Bioinspired Oleic Acid–Triolein Emulsions for Functional Material Design

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Stimuli-responsive nanostructured emulsions can be utilized to innovate tailor-made materials in fields including biotechnology and food materials. This study reports the composition and pH-responsive colloidal and interfacial properties of the naturally abundant oleic acid–tri olein–water emulsions, and applies the knowledge to the sustainable design of nanoemulsions. Small angle X-ray scattering, spinning drop tensiometry, multi-angle dynamic light scattering, cryogenic transmission electron microscopy, and electrophoretic mobility analysis are used to follow pH and ionic strength triggered nanostructural transformations and analyze emulsion particle size and stability. Energy input by vortexing is found sufficient to form emulsions under pH conditions that trigger a decrease in the oil–water interfacial tension. The lipid composition, solution pH, and ionic strength are discovered to influence the protonation of oleic acid, driving pH-triggered colloidal transformations in the emulsions. The findings from this study can guide the design of novel sustainable functional food emulsions at low-energy for instance for the solubilization and delivery of degradation sensitive nutrients.

1. Introduction

Soft colloidal structures formed through the self-assembly of amphiphilic lipids are ubiquitous in nature, and integral to many biological processes and structures, including cellular membranes. They have great potential for applications, for instance as sustainable solvents for chemical or biotechnological processes, drug- and nutrient delivery systems, and functional bio- and food materials.

The structure, composition, and interfacial properties of dietary lipid emulsions were reported to significantly affect lipid digestion and absorption, as well as the bioavailability of lipid-soluble nutrients and drugs from in vitro studies. During the digestion of foods rich in dietary fats, human gastric lipase and human pancreatic lipase hydrolyze long-chain triacylglycerols into di- or monoacylglycerols by cleaving off fatty acid units. Being amphiphilic lipids, these digestion products can self-assemble into a variety of lamellar and non-lamellar lyotropic liquid crystalline structures attractive for drug and nutrient delivery applications. The dynamic composition of the lipolysis products during triglyceride digestion has a profound influence on the physicochemical properties of the colloidal structures. For example, surface-active lipolysis products can alter the interface of the emulsion particles and transfer water into the triglyceride–oil phase. They can further regulate the digestion process by competing with the lipase for the oil–water interface. Their adsorption to the oil–water interface modifies the interfacial tension which, together with shear forces in the digestive tract, can further reduce the emulsion droplet size. The resulting increase in surface area for lipases to access their substrate can ultimately increase the digestion kinetics. Therefore, understanding the structure and properties of these biologically important systems contributes to the development of smarter food materials, which are of great interest for the food industry.

In addition to compositional changes, food colloids are also exposed to varying pH and ionic strength conditions as they travel along the digestive tract. While pH in the stomach can be very acidic, intestinal pH can reach alkaline pH values, with this broad pH range being one of the challenges of oral delivery of nutrients and drugs. Such changes in the local microenvironment influence the ionization state of the carboxyl group of fatty acids. This modifies their solubility in aqueous media or in the hydrophobic interiors of the emulsion droplet, changing its interfacial properties and their potential to self-assemble into nanostructures. The ionization state of acids at a given pH, however, is not exclusively dependent on their molecular structure. While the \( pK_a \) of an isolated carboxyl group of monomeric fatty acid in water is 4.75, different values are reported in literature for the apparent \( pK_a \) of acids in self-assembled systems, strongly affected by a variety of parameters such as intermolecular interactions, presence of neighboring charges, dielectric constant, and ionic strength.

Therefore, these external parameters can indirectly affect the physicochemical properties of dispersions containing molecules that can be deprotonated. For the case of oleic acid (OA) in particular, reported values of apparent \( pK_a \) at different conditions and environments range from 6.1 to 9.4.
Some of the techniques which have been used to determine apparent $pK_a$ include 2-p-toluidinynaphthalene-6-sulfonate (TNS) fluorescence, electrokinetic mobility, surface viscosity and foam stability, and surface tension (gas–liquid).

Interfacial tension (liquid–liquid) measurements have not been widely used to determine the apparent $pK_a$. To the best of our knowledge, this method has only been used to study the apparent $pK_a$ of stearic acid–cyclohexane mixtures in water.[33] Interfacial tensiometry requires relatively simple instrumentation and has the advantage of providing valuable information about the absolute values of the interfacial tension in addition to the apparent $pK_a$. There have been a few studies concerning the effect of the pH at the liquid–liquid interface of fatty acids.[33,34] Contrary to gas–liquid interfaces, no minimum in the interfacial tension curve is observed for liquid–liquid interfaces, as the interfacial tension continues to decrease with increasing pH beyond the $pK_a$. At gas–liquid interfaces, protonated (hydrophobic) fatty acid molecules reside at the surface (or migrate into the bulk as micelles) while at liquid–liquid interfaces, they can migrate to the interior of the nonpolar droplets to avoid interaction with the polar phase.[31]

OA is a monounsaturated fatty acid approved as a food additive by food authorities. It is mostly found in the form of triglyceride esters, representing more than 60% of the composition of common vegetable oils such as peanut oil, olive oil, and rapeseed oil.[35] Triolein is a symmetrical triacylglycerol with OA esters on all three glycerol-OH groups, being often used as a model for vegetable oil in food-related research.[36–38] The amphiphilic nature of OA and its pH-triggered interfacial activity allow this fatty acid to form a variety of self-assembled structures in water, depending on the environment. OA can self-assemble into spherical and cylindrical micelles, vesicles and other lyotropic liquid crystalline phases such as the inverse cubic and the inverse hexagonal phases.[39,39] It can also integrate into the self-assembly of other amphiphilic molecules to form stimulus-responsive supramolecular structures. Some examples of such integration include: monooacylglycerols,[40,41] phospholipids,[42,43] peptides,[15,22] ionic and nonionic surfactants,[44,45] long-chain alcohols, and sucrose esters.[46]

In this work, we study the interfacial tension, colloidal properties, and self-assembled structures of OA–triolein mixtures in water by means of spinning drop tensiometry, small angle X-ray scattering (SAXS), cryogenic transmission electron microscopy (cryo-TEM), multi-angle dynamic light scattering (DLS), and zeta-potential measurements. This combination of techniques provides an insight into the pH-, composition-, and ionic strength driven colloidal transformations in these emulsions.

2. Results and Discussion

2.1. Interfacial Tension and Apparent $pK_a$ of OA–Triolein Mixtures in Water

The pH-dependent interfacial tension between selected OA–triolein mixtures and phosphate buffered saline (PBS) solution at selected pH values are presented in Figure 1. At pH 3.0, when most of the OA is protonated, the interfacial tension values were above 10 mN m$^{-1}$ for all the studied samples. As the pH increased, the interfacial tension progressively decreased, reaching a minimum at pH 9.0 where the interfacial tension was below 1.0 mN m$^{-1}$. For comparison, the measured interfacial tension for pure triolein was mostly independent of the pH, with a value of 22 mN m$^{-1}$ (see Figure S1, Supporting Information). This value was lower than the 30 mN m$^{-1}$ reported in the literature,[47] which may be due to the presence of minor impurities. The experimental data of interfacial tension against pH in the OA-triolein system could be approximated with an equation describing the OA dissociation as a function of pH (Equation 1, represented by the solid lines shown in Figure 1). The point of inflection of these interfacial tension titration experiments represents equal amounts of protonated and deprotonated OA at the interface, corresponding to the situation where pH is equal to the apparent $pK_a$ of the OA in these systems.

The influence of lipid composition on the interfacial tension was also investigated (Figure 1). At pH 3.0, the interfacial tension of the mixture containing 1% of OA in triolein was 16.5 $\pm$ 1.2 mN m$^{-1}$ while the mixture containing 50% OA in triolein exhibited a lower interfacial tension of 11.6 $\pm$ 1.1 mN m$^{-1}$ at this pH. The intermediate OA–triolein ratios presented interfacial tensions within those limits. Increasing the OA content in triolein produced a gradual shift to lower apparent $pK_a$ values: at 1% of OA in triolein the apparent $pK_a$ was 7.1 $\pm$ 0.2, and at 50% OA in triolein, 6.4 $\pm$ 0.1.

In addition to the impact of the pH on the oil–water interfacial tension in this system, the influence of the solution ionic strength was also analyzed. The interfacial tension of the 5% OA in triolein system at different NaCl concentrations in PBS is presented in Figure 2. At pH 3.0, the presence of 2.5 m of NaCl decreased the interfacial tension from 15.8 $\pm$ 1.3 (system without NaCl) to 8.5 $\pm$ 0.1 mN m$^{-1}$. The decrease in interfacial tension was less accentuated for systems containing intermediate concentrations of NaCl, namely 0.5 and 1.0 m. The apparent $pK_a$ values were also estimated for these NaCl-containing systems and it was found to gradually decrease.

![Figure 1](image_url)
from 6.7 ± 0.2 for the system without NaCl to 6.0 ± 0.1 at the highest NaCl concentration (2.5 m). Similar changes in interfacial tension due to presence of NaCl were also observed for the 50% OA in triolein system (Figure S2, Supporting Information) in which interfacial tension at pH 3.0 was found to be 8.2 ± 1.0 mN m⁻¹ and the apparent pKᵦ shifted from 6.4 ± 0.1 to 6.2 ± 0.1 for a salt concentration of 1.0 m.

The decrease in interfacial tension of the OA–tri olein systems was attributed to the protonation state of the carboxylic group of the OA. The accumulation of deprotonated OA at the tri olein–water interface induces a higher local concentration of H₃O⁺ at the interface, leading to locally reduced pH values in this region. This results in the observed apparent pKᵦ values of OA in these emulsions between 6.4 and 7.1. Upon increasing the content of tri olein relative to OA, the intermolecular interactions of the neutral form of OA with tri olein may increase the apparent pKᵦ of OA in the system. Van der Waals interactions between carbon chains have been reported to shift the pKᵦ of interfacial fatty acids to higher values.[36] The observed gap in interfacial tension of 50% OA in tri olein at different pH values, and b) illustrations of the different colloidal structures observed with increasing pH values: normal oil-in-water emulsion emulsified micro-emulsion, vesicles, and multilamellar structures (from bottom to top).

2.2. Nanostructure of Oil-in-Water Emulsions

SAXS curves of the emulsions at 50% OA in tri olein in PBS at different pH values are presented in Figure 3a. At pH 6.1 and 7.2, the SAXS curves mostly indicate a normal oil-in-water emulsion, with a power-law decay in the I(q) at q < 0.07 Å⁻¹ according to q⁻⁴ representing the scattering from the relatively smooth lipid–water interface of the particles. The broad, rather weak correlation peak with a maximum around 0.3 Å⁻¹ indicates local arrangements of triglycerides in the internal phase of the droplet.[53] At pH 7.4, a defined, broad correlation peak with maximum at 0.15 Å⁻¹ was observed with SAXS, indicating the presence of water-swollen inverted-type micelles inside the emulsion droplets. Similar emulsified microemulsion phases observed for dispersions of mono linolein–tetradecane in water were previously studied and electrostatic repulsion between charged headgroups. At higher ionic strengths, the augmented charge screening effect of the salt can reduce the electrostatic repulsion between deprotonated OAs, lowering the energetic penalty for the ionization.[33] This process would result in a shift of the deprotonation equilibrium toward lower apparent pKᵦ values. It is also important to consider that the screening effect of the dissociated salt can reduce the local concentration of H₃O⁺ ions at the interface attracted to the negatively charged deprotonated OA, also inducing ionic displacement of H₂O⁺ by Na⁺, thus increasing the bulk concentration of H₂O⁺, shifting the measured apparent pKᵦ to lower values.[36] The effect of both composition and ionic strength on the apparent pKᵦ reinforces the idea that the ionization state of fatty acids during processes such as triglyceride–oil digestion would depend not only on the pH but also on its microenvironment. By tuning the lipid composition and the salt content (ionic strength) in food formulations, one could tailor the ionization state of fatty acids to exploit the significant changes in surface activity and emulsifying capability that arise due to deprotonation.
characterized with SAXS. In terms of the Treubner–Strey model for microemulsions, the position of the maximum of the microemulsion peak, \( q_{\text{max}} \), is associated to the characteristic distance \( d_c = \frac{2\pi}{q_{\text{max}}} \), which indicates the domain size in these self-assemblies. Increasing the pH from 7.4 to 7.8 gradually shifted the correlation peak to smaller \( q \) values, with the characteristic distance increasing from 42 Å (pH 7.4) to 74 Å (pH 7.8). This gradual change in the characteristic distance with pH could be caused by the increased fraction of deprotonated OA molecules at the lipid–water interface. The resulting charge repulsions among their negatively charged carboxyl groups increase the effective headgroup area at the interface, which favors the formation of larger inverse micelles.

A further change in the SAXS curves occurred when the pH increased from 7.8 to 7.9 (Figure 3a). At pH values between 7.9 and 8.6, the curves displayed a very broad shoulder between \( q \) values of 0.02 and 0.2 Å⁻¹. The power-law decay of the scattering intensity at low \( q \) values (\( q < 0.02 \text{ Å}^{-1} \)) decreased from \( q^{-3.2} \) at pH 7.9 to \( q^{-2.3} \) at pH 8.6. The characteristic power-law decay for unilamellar vesicles is \( q^{-2} \).[56] Furthermore, for this pH range (7.9 to 8.6), very weak peaks at \( q < 0.1 \text{ Å}^{-1} \) are also observed. These rather weak and broad peaks may arise from dispersed liquid crystalline phases, potentially an inverse bicontinuous cubic phase that may coexist with the vesicles.

Representative cryo-TEM image of the dispersion containing 50% OA in triolein at pH 8.6 and 9.5 are shown in Figure 4. At pH 8.6 (Figure 4a), emulsion particles coexisting with vesicles of various sizes and shapes were observed, with some of the vesicles showing either several bilayers on their surface or being entrapped in other vesicles. The presence of co-existing emulsion droplets and vesicles agrees well with the observations from SAXS reported above. At pH 9.5 (Figure 4b), merged vesicles and vesicles with multilamellar shells are present. Large, micrometer-sized particles with a rim of most likely multilamellar structures were also observed. The presence of lamellar stacks is in agreement with the SAXS analysis of this sample, which will be discussed in more details below. Detailed characterization of

Figure 4. Cryo-TEM image of 50% OA in triolein emulsion at a) pH 8.6 and b) pH 9.5. Blue arrow indicates emulsion droplet, red arrows indicate vesicles of various shape and size, some of them merged, and green arrows indicate most likely multilamellar vesicles. Micrometer-sized particles (indexed with a star) appear as coexisting, triolein-rich, emulsion droplets with multilamellar structures on the surface.

Figure 5. a) SAXS curve for the system 50% OA in triolein in PBS at pH 8.6 (scatters) and corresponding fit calculated by IFT method (full line); b) The corresponding pair distance distribution function \( p(r) \) obtained from IFT; c) Thickness pair distance distribution function, \( p_t(r) \), calculated for the vesicle bilayer (blue line) with the predicted \( p_t(r) \) calculated from deconvolution (dashed red line), and; d) Excess electron density profile \( \Delta \rho_t(r) \) of the bilayer-half obtained through deconvolution of \( c) \).
multilamellar structures and the potential internal features of emulsion droplets such as the presence of inverse micelles with cryo-TEM was limited by the resolution and contrast.

The pair distance distribution function $p(r)$ was calculated using the Indirect Fourier Transformation (IFT) method (Figure 5) for the SAXS curve of the sample at pH 8.6. The resulting $p(r)$ was characteristic of vesicles (Figure 5b). The oscillation in the $p(r)$ at $r < 50 \, \text{Å}$ may be caused by the differences in electron density within the dimensions of the bilayer region. The rest of the curve corresponded to vesicles with maximum dimensions beyond the resolution limit of our set-up or around 450 Å. Hence, the $p(r)$ was mathematically truncated at $r = 1000 \, \text{Å}$, which does not reflect the vesicle size. Based on this assumption, the SAXS data were fitted using a bilayer model, providing the thickness pair distance distribution $\rho_t(r)$ of the vesicle bilayer (Figure 5c). The thickness excess electron density $\Delta \rho_t(r)$ relative to the buffer was then estimated by deconvolution of the $\rho_t(r)$ using a convolution-square-root-operation (Figure 5d). The lower $\Delta \rho_t(r)$ at $r < 11 \, \text{Å}$ corresponds to the hydrocarbon region within the bilayer, while the region of higher $\Delta \rho_t(r)$ at $r > 11 \, \text{Å}$ represents the deprotonated carboxyl headgroups with their associated counter-ions. The dimension of the half-bilayer can be estimated by the point where the excess electron density approaches 0, which occurs at $r_t \approx 25 \, \text{Å}$, comparable with previous studies involving OA.

A further increase in the pH to 9.5 led to colloidal transformations into structures with equidistant Bragg peaks at $q = 0.140, 0.279$, and 0.418 Å$^{-1}$ in their SAXS patterns, characteristic of multilamellar phases, in agreement with the cryo-TEM analysis discussed above. The interlamellar distance of around 45 Å was calculated, which agrees with the bilayer spacing reported for the OA–sodium oleate-water system. It is worth mentioning that the viscosity of the system increased noticeably upon reaching pH 9.5, which may be related to the reduced mobility caused by the lamellar stack of bilayers that fill out the space.

SAXS curves were also obtained for the system containing 5% OA in triolein at selected pH values (Figure S3, Supporting Information). The curves presented an intensity power-law decay roughly proportional to $q^{-1.3}$ for $q < 0.05 \, \text{Å}^{-1}$, suggesting a rather smooth oil–water interface as “observed” in normal oil-in-water “emulsions”. At pH 9.5, a weak shoulder centered around 0.03 Å$^{-1}$ can be observed, which indicates the presence of nanostructural features. However, the scattering is too low for detailed analysis, indicating that these structures represent a minor population in the sample only.

The nanostructure of the OA–triolien dispersion at 50% OA content is highly dependent on pH, as summarized in Figure 3. The pH-dependence of the nanostructure can be attributed mainly to two factors: the change in amphiphilicity of OA as it deprotonates, and the modification in the effective headgroup area of OA at the lipid-water interface upon deprotonation, which can alter the packing of the self-assembling molecules in agreement with expectations from the critical packing parameter model. The presence of triolein introduces changes in the observed nanostructures when compared to pure OA self-assemblies. For instance, Bragg reflections of inverse micellar cubic and inverse hexagonal structures that were reported in pure OA water systems were not observed in the 50% OA–triolien mixtures. The integration of the rather bulky triolien molecule into the OA self-assemblies may hinder the formation of these highly ordered liquid crystalline structures. In terms of the critical packing parameter model, triolien integrates mostly into the hydrophobic regions of the OA self-assemblies, increasing their volume and thus modifying the packing geometry to more hydrophobic structure. Indeed, the presence of the inverse microemulsions was observed up to pH ≈7.8 when the system transformed into mostly vesicle-type structures.

### 2.3. Colloidal Stability, Particle Size, and Emulsification Properties

The zeta-potential values for aqueous dispersions of OA and triolien at different composition and pH values are demonstrated in Figure 6. At pH 6.0, the zeta-potential was $-14.0 \pm 1.0$ mV for the 50% OA in triolien dispersion. When increasing the pH to 6.5, a decrease to $-54.5 \pm 1.0$ mV was observed for this sample. Further increasing the pH only led to smaller changes in the zeta-potential, with a measured zeta-potential of $-53.8 \pm 1.0$ mV at pH 8.0. The system containing 25% OA in triolien behaved in a very similar way, although a small increase in zeta-potential occurred as pH was increased from 6.5 to 8.0. The 5% OA in triolien system only reached its plateau ($-49$ mV) at pH 7.0 instead of 6.5. The sample at 1% OA in triolien only reached zeta-potential values beyond $-40$ mV at pH 7.5 and 8.0 while all other systems reached this threshold already at pH 6.5. The decrease in zeta-potential for the 1% OA in triolien system did not present a clear plateau although such decrease is more accentuated from pH 6.0 to 70 than from pH 70 to 8.0.

The decrease in zeta-potential upon pH increase for the OA–triolien systems arises from the deprotonation of the OA molecules and their subsequent accumulation at the oil–water interface. A strong change to more negative zeta-potential values was observed when the pH reached the apparent $pK_a$ of OA in the
system, which agrees to observations in the interfacial tension measurements (Figure 1). The higher pH values required for the system containing 5% OA in triolein to reach the plateau in the zeta-potential than the systems with higher amount of OA in triolein is also in-line with the findings from interfacial tension studies where lower ratios of OA in triolein have a higher apparent pK_a values. The less negative zeta-potential values and absence of a plateau in the system containing 1% OA in triolein indicate that the amount of deprotonated OA was not sufficient to fully cover the oil–water interface up to pH 8.0. These findings are also in agreement with the interfacial tension analysis that resulted in the highest apparent pK_a for this system. Zeta-potential values that are high in magnitude are crucial for the formation of charge-stabilized emulsions.\textsuperscript{[62]} The related Ka values. The less negative zeta-potential values and absence of a plateau in the system containing 1% OA in triolein indicate that the amount of deprotonated OA was not sufficient to fully cover the oil–water interface up to pH 8.0. These findings are also in agreement with the interfacial tension analysis that resulted in the highest apparent pK_a for this system. Zeta-potential values that are high in magnitude are crucial for the formation of charge-stabilized emulsions.\textsuperscript{[62]} The related stability of the dispersions from their visual appearance was in agreement with the zeta-potential analysis. Photographs of the dispersions at 1%, 5%, and 50% OA in triolein at different pH values, taken 1 and 5 days after preparation, are presented in Figures S4–S6 (Supporting Information). For the system with 1% OA in triolein, phase separation occurred already on the 1st day at pH 6.0. On the 5th day, only emulsions at pH ≥ 7.5 do not appear phase-separated. The systems containing 5% and 50% OA in triolein were stable at least for 1 day. On the 5th day, phase-separation occurred for the 5% OA in triolein system at pH 6.0 and 6.5 and partially at pH 7.0. On the other hand, for the system with 50% OA in triolein, phase-separation was only observed on the 5th day for the sample at pH 6.0, while samples at higher pH values remained stable for at least several days.

The particle size of the emulsion containing 5%, 25%, and 50% w/w OA in triolein was further investigated by DLS. For the system with 50% OA in triolein: at pH 6.0, the DLS autocorrelation function showed a multimodal decay, suggesting multiple particle size distributions that were not further analyzed (see Figure S7, Supporting Information). This is most likely a consequence of coalescence owing to the weaker interparticle electrostatic repulsions in agreement with expectations from the zeta-potential measurements (Figure 6). At pH 7.0, the corresponding DLS autocorrelation function presents a single decay only, indicating a rather monomodal particle size distribution (Figure S7, Supporting Information). The hydrodynamic radius, R_H, was found to be 129 nm with a polydispersity index (PDI) of 0.26. Increasing the pH from 7.0 to 8.0 leads to an R_H of 125 nm and PDI of 0.34. The decrease in the apparent R_H with the pH of the buffer agrees to the interfacial tension data reported above. The interfacial tension is proportional to the energy needed to form new interfaces. Hence, systems with lower interfacial tension are expected to result in smaller particle sizes (larger interface area at constant volume) at a given energy input. This is also reflected by the decrease in the apparent R_H values with increasing OA content in triolein with 148 nm (PDI 0.45) at 5%; and 136 nm (PDI 0.31) at 25% OA in triolein at pH 7.0 (Figure S8, Supporting Information).

The low-energy emulsification in the OA–triolein system was further explored by comparing emulsions prepared by shaking with a vortex shaker and tip sonication using multi-angle DLS. The oil phase was 5% OA in triolein and aqueous phase was PBS at pH 7.5. Figure 7 shows the apparent R_H values for the emulsions prepared with the two methods. The angular-dependent R_H values shows a dependence on the emulsification time and method in the scattering angle range of 34.0–144.5°. This is a result of the varying degree of polydispersity and potential multi-modal particle size distribution in the emulsions. In agreement with scattering theory, larger particle sizes were observed at lower q^2 values (lower scattering angles), and smaller at higher q^2 values (larger scattering angles). Increasing the time of vortex stirring or the number of sonication cycles resulted in an overall decrease in particle size at all q^2 values. However, for the vortex-stirred sample (Figure 7a), a limit was reached at 300 s, in which further shaking did not result in significant changes in the particle size. For this sample, apparent R_H values ranging from 98 to 190 nm were observed while values ranging from 101 to 186 nm were found for the ultrasonicated sample at the minimum after 100 cycles (Figure 7b).

To further analyze the emulsion characteristics, the decay rate of the DLS autocorrelation function was plotted against q^2 (Figure S9, Supporting Information). For monomodal particle size distributions with low polydispersity, the decay rate of the DLS autocorrelation function is expected to be proportional to q^2, with the slope of the curve representing the

![Figure 7](https://example.com/figure7)

Figure 7. Apparent R_H values as a function of q^2 from multi-angle DLS for emulsions with 5% OA in triolein prepared at pH 7.5 dispersed by a) shaking using a vortex shaker at varying times; and b) ultrasonication at different sonication times of 3 s per cycle. The corresponding decay-rate versus q^2 plots are shown in Figure S9 (Supporting Information).
apparent diffusion coefficient. Multimodal particle size distributions or polydisperse systems will show a deviation from this linear behavior. The proximity to linearity observed by the decay rate as a function of $q^2$ for the ultrasonicated as well as the vortexed sample at pH 7.5 at their highest sonication cycles/stirring time indicates that both emulsions are more monomodal with lower polydispersity. Figure S10 (Supporting Information) displays the PDI for these emulsions as a function of $q^2$. For both dispersions methods, the PDI values are initially around 0.7 (average), decreasing to values around 0.3 at final stirring times/sonication cycles. This progressive decrease in the PDI with increasing stirring times/sonication cycles is in agreement with the observed behavior for the calculated decay rates (Figure S9, Supporting Information). This demonstrates that the low interfacial tension of ≈2 mN m$^{-1}$ in this system allows the preparation of rather monodisperse OA-triolein emulsion simply by shaking, without the addition of additional emulsifier. The OA acts simultaneously as the oil and the stabilizer at sufficiently high pH values.

The particle size of emulsions dispersed via vortex stirring at different pH values were also examined (Figure 8). The triblock copolymer Pluronic F127, a steric stabilizer, was included to the buffer solution in this comparison to allow measurements at pH values < 6.0. At these lower pH values, the emulsions phase separate nearly immediately without this stabilizer due to the lack of sufficient electrostatic stabilization from deproto- nated OA. Increasing the shaking time at pH 3.0 in presence of F127 resulted in an increase in the particle size. Such behavior was not observed at pH 7.5 where the particle size decreased with shaking time. At 600 s of stirring time, the apparent $R_H$ ranged from 152 to 246 nm at pH 3.0 and from 98 to 183 nm at pH 7.5. It is worth noting that the particle size of the F127-containing emulsions at pH 7.5 was comparable to that in absence of F127 reported above, with 98–190 nm in this angular range (Figure 7). The corresponding plots of the decay rate of the DLS autocorrelation in Figure S11 (Supporting Information) show that in both pH 3.0 and 7.5, the decay rate versus $q^2$ curves become more and more linear over longer stirring times, indicating the decrease of the polydispersity and more monomodal particle size distribution. The changes in PDI with $q^2$ for both pH values are shown in Figure S12 (Supporting Information). PDI values ≈0.7 and 1.0, on average, are observed at 30 s vortex stirring for pH 3.0 and 7.5, respectively. Increasing stirring time to 600 s led to PDI values of 0.4 and 0.3 for pH 3.0 and 7.5, respectively. The scattering intensity at a scattering angle of 89.3° for these systems (Figure S13, Supporting Information) increased with stirring time.

The data obtained from multi-angle DLS measurements revealed that emulsions prepared via vortex stirring at pH 7.5 (Figure 7a) have comparable size and polydispersity as those prepared by ultrasonication (Figure 7b). The particle size characteristics of the emulsions at pH 7.5 in presence and absence of F127 are comparable, see Figures 8b and 7. Contrary, the sample at pH 3.0 showed an increase in $R_H$ values with increasing stirring times, see Figure 8a. OA is mostly proto- nated at this pH, and thus does not act as a strong surfactant or stabilizer (see discussion about interfacial tension above).

As the energy required to increase the oil–water interface by a defined value is proportional to the interfacial tension, the emulsion at pH 3.0 requires more energy to disperse at low particle size. The lower zeta potential also makes it more unstable. Indeed, the scattering intensity at pH 3.0 reached a plateau at 300 s and presented overall smaller intensities than at pH 7.5, most likely owing to less volume of oil dispersed at the lower pH values (Figure S13, Supporting Information).

The results show that lower energy dispersion methods can be employed to prepare stabilizer-free OA-based emulsions, at pH > 7.0. In addition to being interesting for the sustainable preparation of emulsions$^{[63]}$ low-energy emulsification can also trigger the natural emulsification of food oils during digestion, creating a larger interfacial area for the action of lipases. It can also be of interest for the emulsification of formulations with sensitive molecules such as proteins that may degrade or dena- ture upon high-energy emulsification methods.

The findings demonstrate that the addition of OA to triolein emulsions results in stimuli-responsive oil-water interfaces. The response of the interfacial tension, charge, emulsion nanostructure and stability to factors such as the pH, ionic strength, and composition in these systems can be further explored to design advanced food materials. These parameters are modified during the transit of the emulsion through the gastro-intestinal tract. This could be exploited to trigger interactions of the
droplets with digestive tract components such as lipases, bile salts, and tissue, which can ultimately influence the digestion kinetics and the delivery of nutrients.

3. Conclusion

This study analyzed the interfacial tension, nanostructure, and colloidal properties of the nutritionally relevant OA-triolein-water system at different pH values and composition. It further demonstrates the use of interfacial tensiometry to measure the apparent pK_a of long-chain fatty acids at the oil–water interface.

The pH was found to significantly affect the interfacial tension and nanostructure in these systems owing to the pH-driven protonation behavior of OA, with the associated changes in its polarity. The apparent pK_a of OA in triolein was also found to be composition-dependent, ranging from 7.1 ± 0.2 at 1% OA in triolein to 6.4 ± 0.1 at 50% of OA in triolein. Increasing the ionic strength also reduced the interfacial tension in the OA–triolein system. For instance, a drop in the apparent pK_a from 6.7 ± 0.2 to 6.0 ± 0.1 upon addition of 2.5 M of NaCl to the 5% of OA in triolein system was observed. While the presence of the triolein shifted the OA deprotonation equilibrium toward higher apparent pK_a values, the ionic strength of the medium had the inverse effect, lowering the apparent pK_a.

The deprotonation behavior of OA had also an effect on the emulsion particle size, zeta potential and ultimately stability. Smaller and more charged particles were found at higher pH, increasing the emulsion stability. The gradual increase of pH produced different structures, including oil-in-water emulsions, emulsified inverse microemulsions, possibly emulsified inverse-type bicontinuous liquid crystalline structures, followed by vesicles and multimamellar structures upon increasing the pH from 6.1 to 9.5. The low interfacial tension values < 2 mN m⁻¹ in the OA–triolein-water system at pH > 7.0 allowed their emulsification by shaking.

The use of interfacial tensiometry to investigate deprotonation at lipid–water interfaces can open up new possibilities to research the behavior of ionizable, surface-active molecules at lipid–water interfaces. Further, discovery of the pH-driven protonation behavior of OA, with the associated changes in its polarity, can guide the design of sustainable emulsification processes to protect labile nutrients from degradation due to harsh dispersion methods and help the colloidal stabilization.

4. Experimental Section

Emulsion Preparation: Stabilizer-free oil-in-water emulsions of OA (>99% purity, Carl Roth) and triolein (>96% purity, Santa Cruz Biotechnology) were prepared as follows: 1, 5, 10, 25, and 50% w/w OA in triolein mixtures were prepared and then combined with sodium phosphate buffer (sodium dihydrogen phosphate monohydrate, >98% purity, Carl Roth and disodium hydrogen phosphate dihydrate, >98% purity, Sigma–Aldrich) with a buffer strength of 100 × 10⁻³ M at selected pH values in glass vials (20 mL) with a concentration of 10% w/w lipid (OA + triolein) in the buffer. The mixture was then sonicated using a Lab500 NexTgen Ultrasonic platform (SinapTec, Lezennes, France) with a microtip at 20% amplitude (max. power 500W) during 20 cycles (3 s ON, 3 s OFF each cycle). pH was then re-adjusted using 100 × 10⁻³ M NaOH solution (>99% purity, pellets, Sigma–Aldrich). For the samples homogenized via vortex stirring: sample was initially prepared as described above but instead of ultrasonication, different times of stirring were applied with an Ika MS-3 basic vortex stirrer (IKA-Werke, Staufen, Germany) at 3000 rpm to disperse the sample. For the vortex-stirred emulsions containing the triblock copolymer “Pluronic F127” as stabilizer (Sigma–Aldrich). F127 was dissolved in the buffer before emulsification at defined concentrations to result in a final F127/oil mass ratio of 1:20.

Interfacial Tension Measurement Using Spinning Drop Tensiometry (SDT): SDT measurements were performed on a spinning drop tensiometer (Krüss Scientific, Hamburg, Germany) at 25 °C using mixtures of OA in triolein at defined compositions as the “light” phase and the sodium phosphate buffer (100 × 10⁻³ M) at selected pH values as the “heavy” phase. The measurements used a drop volume of 1–4 μL with a surrounding volume of 1 mL, and the interfacial tension was calculated from drop dimensions via the Young–Laplace model. Such drop dimensions were obtained by an integrated camera with optical resolution of 2.3 μm. Calibration of the camera (to convert pixels to real dimensions) was performed on the same day as the measurements. For the measurements at higher ionic strength conditions, NaCl (99.5% purity, Acros Organics) was dissolved in the buffer at varying amounts to achieve the desired concentrations. The interfacial tension values were fitted in function of pH by the sigmoid curve model (Equation 1):

\[ γ(\text{pH}) = A_1 + \frac{A_2 - A_3}{1 + 10^{pK_a - p\text{H}}} \]

where γ is the interfacial tension, A_1 is the top asymptote value of interfacial tension while A_3 is the bottom asymptote value, pK_a is the inflection point of the sigmoid curve which corresponds to the apparent pK_a to be determined, and D is a parameter that controls the slope of the sigmoid curve.

Cryogenic Transmission Electron Microscopy (Cryo-TEM): Cryo-TEM images were made with a Tecnai Spirit BioTwin transmission electron microscope (FEI Europe, Eindhoven, Netherlands). Lacey Carbon Films on 200 Mesh Copper Grids (Agar Scientific, Essex UK) were first glow discharged for 30 s. The dispersion (5 μL of a 1% w/w oil concentration) was allowed to adsorb onto the grid for 30 s, then blotted for 3 s and plunged frozen into liquid ethane and stored in liquid nitrogen. Grids were maintained at sub−170 °C temperatures in a Gatan cryo-holder. Images were recorded via a Veleta (2048 × 2048 px) wide angle camera with TEM Imaging & Analysis (TIA) software with a defocus of −3 μm.

Zeta-Potential: Zeta-potential measurements were carried out on a DelsaMax Pro Zeta Potential Dynamic Light Scattering Analyzer (Beckman Coulter, Indianapolis, USA) which employs Massively Parallel Phase Analysis Light Scattering (MP-PALS) to determine the electrophoretic mobility and then calculate the zeta-potential via the Smoluchowski’s theory (Equation 2):

\[ μ_e = \frac{ε_r ε_0 k}{η} \]

where μ_e is the electrophoretic mobility, ε is the dielectric constant of the medium, ε_0 is the permittivity of vacuum, ε_0 is the zeta-potential, and η is the viscosity of medium. These measurements were performed at 0.01% w/w lipid concentration in buffer at 25 °C.

Dynamic Light Scattering (DLS): Single-angle DLS measurements on ultrasonicated dispersions were performed on a LS Spectrometer (LS Instruments, Fribourg, Switzerland). Scattering was collected at a scattering angle of 90° using a 660 nm laser as excitation source at 25 °C. Accumulations with a measurement time of 20 s per sample were performed. Samples were diluted to 0.01% w/w concentration to minimize multiple-scattering. The hydrodynamic radius and polydispersity index
was calculated from the normalized autocorrelation function (ACF) by means of 2nd order cumulant analysis.\textsuperscript{[63]}

Multi-angle DLS measurements were carried out with a light scattering goniometer (CCS-8F, ALV Langen, Germany) and a solid-state laser (Coherent Verdi V5, 532 nm wavelength, max. power of 5 W) with single-mode fiber detection optics (OZ from GMP, Zurich, Switzerland), 8 fiber-optic detectors and ALV 7004 correlators with fast expansion (ALV, Langen, Germany). Measured angles were in the range between 34.0 and 144.5°. Sample dilution, measurement time, and particle size calculations were performed with the same manner as the single-angle DLS described above.

Small Angle X-ray Scattering (SAXS): SAXS measurements for the system containing 50% OA in triolein were carried out using a NanoMax-IQ (Rigaku Innovative Technologies, Auburn Hills, USA) with a Cu-α source (radiation wavelength of 1.54 Å). Measurements were taken under vacuum at room temperature. 2D scattering data was azimuthally integrated into 1D intensity versus scattering vector \((I(q))\) plots through the Rigaku SAXS Lab software, in which \(q\) corresponds to the length of the scattering vector: \(q = 4\pi/\lambda \sin(\theta/2)\), \(\lambda\) is the wavelength and \(\theta\) the scattering angle. Sample to detector distance was 49.7 cm, with a measurable \(q\)-range from 0.007 to 0.64 Å\(^{-1}\). Samples were prepared at 10% w/w lipid (OA + triolein) content in buffer at the desired pH values and placed in borosilicate glass capillaries (Hilgenberg GmbH, Malsfeld, Germany) with an outside diameter of 1.5 mm that were then wax-sealed. Buffer solution was used for background subtraction.

Additional SAXS measurements for the system containing 5% OA in triolein were carried out in a Bruker Nanostar (Bruker AXS GmbH, Karlsruhe, Germany) with a Cu-α source and a VANTEC-2000 detector. Sample to detector distance was 107 cm, with \(q\)-range between 0.007 to 0.21 Å\(^{-1}\). 1D curves were calculated using the Bruker software DIFFRAC. EVA (Bruker AXS, version 4.1). Sample preparation and data treatment were done in similar way as for 50% OA in triolein system measurements described above.

SAXS Data Analysis: The model-independent indirect Fourier Transformation (IFT) was used to further analyze the SAXS data.\textsuperscript{[67]} With this method, the pair distance distribution function \(p(r)\) is calculated from the \(I(q)\) using Equation 3. This function provides model-free information about the size and shape of the particles.\textsuperscript{[64]}

\[
I(q) = 4\pi \int_0^{2\pi} \langle p(r) \sin qr \rangle dq dr
\]

In which

\[
p(r) = r^2 \Delta \rho^2(r)
\]

Where \(\Delta \rho^2(r)\) being the convolution square of the spatially averaged excess electron density, \(p(r)\). The deconvolution of the \(p(r)\), using a convolution square-root operation, gives the radial excess electron density profile \(\Delta \rho(r)\) relative to the electron density of the buffer. For this, it is necessary to define/assume the symmetry of the system (i.e., spherical, lamellar, or cylindrical).\textsuperscript{[66]} For vesicles, the thickness pair distance distribution function \(p_\ell(r)\) of the vesicle bilayer can be calculated. The deconvolution of \(p_\ell(r)\) provides information about the spatial distribution of the excess electron density.\textsuperscript{[69]}

Statistical Analysis: Interfacial tension and zeta potential data were indicated as mean ± 95% confidence interval. Single-angle and multi-angle DLS measurements are the average of ten accumulations. Presented SAXS curves were averaged over 3 h of accumulation.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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food colloids, lipid self-assemblies, low-energy emulsification, nanoemulsions, oleic acid, pH-triggered interfacial tension, small angle X-ray scattering (SAXS)

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