Experimental report

20/07/2022

Proposal: 9-13-1005 **Council:** 4/2021

Title: Understanding the triolein/aqueousinterface and lipase activity

Research area: Chemistry

This proposal is a resubmission of 9-13-966

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Samples: Polystyrene
Triolein
Silicon Blocks

Instrument	Requested days	Allocated days	From	To
FIGARO User-supplied	4	2	28/06/2021	30/06/2021

Abstract:

The triglyceride aqueous interface has an important role in many processes, none more so than lipase activity. Lipases, such as TLL, are responsible for the degradation of triglycerides to smaller components, such as fatty acids. TLL attracts considerable interest in the food and pharmaceutical industries due to the catalytic behavior it possesses. In spite of the importance of this aqueous triglyceride system for many processes, relatively little is known about the interface compared to that between alkanes and water. Thus, understanding the oil-water interface and how this influences lipolytic activity is of significant importance. Recently, we have studied the aqueous/triolein interface using coarse-grained simulations, spectroscopic ellipsometry and quartz crystal microbalance with dissipation. Although these measurements provided detailed information related to changes in the film thickness and integrity, no information is provided for the change in the internal structure and composition with solvent uptake nor where the lipase activity takes place. Therefore, we propose using neutron reflectometry, which allows both properties to be examined.

Exp. 9-13-1005 "Understanding the triolein/aqueous interface and lipase activity"

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In this experiment we utilised neutron reflectometry to investigated changes in the internal structure of a thin triolein film after lipolysis at pH 7 and pH 8.5.

Samples were prepared on 50 x 80 mm^2 silicon blocks. The silicon blocks were modified with a thin layer (~100 Å) of deuterated polystyrene (dPS) to increase the stability of the meta stable triolein film. After spin coating triolein on top of the dPS film, the surface was immediately installed in a solid liquid cell and exposed to a TRIS buffer solution. Reducing the time between spin coating the triolein film and exposing the surface to the buffer solution has been shown to minimise dewetting of the triolein at the dPS interface. Once exposed to TRIS buffer, the triolein film increases in thickness following the uptake of aqueous solution within the oil phase until an equilibrium thickness is achieved within 60 minutes.

All measurements were performed in a horizontal configuration to eliminate the effects of gravity on the triolein film. Throughout the experiment, static measurements were conducted in D_2O buffer at two angles, 0.64° and 3.77° to cover a Q range from 0.007 to 0.4 $Å^{-1}$ while kinetic measurements were taken throughout the digestion of the triolein film every 30 seconds at a single angle of 0.64° covering the q range 0.007 to 0.04 $Å^{-1}$.

Static measurements were performed on the buffer equilibrated triolein film before 2 ppm *Thermomyces lanuginosus* lipase (TLL) was injected into the solid liquid cell. The kinetics of the lipolysis was tracked for ~2 hour after which a final static measurement was performed.

Several static measurements at various solvent contrasts were performed after the digestion was complete to enable co-refinement of the data. However, it has since been revealed that the solvent exchange process changed the structure of the film where some of the digested products are being removed.

Figure 1 shows the reflectivity curve for the buffer equilibrated triolein film at pH 7 along with a preliminary fit and the corresponding SLD fraction profile.

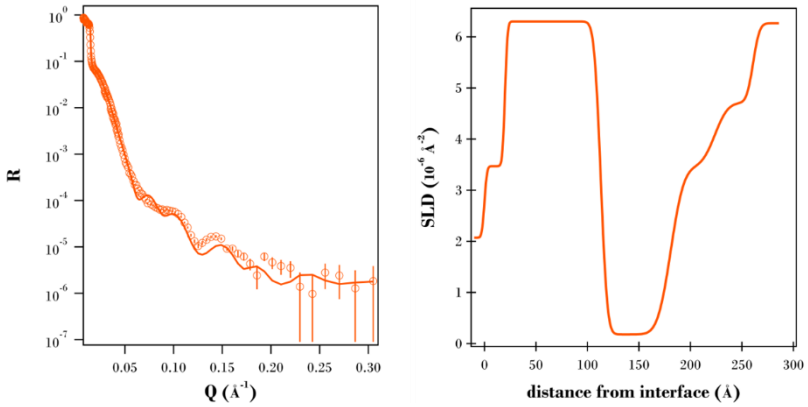

Figure 1. Reflectivity curve and preliminary fit for the equilibrated triolein film in tris buffer at pH 7 with the corresponding SLD profile.

The model included a single slab for both the silica and dPS layers followed by 3 slabs for the triolein film with increasingly more solvent within the triolein film as a function of distance from the substrate. The fitting of the data has additional complications because the

reflectivity curve slopes downward from low Q out to the critical edge. This is due to off specular scattering (OSS) from the film. The OSS has been investigated and a similar pattern can be reproduced using a model assuming small clusters of dry triolein (sub-micron sized in-plane) separated by large areas of D_2O buffer (in the tens of microns range) within the triolein layer(s) as shown in Figure 2.

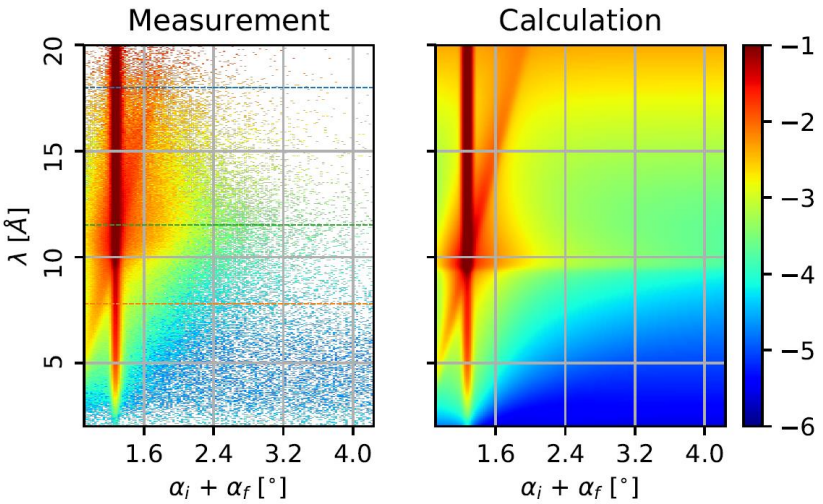

Figure 2. The off specular scattering observed in the measurement and the simulated model showing a similar pattern.

Figure 3 shows the reflectivity curve for the triolein film after TLL lipolysis at pH 7 along with a preliminary fit and the corresponding SLD fraction profile.

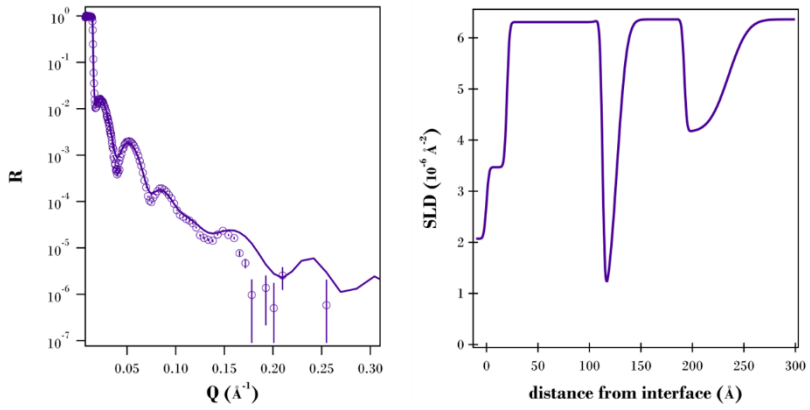

Figure 3. Reflectivity curve and preliminary fit for the triolein film after lipolysis at pH 7 with the corresponding SLD profile.

Once again, the model included a single slab for both the silica and dPS layers followed by 3 slabs for the triolein film however now there is no evidence of any OSS. The SLD profile now reveals an oil rich phase at the substrate followed by a solvent rich region then a layer of solvated oil. We believe this is the separation of the various products formed throughout the digestion of the triolein including oleic acid, calcium oleate complexes, and glycerol.

Figure 4 shows the reflectivity curves for the first 30 minutes of lipolysis after the sample was exposed to TLL. Here, the reflectivity curves reveal the kinetics of the lipolysis are completed within 30 minutes of TLL exposure as can be seen with the curves for 28, 29 and 30 minutes overlaying the curve for the final static measurement taken after lipolysis for the

Q range presented. What is also evident in Figure 4 is that the downward slopping curve prior to the critical edge due to OSS disappears within 3 minutes of TLL exposure.

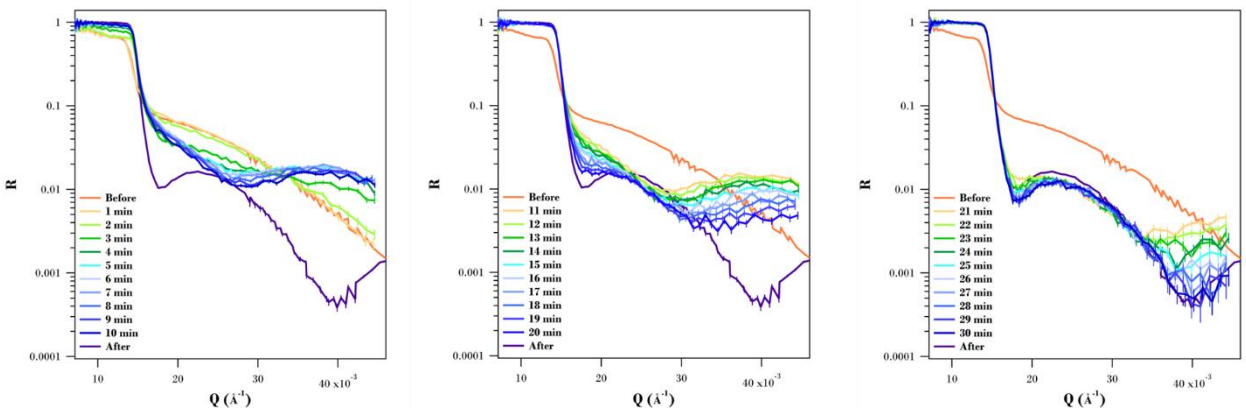

Figure 4. Reflectivity curves for the first 30 minutes of lipolysis split into three 10-minute series.

The next step is to continue refining the model to increase the quality of the fits for the data set presented above. Once an adequate model is defined, the remaining results for the experiments performed at pH 8.5 will be analysed.