Experimental report

20/05/2022

Proposal: 9-11-1998 **Council:** 4/2020

Title: Oleogels formed by plant phytosterols for the replacement of saturated and trans fat in foods

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: mixture of phytosterol (beta-stitosterol) and sterol ester (gamma-oryzanol or lanosterol or cycloartenol) in decane/d-decane

Instrument	Requested days	Allocated days	From	To
D22	2	0		
D33	2	0		
D11	2	2	05/06/2021	07/06/2021

Abstract:

Saturated and trans fats in foods increase in blood serum cholesterol levels, a risk factor for cardiovascular disease. Strategies for replacement of fats exist, but often fat-free or fat-reduced products are viewed as inferior by the consumer. One potential way to replace saturated and trans fats is to use olegelation, where healthier polyunsaturated oils are solidified using low molecular weight gelators. One method we have studied in some detail, exploits the self-association of the phytosterol β-sitosterol (SIT) and sterol ester γ-oryzanol (ORY) to form nm diameter tubules. A technical difficulty that hinders the development of phytosterol oleogel based foods is that under quiescent conditions supercooling occurs and nucleation and growth of the SIT + ORY tubules can take a considerable time after cooling. Gelation can be induced by shearing, but how to control the process is poorly understood. Our goal is to carry out, in future, rheoSANS experiments to understand how shear affects the gel structure. However, characterisation of the oleogels structure is needed, prior to the rheoSANS measurements.

Oleogels formed by plant phytosterols for the replacement of saturated and *trans* fat in foods

Background: Saturated and *trans* fats in foods are considered detrimental to health since they are associated with an increase in blood serum cholesterol levels, a risk factor for cardiovascular disease. Strategies for replacement of fats exist, but often fat-free or fat-reduced products are viewed as inferior by the consumer. One potential way to replace saturated and *trans* fats is to use oleogelation, where healthier polyunsaturated oils are solidified using low molecular weight gelators to form a self-associated gel structure. One method we have studied in some detail[1-5], exploits the self-association of the phytosterol β-sitosterol (SIT) and sterol ester γ-oryzanol (ORY) to form helical tubules of approximately 10 nm diameter when dispersed in polyunsaturated triacylglycerol (TAG). The appeal of the ORY+SIT system is two-fold in that these sterols also have an inherent cholesterol lowering ability, and thus would have a dual cholesterol reducing effect.

For early SANS experiments by Bot et al.[6], the scattering patterns were modelled by accounting for both inner and outer shells having different hydrogen density (Figure 1?). However, their fits show evidence for even more complex structures. For example, it was noted that discrepancies between experimental data and model calculations may be due to neglecting contributions of alkyl chains protruding at the inside of the tubules, in the organic phase. As suggested by these authors, further contrast variation studies as conducted here can provide additional information on the self-assembly in these systems and this might help understanding the kinetics of tubule formation.

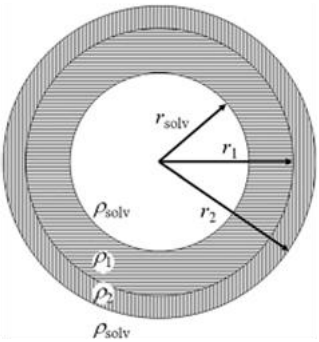

Figure 1 Schematic representation of the double-walled tubule structure

Experimental

ORY+SIT gels in decane + deuterated decane (d-decane) mixtures were made at 1:1 molar ratio of the two sterols, varied total sterol concentration (10, 20 or 30%w/w) and varied proportion of decane:d-decane as summarised in Table 1. The d-decane proportions were chosen to contrast match various regions of the tubule or of the individual sterol molecules (Table 1, Figure 1 (from ref 7)). Using a partially deuterated SIT ($C_{10}H_{11}D_7$) we were also able to carry SANS measurements at contrast match conditions for the alkyl chains of SIT (49% d-decane).

Table 1

Region contrast matched	% d-decane	Scattering length density	Concentration of Sterol used (%w/w)
SIT	9	1.68×10^{-7}	10, 20 & 30
Inner shell of tubule	13	4.32×10^{-7}	10 & 30
ORY	17	7.21×10^{-7}	10 & 30
Outer shell of tubule	31	1.71×10^{-6}	10 & 30
Alkyl chains of d7-SIT	49	2.98×10^{-6}	10 & 30
	100	6.58×10^{-6}	10 & 30

SANS measurements were carried out on D11 at 3 sample-to-detector distances and the overlapped data for a sample containing 30 wt% sterol in d-decane are shown in Figure 2, with both single and

two-shell fits. Preliminary fitting was carried out using SASView and both a core-shell as well as a core-multi-shell model (using two shells) developed by Richard Heenan. Deviations at low Q were modelled by a Q^4 power law. Figure 2 shows a preliminary fit for a 30 wt% sterol oleogel with 100% d-decane as the solvent phase. The fitting parameters from SASView for the data in Figure 2 for the single shell model show a core radius in the range 20.7-21.8 Å and a shell thickness of 25.7-27.8 Å (total tubule radius of 47.4-49.1 Å). The fitting to a 2-shell model is consistent with the single shell model, with a slightly lower core radius of 19.4-20.3 Å, an inner shell of 15.7-21.1 Å and an outer shell of 6.2-11.5 Å (total shell thickness of 27.1-27.3 Å and total tubule radius of 46.6-47.7 Å). For other contrasts only a fit to the single shell model has been attempted so far. The 13% and 17% d-decane samples show very little scattering, and fits that do not conform to the expected tubule dimensions. For the 9% d-decane sample, where we are contrast matching beta-sitosterol we might expect to see an increase in the core radius since part of the SIT molecule extends into the core. We do see an increase in core radius to 44.4-46.0 Å and also a very thin shell (0.6-1.0 Å). Even though the overall tubule radius for the 9% d-decane samples (45.0-46.8 Å) is similar to that for the 100% d-decane sample, the core radius seems too large and shell thickness too small considering we should still be seeing scattering from the ORY molecules in the tubule.

For the 31% d-decane contrast, where we contrast match the outer shell we would expect the core radius to stay largely unchanged, and the shell thickness to decrease. We observe an increase in core radius to 36.0-36.9 Å, with a decreased shell thickness of 1.0 Å. The overall core + (inner) shell thickness of 37.0-37.9 Å is close to that predicted from the 2 shell model with the 100% d-decane (35.0-41.5 Å) although again the core radius would seem too large and the shell thickness to small.

For the 49% d-decane contrast a partially deuterated d7-SIT is used. This allows contrast matching of the alkyl chain of the SIT which we believe sits towards the core of the tubule. Thus, we might expect the core radius to increase slightly, with the shell thickness (comprised of the sterane core of the SIT and the ORY) to remain largely unchanged. We do see an increased core radius (38.6 Å) but also a very thin shell (1.4-3.0 Å), a result that again does not fit with our expected dimensions of the tubule.

The initial estimates of tubule core radius and shell thickness from the 100% d-decane samples are reasonable and consistent with the earlier results of Bot et al.[7] Additional data from the fits for samples where we contrast match various regions of the tubule require further work to refine the fitting parameters as some results do not match our expected model for the tubule.

References

1. Matheson, A, Koutsos V, Dalkas, G, Euston, SR, Clegg, PS, *Langmuir*, **33**, 4537, 2017.
2. Matheson, A, Dalkas, G, Gromov, A, Euston, SR, Clegg, PS *Food & Function*, **8**, 4547, 2017.
3. Matheson, A., Dalkas, G., Mears, R. Euston, SR, Clegg, PS *Soft Matter*, **14**, 2044, 2018.
4. Dalkas, G, Matheson, AB, Vass, H, Gromov, A, Lloyd, GO, Koutsos, V, Clegg, PS, Euston, SR *Langmuir*, **34**, 8629, 2018.
5. Matheson, A, Dalkas, G, Clegg, PS, Euston, SR *Nutrition Bulletin*, **43**, 189, 2018.
7. Bot, A, Gilbert, EP, Bouwman, WG, Sawalha, H, Den Adel, R, Garamus, VM, Venema, P, van Der Linden, E, Flöter, E *Faraday Discussions* **158**, 223, 2012

Figure 2 – SANS data of 30 wt% sterol in 100% d-decane and fits using (a) the core-single shell model plus power law and (b) core-2-shell + power law model.