Experimental report

20/02/2018

Proposal: 9-10-1488 **Council:** 10/2016

Title: Understanding the interfacial behaviour of bile salts to better engineer lipid emulsions

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: DPPC
d-DPPC
Sodium taurocholate hydrate
Sodium taurodeoxycholate hydrate

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	3	3	17/02/2017	20/02/2017

Abstract:

The current project aims at elucidating how bile salts (BS) structure influences their interfacial behaviour and their impact on lipid (fat) digestion. Although the characterisation of BS interfacial behaviour is beginning to garner interest, the structure-function relationships governing their interfacial behaviour remain unknown. Neutron reflectivity (NR) is the only technique which can provide a detailed molecular picture of the BS/lipids interface, over time and at equilibrium. We propose here to measure the interfacial interactions of two distinct BS, sodium taurocholate hydrate (NaTC) and sodium taurodeoxycholate hydrate (NaTDC), with a 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) monolayer, using a Langmuir trough (LT). NR measurements will be employed to obtain information on the organisation of these systems at the air/water interface, monitor changes in the lipid monolayer and establish, with precision, the localisation of each BS at the interface.

Understanding the interfacial behaviour of bile salts to better engineer lipid emulsions

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Backgrounds. Bile salts (BS) are biosurfactants produced in the liver and released into the small intestine (duodenum), which play key roles in lipid (fat) digestion and absorption[1,2]. BS facilitate the adsorption of the lipase/co-lipase complex to fat droplet interfaces, thus promoting enzyme-catalysed lipolysis, and they also desorb from the interface and shuttle insoluble lipolysis products to the gut mucosa in mixed micelles, to facilitate absorption (Fig. 1).

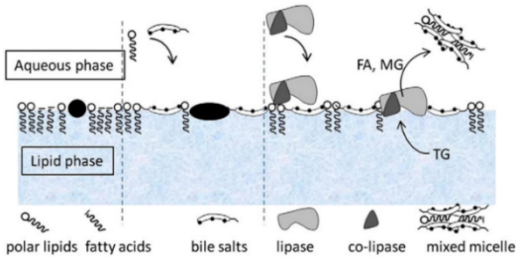

Figure 1: Duodenal lipolysis mechanism[2]

The present experiment aimed to improve our understanding of the interfacial behaviour of BS and thus their role in lipid digestion. In a second phase of the project, we will examine lipid emulsifiers that compete with BS for adsorption at interfaces, thus slowing down lipolysis, and which could therefore be used to regulate fat digestion and go some way to addressing the severe health problems associated with obesity.

BS have a variety of structures (Fig. 2), with very minor variations. Our working hypothesis is that their contrasting roles arise from their natural diversity, i.e., BS with different structures carry out different functions in fat digestion[3]. Indeed, it has been shown previously that cholate, chenodeoxycholate and deoxycholate derivatives display different interfacial adsorption and desorption kinetics[3]. Nevertheless, to date, no study has elucidated how BS structure influences their interfacial behaviour and their impact on lipolysis. Although the characterisation of BS interfacial behaviour is beginning to garner interest[3], the structure-function relationships governing their interfacial behaviour remain unknown. Neutron reflectivity (NR) is the only technique which can provide a detailed molecular picture of the BS/lipids interface, over time and at equilibrium.

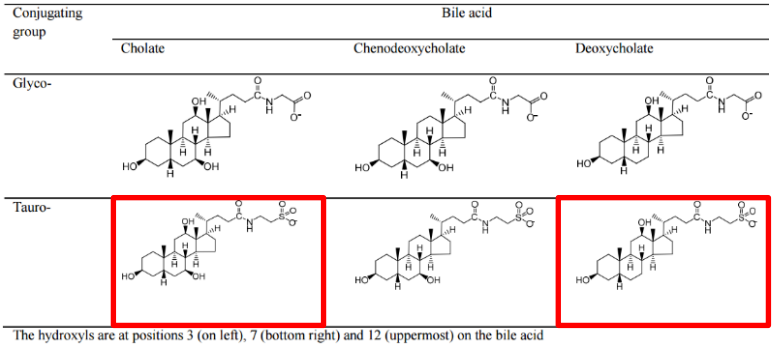

Figure 2: BS structures[3]

Preliminary studies. Prior to our NR measurements, the interfacial interactions of two distinct BS, sodium taurocholate (NaTC) and sodium taurodeoxycholate (NaTDC) (Fig. 2), with a 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) monolayer were measured, using a Langmuir trough (LT). The results of these experiments clearly show that NaTC (Fig. 3A) has a higher affinity for the interface, and would thus be more effective at promoting lipolysis, than NaTDC (Fig. 3B), which desorbs rapidly and thus may more efficiently promote the displacement and solubilisation of lipolysis products.

Building upon the LT studies, we have used NR to obtain information on the molecular-level organisation of these systems at the air/water interface. More specifically, we have reproduced the same LT experiments and monitored the structural changes of the interfacial film over time to establish the localisation of each BS at the interface.

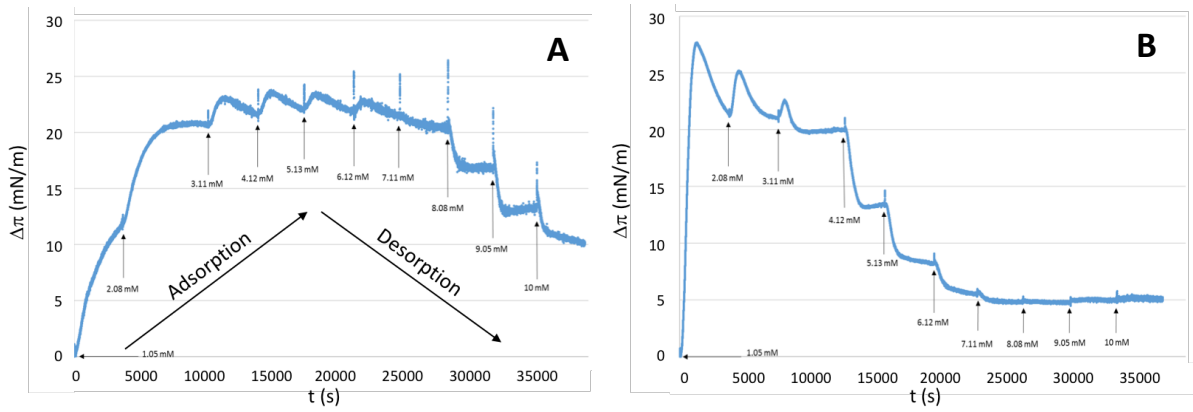

Figure 3: Evolution of the surface pressure ($\Delta\pi = \pi(t) - \pi_{DPPC}$) over time, upon successive addition of BS (A. NaTC, B. NaTDC) into the subphase. The lipids were spread onto water at π_{DPPC} = 25.0 ± 2.0 mN/m at 23°C. Each addition is shown by an arrow, together with the corresponding $[BS]_{subphase}$ achieved. An increase in surface pressure means that BS adsorbs at the interface, whereas a decrease in the same parameter indicates desorption.

Experimental plan. NR experiments were performed on protonated and fully deuterated DPPC monolayers (DPPC and d_{75}-DPPC, respectively), at a lateral pressure of 25 mN/m and at 23°C. After the characterisation of the pristine lipid monolayers in air-contrast matched water (ACMW) and D_2O, BS were injected into the subphase. Then, both kinetic and static studies were carried out, using three different contrasts (Table 1). Three different BS concentrations below, at, and above the critical micelle concentration (CMC) were successively added, because different interfacial behaviours were observed at these values. With kinetic studies, successive BS injections into the ACMW and D_2O subphases were carried out, and the intensity at one incident angle (in the low-q region) was monitored over time to measure the time evolution of the surface excess. Once equilibrium was reached, reflectivity was measured at an additional incident angle to cover a larger q range. All the scattering length densities (SLD) are summarized in Table 2.

Lipid	Subphase	BS
DPPC	D_2O	NaTC or NaTDC
d_{75}-DPPC	D_2O	NaTC or NaTDC
d_{75}-DPPC	ACMW	NaTC or NaTDC

Table 1: Kinetic and static studies

DPPC	SLD (Å^{-2})	d_{75}-DPPC	SLD (Å^{-2})
h_{62}-tail	-4.05E-07	d_{62}-tail	7.66E-06
h_{18}-head group	1.75E-06	d_{13}-head group	5.68E-06
Subphase	**SLD (Å^{-2})**	**BS**	**SLD (Å^{-2})**
ACMW	0.00E+00	NaTC	9.52E-07
D_2O	6.33E-06	NaTDC	8.96E-07

Table 2: SLD values of each component

Results. The reflectivity curves (Fig. 4) have been analysed using the software Aurore®, and the SLD profile of each lipids layer has been established as a function of the distance from the interface.

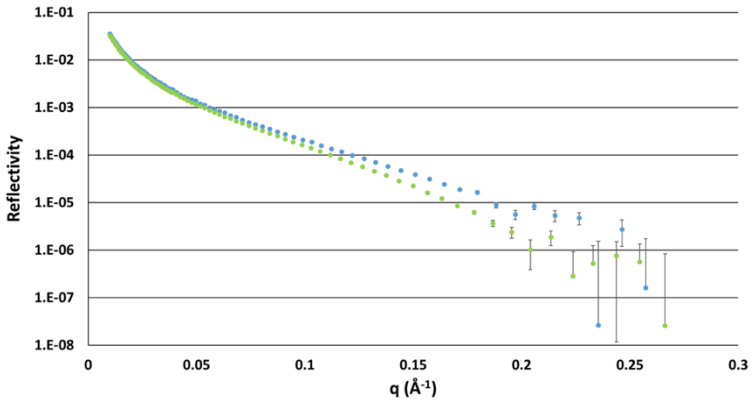

Figure 4: Reflectivity profiles for a d_{75}-DPPC monolayer spread onto the ACMW subphase, before (•) and after injection of 5.0 mM NaTC (•), at 23°C. These curves are the typical reflectivity curves that have been obtained.

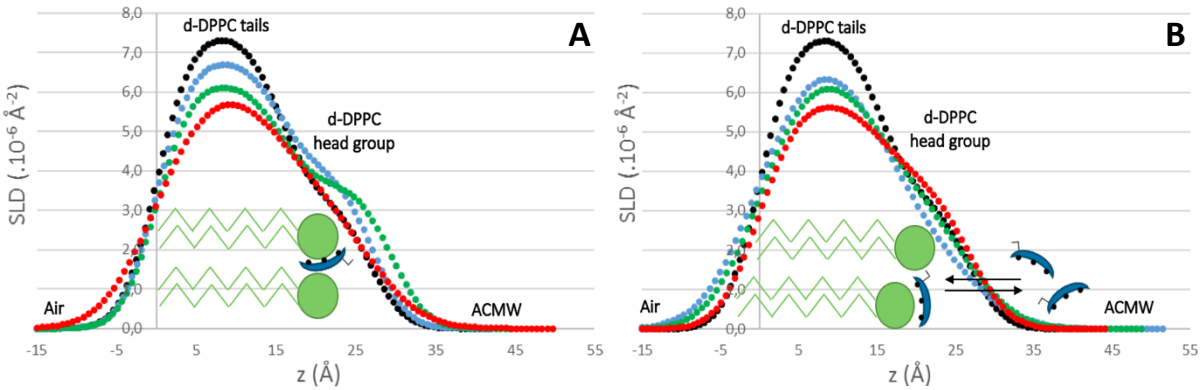

Figure 5: SLD profiles for a d_{75}-DPPC monolayer spread onto the ACMW subphase, before (•) and after successive injections of 1.0 (•), 5.0 (•), and 10 (•) mM of BS (A. NaTC, B. NaTDC), into the subphase, at 23°C. The interfacial behaviour of each BS is depicted by a schematic representation.

For now, only the static results obtained when using d_{75}-DPPC and ACMW have been fitted (Fig. 5), and the SLD profile of the pure d_{75}-DPPC monolayer formed onto ACMW has been established considering the film as homogeneous and compacted (Fig. 5).

Upon addition of 1.0 and 5.0 mM NaTC (Fig. 5A), the water content in the head groups layer significantly decreases, whereas the amount of BS in the same region increases, which suggests that NaTC molecules strongly penetrate the head groups layer and replace water. From 10 mM NaTC (Fig. 5A), the amount of BS at the interface decreases while the air content in the tails region further increases; this result shows that NaTC may desorb from the interface and remove some lipids upon desorption. Regarding NaTDC (Fig. 5B), the less defined tails and head groups layers and the significant increase in interfacial roughness suggest that NaTDC readily exchanges with the interface and leads to a much more disordered system. Therefore, NaTC (Fig. 5A) seems to form a more favourable complex with DPPC than NaTDC (Fig. 5B), thus confirming that NaTC has a higher affinity for the interface, and could thus facilitate lipase adsorption, whereas NaTDC (Fig. 5B) could be more effective at displacing lipolysis products from the interface, through dynamic exchange.

References. [1] Maldonado-Valderrama *et al.*, *Adv. Colloid Interface Sci.*, **2011**, *165*, 36-46 [2] Wilde & Chu, *Adv. Colloid Interface Sci.*, **2011**, *165*, 14-22 [3] Parker *et al.*, *Soft Matter*, **2014**, *10*, 6457-6466 [4] McClements & Li, *Food Funct.*, **2010**, *1*, 32-59