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Phospholipid-Based Reverse Micelle Structures in Vegetable Oil Modified by Water Content, Free Fatty Acid, and Temperature

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ABSTRACT: Colloidal assemblies of phospholipids in oil are known to be highly sensitive to changes in system composition and temperature. Despite the fundamental biological and high industrial relevance of these aggregates, the mechanisms behind the structural changes, especially in real oils, are not well understood. In this work, small-angle X-ray scattering (SAXS) was combined with molecular dynamics simulations to characterize the effects of oleic acid, water, and temperature on self-assembled structures formed by lecithin in rapeseed oil. SAXS showed that adding water to the mixtures caused the precipitation of liquid-crystalline phases with lamellar or hexagonal geometry. The combination of SAXS and molecular dynamics simulations revealed that stable spherical reverse micelles in oil had a core radius of about 2 nm and consisted of approximately 60 phospholipids centered around a core containing water and sugars. The presence of oleic acid improved the stability of reverse micelles against precipitation due to the increase in the water concentration in oil by allowing the reverse micelle cores to expand and accommodate more water. The shape and size of the reverse micelles changed at high temperatures, and irreversible elongation was observed, especially in the presence of oleic acid. The findings show the interdependency of the structure of the reverse micellar aggregates on system composition, in particular, oleic acid and water, as well as temperature. The revealed characteristics of the self-assembled structures have significance in understanding and tuning the properties of vegetable oil-based emulsions, food products, oil purification, and drug delivery systems.

INTRODUCTION

The self-assembly of amphiphilic surfactants in solution is an interesting phenomenon that has wide significance in fields ranging from fundamental biology to chemical engineering and biotechnology. Particularly interesting biological surfactant self-assemblies are formed by phospholipids. Besides their essential role in the cell membrane of most organisms, phospholipids are present and form various aggregates and colloidal assemblies in many bioproducts, including all plant oils and their derivatives, and have various industrial applications as surface-active components. A commonly employed phospholipid surfactant is lecithin, which is an amphiphilic substance mostly consisting of glycerol-based phospholipids extracted from food sources such as eggs and soybeans. This makes it biocompatible and nontoxic.

Most widely studied and applied self-assembling lecithin systems include ternary mixtures of lecithin, water, and an organic solvent of low polarity (oil), which typically take the form of an emulsion with the hydrophobic tail of the lecithin molecule residing in the nonpolar oil phase and the hydrophilic headgroup being in contact with the water. Especially in water-in-oil systems at very low water concentrations, lecithin molecules assemble into spherical or cylindrical reverse micelles. Lecithin/water/oil systems have a wide range of applications as food stabilizers, drug carriers, and nano-reactors, among other things.

A characteristic feature of lecithin/water/oil systems is their sensitivity to the concentration of water. Initially low water concentrations, even a minor increase in the water content can lead to drastic changes in the reverse micelle morphology or a phase separation. Although this property can be utilized as the basis of oil degumming, it might cause problems in other industrial processes such as the purification of vegetable oils by filtration. One way to modify the system’s response to chemical or physical changes is to supply it with an additive that modifies or stabilizes the assembly of the surfactants. In a recent work, oleic acid was reported to stabilize reverse micelles in systems of lecithin and water in rapeseed oil. However, the exact nature of the micellar structures and their temperature behavior remain unclear.

A common challenge in the study of self-assembled surfactant structures, especially in oily systems, is the lack of suitable characterization methods. In particular, many methods that work well for water-based systems, such as light scattering and surface tension measurements, suffer from the nonpolarity, low viscosity, and high turbidity of oil. On the other hand, methods such as transmission electron microscopy cannot be used for real-time monitoring of changes in the system caused by water content and temperature.
by external conditions such as temperature. Small-angle scattering of either X-rays or neutrons is therefore an excellent tool for studying the nanostructure of micellar solutions, allowing studies under various conditions and without practically any sample preparation. Moreover, scattering methods enable the determination of an average nanoscale structure from a large ensemble of particles, which is a considerable advantage over microscopy methods. Even though small-angle scattering has been widely used to elucidate the morphology and morphological changes of lecithin-based systems in organic solvents such as cyclohexane or n-decane,7,10−14 significantly less is known about the morphology of reverse micelles in real vegetable oils.

Although limited in time scales and system sizes, computational approaches provide complementary approaches to the structural characterization of lecithin structures in apolar media on the molecular level. Atomistic detail approaches to the characterization of phospholipid reverse micelles concentrate on the structural characterization of the aggregates15−17 and model assessment18 in cyclohexane or benzene solvents. More recently, phospholipid reverse micelles in triglyceride solvent have been characterized in coarse-grained detail,18 and atomistic detail approaches to capturing reverse micellar aggregate size distributions and assembly mechanisms in triglyceride solvent exist.19

In this study, small-angle X-ray scattering (SAXS) was applied to better understand the role of oleic acid and water addition in mixtures of lecithin, water, and rapeseed oil. In particular, the stabilizing effect of oleic acid on the micellar structures at increased water content and the temperature behavior of these systems were addressed. Complementary information on the molecular assembly of lecithin and water in the oil was obtained with atomistic detail molecular dynamics simulations. Rapeseed oil was chosen as the apolar solvent because it is a major cooking oil in Europe and has an overall significant role in food processing, biobased fuels, cosmetics, and personal care products. The global production of rapeseed is second only to soybean oil.20 Furthermore, lecithin is naturally present in rapeseed oil and forms colloidal aggregates that influence the oil characteristics.21

■ EXPERIMENTAL SECTION

Materials. Rapeseed oil (K-brand, Finland) was purchased from a supermarket and contained 96.9 wt % triglycerides, 0.6 wt % free fatty acids, and less than 0.6 ppm phosphorus, as determined with gas chromatography according to the ISO15304 M standard. Oleic acid from VWR Chemicals (Radnor, PA, USA) and consisted of 84.5 wt % oleic acid, 12.5 wt % linoleic acid, and 3 wt % other fatty acids, mainly palmitic acid and stearic acid. i-α-Lecithin (from soybean, CAS number 8002-43-5) was obtained from Acros Organics (Morris Plains, NJ, USA). The lecithin composition, according to the producer, was 23 wt % phosphatidyl choline, 20 wt % phosphatidyl ethanolamine, 14 wt % phosphatidyl inositol, 8 wt % phosphatidic acid, 8 wt % other phospholipids, 8 wt % sugars, 15 wt % glycolipids, and 3 wt % triglycerides as well as a small amount of moisture. The water used in the experiments was Millipore water produced using a Synergy UV water purification system (Millipore SAS, Molsheim, France).

Rapeseed oil and oleic acid were dried using 3 Å molecular sieves from Sigma-Aldrich (St. Louis, MO, USA). The molecular sieves were first dried overnight (20 h) at 175 °C, after which they were inserted into rapeseed oil and oleic acid at a concentration of about 3 wt %. The rapeseed oil was dried in a heating cabinet at 40 °C overnight in order to reduce the viscosity and enhance drying, whereas the oleic acid was dried at room temperature. Lecithin was dried in a vacuum desiccator using regenerated silica granules.

The moisture content of the materials was determined using the Karl Fischer titration method at room temperature (Mettler-Toledo Titrator DL35). Lecithin was dissolved in chloroform before measurements. The average value of these results was calculated, and the standard deviation based on three repetitions was used as an error estimate. The obtained moisture contents after drying were 0.015 ± 0.002 wt % for the rapeseed oil, 0.397 ± 0.004 wt % for the lecithin, and 0.028 ± 0.001 wt % for the oleic acid.

Preparation of Micellar Solutions. To prepare the micellar solutions (Table 1), dried rapeseed oil was mixed with a specific amount of oleic acid (0, 5, 10, or 20 wt %) and 1 wt % lecithin. The solutions were mixed for 3 h at 70 °C, after which appropriate amounts of Millipore water were added. To break down the water droplets and better disperse the water, the solutions were sonicated for a few minutes with a 0.2 s pulse at medium amplitude (Branson S-450 digital sonicator). After sample preparation, the samples were agitated in a heating cabinet rotator at room temperature for 5 h and centrifuged to induce macroscopic phase separation. In the samples that showed phase separation after centrifugation (indicated in Table 1), the precipitated phase and the corresponding supernatant were characterized separately with SAXS.

Small-Angle X-ray Scattering. Synchrotron—SAXS measurements were carried out at beamline ID02 of the European Synchrotron Radiation Facility (ESRF). The wavelength of the X-rays was λ = 0.995 Å, and the SAXS patterns were collected with a Rayonix MX-170HS detector. For measurements at room temperature, about 100−200 μL of the sample solution was inserted into a glass capillary having an outer diameter of 2 mm, and the capillary was sealed with thermoplastic adhesive. The capillary was placed in the X-ray beam, and SAXS data were recorded at sample-to-detector distances of 1.3 and 10 m. A temperature series was carried out for selected samples sealed in aluminum cells (thickness 2 mm) with mica windows using only the detector distance of 1.3 m. The temperature was varied from 20 to 100 °C and back to 20 °C, with monitoring of the changes in the structure of the samples at intervals of 10 to 20 °C. The data were normalized for the transmission of the direct beam, corrected for the dark field and flat field, and scaled to absolute intensity using water as a secondary calibration standard.22,23 Subsequent treatment of the data, including the averaging of frames, subtraction of the scattering of an empty capillary or sample cell, merging of data from different detector distances, and rebinning was carried out using the SAXSutilities software.

The SAXS intensities from micellar solutions after background subtraction (as explained in the Results and Discussion section) were approximated with the function

$$I(q) = NV^2(\Delta \rho)^2 P(q)$$  

(Table 1. Total Weight Fractions of Water and Oleic Acid in Mixtures of Lecithin (1 wt %) and Rapeseed Oil and Whether the System Phase Separates*)
where \( N, V, \) and \( P(q) \) are the number density, volume, and form factor of the particles, respectively, and \( \Delta P \) is the difference in the scattering length density between the particle and the solvent. In the current case, \( P(q, R, \epsilon) \) is the form factor of a prolate ellipsoid (ellipsoid of revolution) with equatorial and polar semiaxes \( R \) and \( \epsilon R \) respectively,\(^{24}\)

\[
P(q, R, \epsilon) = \int_0^{\pi/2} F^2[q, r(R, \epsilon, \alpha)] \sin \alpha \, d\alpha
\]

where

\[
r(R, \epsilon, \alpha) = R(\sin^2 \alpha + \epsilon^2 \cos^2 \alpha)^{1/2}
\]

and

\[
F(q, R) = \frac{3[qR - qR \cos(qR)]}{(qR)^3}
\]

The fitting of eq 1 with the form factor of eq 2 to the SAXS intensities was done with Python scripts and weights proportional to the intensity at each data point.

Computational Methods. The GROMACS 5.1 simulation package\(^{35}\) with the empirical CHARMM biomolecular force field\(^{36}\) was used for the simulations. To simplify the experimental composition, vegetable oil was modeled as triolein (because of oleic acid being a major component of rapeseed oil), lecithin was modeled as a pure 1,2-diolenylphosphatidylcholine (DOPC), and sugar was modeled as sucrose (because of sucrose being a major component of the free sugar fraction of soybean lecithin\(^{16}\)). Other solution species were considered to be minority species and were not included in the modeling. The DOPC was modeled using the CHARMM27 lipid model\(^{37,38}\), while triolein was constructed from the DOPC by replacing the phospholipid group with an additional oleic acid residue. To speed up simulations, the united atom hydrocarbon tail parameters of Henin et al. were used for the solvent.\(^{39}\) The Glycan module\(^{11,13}\) of CHARMM-GUI\(^{33,34}\) was used to obtain the parameters for sucrose.

The simulations were performed in the NPT ensemble. Temperature was controlled with the Bussi et al. thermostat\(^{35}\) \((T_{ref} = 70 \, ^\circ\text{C}, \, \tau_T = 0.5 \, \text{ps})\), and pressure was controlled by the Parrinello–Rahman barostat\(^{36}\) \((P_{ref} = 1 \, \text{bar}, \, \tau_P = 2 \, \text{ps})\). Standard long-range electrostatics treatment \((\text{PME})\)\(^{37}\), 1.2 nm cutoff, \(\sim 0.12 \, \text{nm grid spacing, and fourth-order interpolation}\) and the Lennard-Jones (smoothly switched to zero between 1.0 and 1.2 nm) cutoff scheme of CHARMM were used. The system configuration was propagated by a leapfrog integrator \((2 \, \text{fs time step})\) with bonds involving hydrogens constrained via LINCS\(^{39}\) and SETTLE\(^{40}\) algorithms.

Preassembled reverse micelles characterized by their aggregation number \(N_{agg} = n_{DOPC}\), water-to-surfactant mole ratio \(W_0 = n_{H_2O}/n_{DOPC}\), and sugar-to-surfactant mole ratio \(S_0 = n_{sugar}/n_{DOPC}\) were constructed using the Packmol software\(^{41}\) (Table 2). First, a spherical polar core composed of \(n_{H_2O}\) water and \(n_{sugar}\) sucrose molecules was created. The radius of the sphere was calculated by assuming the effective volumes of water and sucrose molecules to be 31 and 358 Å\(^3\), respectively. Second, a shell of DOPC molecules was set around this polar core with the DOPC tails pointing radially outward. Third, to pre-equilibrate the DOPC reverse micelle and its polar core, the system was solvated with dodecane, and an NPT simulation was run with position restraints for 1 ns followed by 10 ns without restraints. Finally, the pre-equilibrated reverse micelle was set without the surrounding dodecane solvation shell into a pre-equilibrated bulk triolein simulation box, where a spherical cavity corresponding to the radius of gyration of the reverse micelle polar core had been constructed. To initiate the equilibration of the system, the aggregate position was restrained inside the cavity and the triolein solvent was brought into contact with the aggregate via a brief 50 ps \(\lambda\)-coupling simulation. Finally, unrestrained production simulations were run for 200 to 500 ns (Table 2), with the first 100 ns discarded as the relaxation period.

### RESULTS AND DISCUSSION

#### Phase Behavior of the Mixtures at Room Temperature

The SAXS intensities of the mixtures of lecithin, water, and oleic acid in oil, as well as pure rapeseed oil and water as references, all measured at room temperature, are shown in Figure 1. The intensity from pure rapeseed oil showed no signs of ordered structures but instead showed a broad maximum

![Figure 1](image-url)
centered roughly around \( q = 2.8 \text{ nm}^{-1} \) and a sharp increase toward the lower \( q \) values. A similar increase at low \( q \) was also observed in the SAXS intensity of bulk water, which on the other hand was almost constant above \( q = 0.5 \text{ nm}^{-1} \). Therefore, all other features in the SAXS intensities were assumed to originate from structures involving lecithin, possibly accompanied by some minor impurities present in the rapeseed oil. In particular, the presence of nanoscale micellar structures in solution resulted in a broad intensity distribution centered at \( q = 1.1 \text{ nm}^{-1} \) (such as in the \( W_d(0.8) + O_d(13) \) sample), whereas ordered liquid crystal phases produced sharp diffraction peaks characteristic of that particular phase (e.g., sample \( W_d(3) \)). Self-assembled surfactant structures either in solution or as ordered phases could be detected in all nonprecipitating samples as well as in all of the precipitated phases. From the supernatant fractions of the precipitating samples, only the samples with 0.07 wt % water (\( W_d(3) \) and \( W_d(3) + O_d(13) \)) contained some reverse micelles in solution. In sample \( W_d(3) + O_d(13) \), the precipitation was weak and no ordered liquid crystal structures were observed in the lower part (precipitate) of the sample. The ordered structures from the precipitated ordered phases, as determined from the locations of the diffraction peaks, are presented in Table 3.

<table>
<thead>
<tr>
<th>sample</th>
<th>order of reflection</th>
<th>( q ) (nm(^{-1}))</th>
<th>structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>( W_d(3) )</td>
<td>1</td>
<td>1.227</td>
<td>lamellar, ( d = 5.121 \pm 0.001 \text{ nm} )</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.454</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.682</td>
<td></td>
</tr>
<tr>
<td>( W_d(13) )</td>
<td>1 (L)</td>
<td>1.137</td>
<td>lamellar, ( d = 5.5242 \pm 0.0003 \text{ nm} )</td>
</tr>
<tr>
<td></td>
<td>2 (L)</td>
<td>2.275</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (L)</td>
<td>3.412</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 (H)</td>
<td>0.943</td>
<td>hexagonal, ( d = 7.691 \pm 0.001 \text{ nm} )</td>
</tr>
<tr>
<td></td>
<td>11 (H)</td>
<td>1.634</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 (H)</td>
<td>1.887</td>
<td></td>
</tr>
<tr>
<td>( W_d(40) + O_d(13) )</td>
<td>10</td>
<td>0.728</td>
<td>hexagonal, ( d = 9.95 \pm 0.01 \text{ nm} )</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>1.263</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.460</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1.929</td>
<td></td>
</tr>
<tr>
<td>( W_d(100) + O_d(13) )</td>
<td>10</td>
<td>0.672</td>
<td>hexagonal, ( d = 10.791 \pm 0.008 \text{ nm} )</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>1.165</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.346</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1.777</td>
<td></td>
</tr>
</tbody>
</table>

"The distance \( d \) refers to the lamellar period in a lamellar crystal and to the nearest-neighbor distance between cylinders in a hexagonal crystal."
system was approximated to consist of two phases with distinct mean electron densities: reverse micelle cores and a surrounding oil phase that also accommodates the main portion of the phospholipid tails. The justification for this assumption will be given later on the basis of the molecular dynamics simulations results. The peak from a lamellar structure in the samples without oleic acid was excluded from the fitting range.

The optimized fitting parameters (Table 4) showed an almost perfectly spherical shape ($\epsilon = 1$) with a radius of around 2 nm for reverse micelles in all samples with 5 wt % or no oleic acid, whereas more elongated shapes ($\epsilon > 1$) were observed in samples with 10 and 20 wt % oleic acid. The fits to the samples without oleic acid ($W_6(0.8)$ and the supernatant of $W_6(3)$) yielded identical parameters for the reverse micelles, indicating that the small amount of added water was included in the precipitated lamellar phase rather than in the reverse micelles in solution. On the other hand, in the presence of 5 wt % oleic acid, the addition of an equal amount of water (from $W_6 = 0.8$ to 3) yielded an increase of about 15% in the reverse micelle radius (compare samples $W_6(0.8) + O_6(13)$ and $W_6(3) + O_6(13)$). This suggests that oleic acid acted as a cosurfactant and allowed the core region of the reverse micelle to expand and accommodate more water molecules. The same idea was also proposed by Lehtinen et al., and it is in agreement with the results of Sugiura et al.12 for a system with phosphatidyl choline in ethyl oleate and similar oleic acid concentrations. In addition, the reverse micelles at the lowest water content appeared smaller in the presence of 5 wt % oleic acid (1.9 nm, sample $W_6(0.8) + O_6(13)$) than in the absence of it (2.1 nm, sample $W_6(0.8)$), which is in line with the decreasing trend of reverse micelle radius with oleic acid concentration reported by Sugiura et al.12 This could be explained by the proposed dual function of oleic acid and, more specifically, its ability to solubilize phospholipids in oil at low water concentrations.6 In this case, the slightly different solubility of each phospholipid component would cause the formation of reverse micelles with slightly different interfacial curvature. At considerably higher oleic acid concentrations (samples $W_6(0.8) + O_6(27)$ and $W_6(0.9) + O_6(52)$), the reverse micelles started to merge into each other to form elongated structures, thereby decreasing the total interfacial area between the reverse micelles and oil. This is probably a more drastic effect of the oleic acid-induced solubilization of lecithin.

**Detailed Structure of Lecithin-Based Reverse Micelles.** To better understand the assembly of lecithin in the micellar structures, molecular dynamics simulations of reverse micelles in oil were conducted. The significance of the simulations for the study is 2-fold: (i) to justify the employed two-phase SAXS analysis by mapping the characteristic density profiles of the formed reverse micellar aggregates at the molecular level and (ii) to provide an explanation of why the SAXS-based core radius of 2.1 nm (sample $W_6(0.8)$ in Table 4) significantly exceeds what would be expected if the reverse micelle cores were composed of densely packed lipid headgroups.15 Such dense packing of the headgroups could be expected at low hydration, when there is a very low amount of water in the reverse micelle cores. Specifically, because the core radius measured with SAXS is significantly larger than the extended length of common lipid headgroups (around 1.3 nm), it is relevant to examine the molecular-level structure of the formed reverse micellar aggregates in more detail with molecular dynamics simulations.

To resolve these two questions via molecular modeling, first a realistic aggregate size for the simulated reverse micelles was determined by comparing the mean dimensions of a series of

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**Table 4. Results from Fits of Equation 1 with the Form Factor of Prolate Spheroids (Equation 2) to the SAXS Intensities from Micellar Solutions of Lecithin, Water, and Oleic Acid in Rapeseed Oil Measured at Room Temperature**

<table>
<thead>
<tr>
<th>sample</th>
<th>$NV^2(\Delta \rho)^2$ (cm$^{-1}$)</th>
<th>$R$ (nm)</th>
<th>$\epsilon$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$W_6(0.8)$</td>
<td>0.3156 ± 0.0006</td>
<td>2.119 ± 0.002</td>
<td>1.00$^{a}$</td>
</tr>
<tr>
<td>$W_6(3)$, supern.</td>
<td>0.0589 ± 0.0001</td>
<td>2.111 ± 0.002</td>
<td>1.00$^{a}$</td>
</tr>
<tr>
<td>$W_6(0.8) + O_6(13)$</td>
<td>0.2523 ± 0.0006</td>
<td>1.915 ± 0.003</td>
<td>1.00$^{a}$</td>
</tr>
<tr>
<td>$W_6(3) + O_6(13)$, supern.</td>
<td>0.2301 ± 0.0005</td>
<td>2.235 ± 0.003</td>
<td>1.00$^{b}$</td>
</tr>
<tr>
<td>$W_6(3) + O_6(13)$, precip.</td>
<td>0.409 ± 0.002</td>
<td>2.238 ± 0.005</td>
<td>1.00$^{b}$</td>
</tr>
<tr>
<td>$W_6(0.8) + O_6(27)$</td>
<td>0.2715 ± 0.0005</td>
<td>1.79 ± 0.01</td>
<td>1.48 ± 0.03</td>
</tr>
<tr>
<td>$W_6(0.9) + O_6(52)$</td>
<td>0.3134 ± 0.0005</td>
<td>1.88 ± 0.01</td>
<td>1.63 ± 0.02</td>
</tr>
</tbody>
</table>

$^{a}$Parameters $R$ and $\epsilon R$ correspond to the equatorial and polar radii, respectively. $^{b}$Value fixed in order to obtain a realistic error estimate for $R$. 

---

**Figure 2.** SAXS intensities (circles) of mixtures of lecithin, water, and oleic acid in rapeseed oil, measured at room temperature and corrected for the oil background by subtracting the intensity of pure oil at high $q$ and a power law at low $q$. Fits of an ellipsoid form factor (eqs 1 and 2), corresponding to the parameters shown in Table 4, are shown with a solid line on the fitting range and with a dashed line outside of it. A power law with an exponent of $-4$ is shown with a thin dashed line at high $q$ for comparison.
preassembled aggregates at $W_0(1)$ to the aggregate radius obtained from SAXS analysis (Figure 3a). The simulations showed a systematic increase in both the polar core and the overall aggregate radius with increasing aggregation number $N_{agg}$. The presence of sucrose (an amount corresponding to 8 wt % of lecithin) did not have much effect on the scaling characteristics of the aggregate size. However, the simulations showed that sucrose packs in the reverse micelle core, enabling the formation of larger aggregates. This influenced the overall molecular packing in the aggregates. In the simulations without sucrose, the DOPCs aggregated in multiple concentric spheroidal layers at $N_{agg} = 23$ to 46. This is due to a lack of space for lipid headgroups in the micelle cores, which prevented the formation of large single-layer reverse micelles. However, the presence of sucrose changed the core packing such that monolayer adsorption and consequently the formation of larger reverse micelles is feasible. In total, the finding suggests that sucrose affects not only the aggregate size but also the packing order in the aggregates. The best match of the simulated micelle dimensions and the SAXS measured core size data was obtained with $N_{agg} = 67$ and sucrose solubilized in the core.

To justify the assumption of a two-phase system used in the SAXS analysis and to estimate the electron density difference between the two phases, the radial electron density profile resulting from the simulations of a reverse micellar aggregate with $N_{agg} = 67$ and sucrose solubilized in the core was plotted (Figure 3b). The figure also shows a two-phase approximation of the electron density, which was obtained by fitting a step function to the simulated electron density profile. The location of the step (the distance from the reverse micelle center point) in the fitted step function corresponds to the radius of the simulated reverse micelle core, when a two-phase system is assumed. The radius resulting from the fit is in good agreement with the radius resulting from the SAXS analysis (included in Figure 3b for comparison). This match shows that the chosen approach to the SAXS analysis is fully supported by the molecular-level structure of the reverse micelles, as predicted by the modeling. Furthermore, the simulation data enables the determination of an electron density difference of 0.138 e/Å$^3$ between the reverse micelle core and the solvent. The estimate is based on the step height in the fit to the simulated electron density profile. Similar electron density differences were also observed in the other simulated systems, but noise in the density profiles excluded quantitative comparison.

The simulations also enabled the characterization of the aggregate structure and the location of the different components in detail, as shown by the density plots in Figure 3c. The figure shows the density distribution of lecithin headgroups, water, and sucrose in the core of the aggregate and lecithin tails in the corona. Notably, sucrose, when present, resides in the core region, with water and lecithin headgroup moieties forming the outer layer of the core region. The core–corona interface has a degree of roughness associated with it, as evidenced by the overlap between solvent and lecithin moieties in the density distribution graph. Notably, the glycerol groups of the triglyceride solvent are enriched at the core–corona interface of the aggregates, indicating significant solvent penetration to the tail region as well as favorable interaction between the polar glycerol backbones of triolein and lecithin. The 2.1 nm radius determined with SAXS is located in the same region, close to the core–corona interface, solidifying the interpretation that it corresponds to the radius of the reverse micelle core.

In addition to the simulation-based approach of Figure 3a and the related discussion, an experimental estimate of the
aggregation number $N_{agg}$ can be obtained from the SAXS data. This can be done using the expression

$$N_{agg} = \frac{cN_A}{N}$$

where $c$ is the molar concentration of lecithin, $N_A$ is Avogadro’s number, and $N$ is the number density of the reverse micelles. The value of $N$ can be obtained from the fits of eq 1 when the volume of the reverse micelles ($V$ in eq 1) and the scattering
length density difference between them and the surrounding solvent ($\Delta \rho$ in eq 1) are known. Here $\Delta \rho = r_e \Delta \rho_e$, with $r_e$ being the classical electron radius and $\Delta \rho_e$ being the electron density difference between the two phases. By using a value of $\Delta \rho_e = 0.138 \text{ e/A}^3$ for the electron density difference, as determined from the molecular dynamics simulations (Figure 3b), and the volume calculated from the parameters in Table 4, an aggregation number of $N_{agg} = 56$ is obtained for sample $W_0(0.8)$. This aggregation number is within the range previously reported for egg lecithin in various organic solvents, and it is in good agreement with the simulation results (Figure 3a).

Effects of Heating on the Reverse Micelles. To investigate the effects of temperature on the reverse micellar structures, SAXS from selected samples was measured at various temperatures, first by heating the samples from 20 to 100 °C and then by cooling back to 20 °C. The background subtraction for the heated samples was carried out similarly as for those measured at room temperature, except that the power-law exponent at low $q$ was fixed to $-4$. The form factor of prolate ellipsoids (eqs 1 and 2) could be successfully fitted to all samples at most of the temperature points (Figure 4).

Only the three last temperature points of the sample with the highest oleic acid content ($W_0(0.9) + O_6(52)$) could not be fitted because of an emerging peak at around $q = 0.75 \text{ nm}^{-1}$, which shifted to slightly higher $q$ values when the sample was returned to 20 °C (Figure 4d). A similar peak has been observed before and assigned to a weak repulsive interaction between the reverse micelles, making fitting unreliable without taking into account the particle–particle interaction by means of a structure factor.

According to the fitting results of Figure 4a, the spherical reverse micelles in sample $W_0(0.8)$ retained their shape throughout the temperature cycle but shrank in size at higher temperatures. The shrinkage was almost reversible, meaning that the reverse micelles obtained almost the same size again upon cooling. This type of shrinkage at elevated temperatures is often observed in reverse micelle systems and is explained by an enhanced penetration of oil in the surfactant tail, which increases the effective volume of the tail. At the same time, the aggregation number, $N_{agg}$, is expected to decrease, which was observed in our current data as a reversible increase in the number density of the reverse micelles ($N$) by >30% (calculated from $N N_0^2(\Delta \rho)^2$ assuming constant $\Delta \rho$). Therefore, in the absence of added oleic acid (sample $W_0(0.8)$), the size of the reverse micelles decreased at higher temperatures because of reorganization of the phospholipids into smaller reverse micelles, which took place in order to compensate for the larger effective tail volume of the phospholipids.

The temperature dependence of the samples containing oleic acid was substantially different. The samples with 5 wt % oleic acid ($W_0(0.8) + O_6(13)$ and $W_0(3) + O_6(13)$ in Figure 4b-c, respectively) showed spherical reverse micelles shrinking at temperatures of up to 60 °C, after which they started to elongate to prolate ellipsoids. A major difference between the two samples was that in the absence of added water (sample $W_0(0.8) + O_6(13)$) the new prolate shape was preserved even after decreasing the temperature, whereas in the presence of more water (sample $W_0(3) + O_6(13)$) the reverse micelles returned to close to their original, spherical shape. The most dramatic changes with temperature were observed in sample $W_0(0.9) + O_6(52)$ (Figure 4d), in which the originally slightly prolate ellipsoids grew both in length and width during heating and started to interact with each other with a packing distance of about 8 nm during cooling. No signs of immediate precipitation were observed in this sample after heating.

Because nonionic reverse micelles in nonaqueous media normally exhibit shrinkage and a prolate-to-spherical transformation at higher temperatures, the different behavior observed in the current data was attributed to the presence of oleic acid. The presence of free fatty acids such as oleic acid is known to enhance lipid oxidation, and the intensity of these processes increases with temperature. Such reactions could modify the chemical structure of both the lecithin and oil components, which would lead to differences in the size and shape of the reverse micelles formed in the system. In particular, a decrease in the chain length of the phospholipid can induce the formation of reverse micelles with more elongated geometry. Such a mechanism could also be behind the oleic acid-induced changes in the temperature dependence of the reverse micelles in the current study. In particular, the irreversible behavior in the samples with oleic acid and no added water (samples $W_0(0.8) + O_6(13)$ and $W_0(0.9) + O_6(52)$) suggests that the chemical composition of the system was altered as a consequence of heating in the presence of oleic acid. On the other hand, the presence of added water in sample $W_0(3) + O_6(13)$ possibly allowed the reverse micelles to break into smaller ones during heating, which might have had an effect on the chemical reactions taking place. On the basis of the results of Kittipongpittaya et al., the observed effects of oleic acid could also be common to other similar fatty acids.

The studied system is a fascinating example of colloidal aggregate formation in plant oils and the response of such aggregates to changes in the system composition and temperature. The formation and characteristics of colloidal aggregates have many implications for the use of the assemblies as solubilization and drug transport systems as confined reaction platforms, and in general for the preservation and characteristics of vegetable oil-based formulations. The findings show that the self-assembly mechanisms in apolar solvents and, in particular, this real rapeseed oil system can be highly sensitive to changes in composition and temperature. The sensitivity of the colloidal aggregate structures can radically enhance or diminish the material performance in certain applications (e.g., reverse micellar nanoreactors, the control of solubility, and drug release in pharmaceutical formulations). Elucidating the changes caused by variations in the systems, such as here, is hence of high importance in both understanding the mechanisms behind the changes and in the enhanced use of reverse micellar systems in applications.

CONCLUSIONS

Self-assembled colloidal structures of lecithin and other phospholipids play important roles in the processing and applications of vegetable oils. These structures are highly sensitive to the composition and temperature of the system, which can cause various problems related to material characteristics and the function of the colloidal species in them. In this work, the water-induced precipitation of lecithin-based structures and phase separation in rapeseed oil with lecithin could be prevented by the addition of free fatty acid, which allowed the lecithin reverse micelles to expand and accommodate more water in their core regions. On the other hand, larger amounts of the fatty acid and high temperatures induced an irreversible change in the reverse micelle geometry from spherical to elongated. The results demonstrate the
delicate balance of multiple factors related to the structure formation of colloidal aggregates in vegetable oils and highlight the importance of understanding structural changes in these systems.

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Notes
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References


