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# Bile salts at the air–water interface: Adsorption and desorption

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ABSTRACT

Bile salts (BS) are bio-surfactants which constitute a vital component in the process of fat digestion. Despite the importance of the interfacial properties in their biological role, these have been scarcely studied in the literature. In this work, we present the adsorption–desorption profiles of two BS (NaTC and NaGDC) including dilatational rheology. Findings from this study reveal very different surface properties of NaTC and NaGDC which originate from different complexation properties relevant to the digestion process. Dynamic adsorption curves show higher adsorption rates for NaTC and suggest the existence of various conformational regimes in contrast to NaGDC which presents only one conformational regime. This is corroborated by analysis of the adsorption isotherms and more in detail by the rheological behaviour. Accordingly, the dilatational response at 1 Hz displays two maxima of the dilatational modulus for NaTC as a function of bulk concentration, in contrast to NaGDC which displays only one maximum. The desorption profiles reveal that NaTC adopts an irreversibly adsorbed form at high surface coverage whereas NaGDC fully desorbs from the surface within the whole range of concentrations used. Analysis of the adsorption–desorption profiles provides new insight into the surface properties of BS, suggesting a surface complexation of NaTC. This knowledge can be useful since through interfacial engineering we might control the extent of lipolysis providing the basis for the rational design of food products with tailored digestibility.



## 1. Introduction

Bile salts (BS) are bio-surfactants present in the gastrointestinal tract that play a crucial role in digestion and absorption of nutrients [1]. The importance of BS for controlled release and transport of lipid soluble nutrients/drugs has recently stimulated the scientific interest in these physiological compounds. BS are a peculiar type of surfactants with unusual properties. In contrast to classical surfactants, BS do not have a well defined tail and head group but they exhibit instead a planar polarity. Chemically, BS are rigid, almost flat molecules with weakly separated hydrophilic and hydrophobic faces [2]. This peculiar molecular structure facilitates the formation of dynamic aggregates able to solubilise and transport lipid soluble compounds, hence, constituting a vital component in the process of fat digestion. Most lipid digestion in humans occurs in the duodenum. The reason for this is principally due to BS, which adsorb onto and remove other materials such as proteins and emulsifiers from the lipid surface [3,4]. This allows lipase and its cofactor co-lipase to adsorb onto the lipid surface and instigate lipolysis. The unusual surface behaviour of BS is directly related to their intriguing molecular structure and further knowledge could provide an improved understanding and rational control of lipid digestion.

The detergent nature of BS has been studied in the literature, mostly concentrating on the self-assembly behaviour of BS in solution. Many literature reviews provide interesting information about the current knowledge of self-assembly of BS in solution emphasizing the peculiar properties of BS aggregates as compared to classical surfactants [2,5–7]. Also, recent studies with computer simulations have provided new insight into the formation mechanisms of micelles [8,9]. As a result, the self-assembly and aggregation mechanisms of BS in solution are rather well understood nowadays. Interaction of BS with proteins and lipids has also been a subject of intensive research. In particular, there are some recent works dealing with interactions between interfacial material on oil droplets and BS such as proteins [4,10,11], aminoacids [12] or lipids [13]. In contrast, studies dealing directly with the interfacial behaviour of BS are very limited in the literature [1,14]. Although investigation of the adsorption behaviour of classical surfactants

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onto air–water and oil–water interfaces has proven to be extremely useful in improved understanding of their diffusion and aggregation behaviour, the interfacial tension profiles of BS are often not reported in the literature and even less studies deal with surface rheological characterisation. Another important innovation of the present work are the desorption profiles. In order to proceed with the transport and absorption of lipid nutrients, the ability to desorb from the lipid surface plays a crucial role. The lipolysis reaction which occurs at the interface involves complex equilibria between adsorption–desorption processes and conformational changes at the interface. Accordingly, studying the reversibility of adsorption of BS onto hydrophobic surfaces definitely provides interesting information applicable to the role of BS in lipid digestion as already spotted in some preliminary measurements in previous works [1].

The present work combines novel surface characterisation methods to study the surface properties of two bile salts: NaTC and NaGDC. We present a systematic study comprising dynamic adsorption curves, surface tension isotherms, dilatational rheology and desorption profiles. Such combination has not been reported to date on bile salts and reveals interesting differences between adsorbed layers formed by NaTC and NaGDC, hence importantly improving our understanding of adsorbed layers of BS. The efficacy of lipid digestion depends closely on the physical-chemistry of the interface and on the unconventional behaviour of BS. Hence, a better understanding of surface activity of BS can facilitate manipulation of physico-chemical and interfacial properties to modulate lipid digestion, improve bioavailability of lipid soluble nutrients and reduce absorption of saturated fats, cholesterol and trans fats.

## 2. Materials and methods

### 2.1. Materials

Sodium glycodeoxycholate (NaGDC, >97% TLC, cat n. G9910, lot no. 039K0308), and sodium taurocholate (NaTC, >97% TLC, cat n. 86339, lot no. BCBG6336V) were obtained from Sigma-Aldrich® and used as received. Both bile salts are negatively charged, and their molecular weights are 537.68 Da (NaTC) and 471.6 Da (NaGDC). BS are a family of soluble amphiphilic molecules with an unusual molecular structure exhibiting planar polarity. Bile acids comprise two connecting units, a rigid steroid backbone with a hydrophobic and a hydrophilic face to which a flexible aliphatic tail is attached [15]. Fig. 1 shows a schematic representation of a bile salt molecule and the molecular formula of NaTC and NaGDC. The hydrophobic surface lies on the convex side of the rigid steroid ring system. The concave side of the molecule contains one two or three hydroxyl groups and an amino group that can be conjugated with taurine, glycine or other aminoacids. Different BS differ in the number, position and stereochemistry of the hydroxyl group as well as on the conjugated amino acid; glycine (75%) or taurine (25%).

The buffer used in all solutions was $2 \times 10^{-3}$ M Bis Tris (Sigma-Aldrich®, ≥99.0%, cat n. 148779) 0.15 M NaCl, 0.01 M $CaCl_2$, adjusted to pH 7 with HCl. This buffer mimics the physiological conditions in the duodenum. Bile salts solutions were prepared daily by dilution from a stock solution of 0.1 M. The stock solution was prepared by dissolving the bile salt directly in the duodenal buffer for 1 h under mild agitation. Dilutions were kept under mild agitation for 30 min before use.

Ultrapure water, cleaned using a Milli-Q water purification system (0.054 μS), was used for the preparation of buffer solutions. All glassware was washed with 10% Micro-90 cleaning solution and exhaustively rinsed with tap water, isopropanol, deionised water, and ultrapure water in this sequence. All other chemicals used were of analytical grades and used as received. The temperature was adjusted to 20 °C with an external temperature control and surface tension of the clean air–water interface was measured before every experiment, in order to confirm the absence of surface-active contaminants, yielding values of 72.8 ± 0.2 mN/m at 20 °C.

### 2.2. The OCTOPUS

All the measurements were made in The OCTOPUS; a Pendant Drop Surface Film Balance equipped with a subphase multi-exchange device which has been fully designed and assembled at the University of Granada (UGR) (patent *submitted* P201001588) and is described in detail in [16]. The pendant drop is placed on a three axis micro-positioner and is immersed in a glass cuvette (Hellma) which is kept in a thermostatically-controlled cell. Drop images are captured by a CCD camera (Pixelink®) connected to an optical microscope (Edmund Optics®). The computer program DINATEN© fits experimental drop profiles, extracted from digital drop micrographs, to the Young–Laplace equation of capillarity by using ADSA (Axisymmetric Drop Shape Analysis), and provides as outputs the volume ($V$), the surface tension ($\gamma$), and the interfacial area ($A$) of the pendant drop. The OCTOPUS allows also measuring the dilatational rheology of the surface layers at different oscillating frequencies by recording the response of the surface tension to a triangular area deformation [17]. In a general case, the dilatational modulus ($E$) is a complex quantity that contains a real and an imaginary part:

$$E = E' + iE'' = \varepsilon + i\nu\eta \quad (1)$$

where $E'$ is the storage modulus that accounts for the elasticity of the interfacial layer ($\varepsilon$), $E''$ is the loss modulus that accounts for the viscosity of the interfacial layer ($\eta$) and $\nu$ is the angular frequency of the applied oscillation. The applied oscillations in interfacial area are maintained at amplitude values of less than 5%, in order to avoid excessive perturbation of the interfacial layer. The measurement frequency ($\nu$) can be changed between 0.01 and 2 Hz depending on the requirements of the system. Computer software DINATEN© records the images of the dilatational experiments in real time and these are then analysed and processed by computer software CONTACTO©, which provides as outputs the mean surface tension ($\gamma$), the surface elasticity ($\varepsilon$), the surface viscosity ($\eta$), the storage modulus ($E'$), the loss modulus ($E''$) and the dilatational modulus ($E$) of the adsorbed layer.

Automatic adsorption and dilatational rheology measurements can be made of up to 12 solutions adjusting the surface area, the total adsorption time and the oscillating frequency. In this work, we have measured the adsorption process at 30 $mm^2$, for 1 h and the dilatational rheology at fixed periods of 10 s, 5 s and 1 s. The concentrations used range between $10^{-6}$ and 0.1 M. The OCTOPUS software automatically discards the solution once the measurement is over, injects the new solution, creates a new droplet and starts the new measurement. This can be done automatically up to 12 times.

The desorption experiments are carried out by means of the double capillary [18] which allows an automatic, non-invasive and complete exchange of the subphase of the drop preserving the pendant drop volume and surface area during the subphase exchange. In the case of soluble surfactants, exchanging the bulk subphase by pure buffer enables the study of the desorption profile of the adsorbed surfactants [19]. In this case, the evolution of the surface tension of the surface layer increases as the bulk solution is depleted of bile salts, which is done after the surface layer has equilibrated at constant surface area (Fig. 2). In order to accurately obtain a desorption profile, the conditions of the subphase exchange need to be optimised prior to the experiment, establishing a complete subphase exchange when the changes in interfacial tension are negligible upon further exchanges of the subphase of