Advanced Photon Source. *J Appl Cryst* 2012;45:1318–20.
- [44] Ilavsky J, Jemian PR, Allen AJ, Zhang F, Levine LE, Long GG. Ultra-small-angle X-ray scattering at the Advanced Photon Source. *J Appl Cryst* 2009;42:469–79.
- [45] Long GG, Jemian PR, Weertman JR, Black DR, Burdette HE, Spal R. High-resolution small-angle X-ray scattering camera for anomalous scattering. *J Appl Cryst* 1991;24:30–7.
- [46] Ilavsky J, Zhang F, Allen A, Levine L, Jemian P, Long G. Ultra-Small-Angle X-ray Scattering Instrument at the Advanced Photon Source: History, Recent Development, and Current Status. *Metall Mater Trans A* 2013;44:68–76.
- [47] Lake JA. An Iterative Method of Slit-Correcting Small Angle X-ray Data. *Acta Crystallogr* 1967;23:191–4.
- [48] Ilavsky J, Jemian PR. Irena: tool suite for modeling and analysis of small-angle scattering. *J Appl Cryst* 2009;42:347–53.
- [49] Beaucage G. Approximations Leading to a Unified Exponential/Power-Law Approach to Small-Angle Scattering . *J Appl Crystallogr* 1995;28:717–28.
- [50] Beaucage G. Small-Angle Scattering from Polymeric Mass Fractals of Arbitrary Mass-Fractal Dimension. *J Appl Crystallogr* 1996;29:134–46.
- [51] Hammouda B. A new Guinier–Porod model. *J Appl Crystallogr* 2010;43:716–9.

- [52] Kammler HK, Beaucage G, Kohls DJ, Agashe N, Ilavsky J. Monitoring simultaneously the growth of nanoparticles and aggregates by in situ ultra- small- angle x-ray scattering. *J Appl Phys* 2004;97:54309.
- [53] Schaefer DW, Kohls D, Feinblum E. Morphology of Highly Dispersing Precipitated Silica: Impact of Drying and Sonication. *J Inorg Organomet Polym Mater* 2012;22:617–23.
- [54] Porod G. Die Röntgenkleinwinkelstreuung von dichtgepackten kolloiden Systemen. *Kolloid-Z* 1951;124:83–114.
- [55] Martin JE, Hurd AJ. Scattering from fractals. *J Appl Crystallogr* 1987;20:61–78.
- [56] Peyronel F, Quinn B, Marangoni, Alejandro G. Pink DA. Ultra Small Angle X-Ray Scattering for pure Tristearin and Tripalmitin: model predictions and experimental results. *Food Biophys* 2014. Submitted.
- [57] Peyronel F, Quinn B, Marangoni AG, Pink DA. Ultra Small Angle X-Ray Scattering in Complex Mixtures of Triacylglycerols. *J Phys Condens Matter* 2014;Submitted.
- [58] Meakin P. Formation of Fractal Clusters and Networks by Irreversible Diffusion-Limited Aggregation. *Phys Rev Lett* 1983;51:1119–22.
- [59] Meakin P. Models for Colloidal Aggregation. *Annu Rev Phys Chem* 1988;39:237–67.
- [60] Stanley Smith C. Some Elementary Principle of Polycrystalline Microstructure. *Metall Rev* 1964;9:1–48.
- [61] Burke JE. Some factors affecting the rate of grain growth in metals. *Trans Met Soc A I M E* 1949;180:73.
- [62] Burke JE, Turnbull D. Recrystallization and Grain Growth. *Prog Met Phys* 1952;3:220–92.
- [63] Humphreys FJ, Hatherly M. Recrystallization and Related Annealing Phenomena. Oxford: Elsevier Science Ltd; 1995.
- [64] Quinn B, Peyronel F, Gordon T, Marangoni A, Hanna CB, Pink DA. Aggregation in Complex Triacylglycerol Oils: Coarse-Grained Models, Nanophase Separation, and Predicted X-ray Intensities. *J Phys Condens Matter* 2014. Submitted.