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Effect of emulsifier type against the action of bile salts at oil–water interfaces

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ABSTRACT

Bile salts (BS) are important agents in lipid digestion and absorption. This biological process involves high amounts of BS micelles and this could induce depletion flocculation of oil-in-water (O/W) emulsions by non-adsorbed micelles that are excluded from the interstitial space. In a previous work we observed that the emulsifier type – Pluronic F68 (non-ionic) and phospholipids Epikuron 145 V (anionic) – provided different emulsion stability in the presence of a bile salt (sodium taurodeoxycholate, NaTDC). Namely, Pluronic provided higher stability than Epikuron against the action of BS. In order to elucidate these results observed in O/W emulsions, the aim of the current study is to probe the effect of NaTDC on the interfacial behavior of such emulsifiers. The interfacial properties were measured with a pendant drop film balance equipped with a subphase exchange technique, which allows adding the bile salt directly into the subphase once the surfactant has been pre-adsorbed onto the oil–water interface. We can hence monitor in-situ the effect of the BS on the interfacial layer by comparing with the behavior of the individual systems. Interfacial tension showed lower adsorption rates for BS onto Pluronic-covered interface, as compared to pre-adsorbed Epikuron layer. Finally, in order to gain structural information we have fitted the experimental data with the Frumkin adsorption Isotherm by using software package IsoFit. As a result, we get that the molecular area and molecular interaction decrease in the following order: Pluronic > NaTDC > Epikuron within the interfacial layer. Therefore, we prove that the Pluronic adsorbed layer is more resistant to bile salt adsorption than the Epikuron interfacial layer. This correlates with the physicochemical properties of O/W emulsions. The use of interfacial techniques provides new insight into the action of BS on O/W emulsion, which constitutes one of the main challenges in order to clarify the mechanisms involved in lipid digestion and absorption.

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1. Introduction

Bile salts (BS) are important agents in lipid digestion and absorption (Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011; Wickham, Garrood, Leney, Wilson, & Fillery-Travis, 1998). In the literature, it is possible to find different descriptions of the effect of BS on such processes. For instance, bile salts are considered to displace lipase at oil–water interfaces in the absence of co-lipase; however, they also promote desorption of other surfactants from the interface, so that lipase/co-lipase complexes can adsorb onto the interface promoting lipid digestion (Mun, Decker, & McClements, 2007). Other authors show that bile salts reduce the penetration of the lipase due to the electrostatic repulsion between the negatively charged BS adsorbed at the interface and the lipase (Wickham et al., 1998). The interfacial properties of BS will certainly play a crucial role in these phenomena. The importance of this aspect has boosted the publications dealing with this topic recently (Euston, Bellstedt, Schillbach, & Hughes, 2011; Fernández-Leyes, Messina, & Schulz, 2011; He et al., 2011) However, specific work on the fundamental surface properties of BS is still very scarce in the literature as reviewed recently (Maldonado-Valderrama et al., 2011). Bearing this in mind, improving the current understanding of the behavior of BS at oil–water interfaces is one of the main challenges in order to clarify the mechanisms involved in lipid digestion and absorption.

In a previous work (Jódar-Reyes, Torcello-Gómez, Wulff-Pérez, Gálvez-Ruiz, & Martín-Rodríguez, 2010), we studied the stability, the electrokinetic behavior and droplet size of surfactant-stabilized olive oil-in-water (O/W) emulsions after the addition of different bile salt concentrations. Adsorption of BS onto lipid droplet surfaces allowed us to explain the higher stability at low bile salt concentrations as well as the more negative electrophoretic mobility when comparing with the emulsion in the absence of BS. Differently, BS micelles induced depletion flocculation of O/W emulsions at high bile salt concentrations. Effects of the type of emulsifier present at the oil–water interface were also observed. However, further work became necessary in order to ascertain which interfacial mechanism (i.e. displacement of the emulsifier by the bile salt and/or co-adsorption at the emulsion interface) was taking place.

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In this sense, the importance of the interfacial mechanisms underlying the behavior of emulsions is well-known. However, details about the specific interaction between digestion compounds and emulsifiers at the interface are still unclear (Maldonado-Valderrama et al., 2011). Accordingly, the aim of this work was to analyze the behavior of a bile salt at an oil–water interface in the presence of two types of surfactants, which were used as emulsifiers in our previous study (Jódar-Reyes et al., 2010), and to correlate it with the physicochemical properties observed in emulsions. Concretely, we elucidate the interfacial interactions taking place between bile salt and surfactant molecules, providing a better understanding of the different emulsion stability induced by each emulsifier. This will be achieved by using state of the art surface tension techniques. Hence, the evolution of the interfacial composition was monitored by looking at its mechanical properties (interfacial dilatational rheology) (Torcello-Gómez, Maldonado-Valderrama, de Vicente, Cabrerizo-Vílchez & Gálvez-Ruiz et al., 2011). Despite the variety of BS, they all behave in a qualitative similar manner (Maldonado-Valderrama et al., 2011). Sodium taurodeoxycholate (NaTDC) is one of the main components in human bile salt (Arleth et al., 2003; Friesen et al., 2008). For this reason, we chose NaTDC as a BS model in our study. Two different surfactants were used to study the effect of the emulsifier type on BS adsorption. Namely, one synthetic, polymeric, uncharged (Pluronic F68), and one natural, charged small molecule (Epikuron 145 V). These, instead of proteins, were chosen as emulsifiers because they provide greater protection against lipase-induced destabilization in the presence of bile salts (Mun et al., 2007; Torcello-Gómez, Maldonado-Valderrama, Martín-Rodríguez, & McClements, 2011; Wulff-Pérez, Gálvez-Ruiz, de Vicente, & Martín-Rodríguez, 2010).

Dilatational rheology and interfacial tension are sensitive tools to the intermolecular interactions and adsorption providing information about the interfacial structure (Maldonado-Valderrama & Rodríguez Patino, 2010). However, it is sometimes difficult to interpret at the molecular level. In this work we have applied a theoretical model to the adsorption data in order to explain the data, so as to obtain further information about the structure of the interfacial layers. This same procedure has been recently applied to study interfacial properties of protein layers under in-vitro gastric conditions (Maldonado-Valderrama, Miller, Fainerman, Wilde, & Morris, 2010). The application of a theoretical model to study this biological system provides a comprehensive analysis and new information of the role of molecular interactions on the properties of the interfacial layers formed by different emulsifiers. Moreover, by combining experiments and theory original insights have been gleaned into the role of interfacial structuring against the action of bile salts, relevant to physiological studies. Despite the fact that this system does not reflect realistic conditions in the gastrointestinal tract, the generic information obtained in this study can be extrapolated to duodenal conditions (Torcello-Gómez, Maldonado-Valderrama & de Vicente et al., 2011; Torcello-Gómez, Maldonado-Valderrama & Martín-Rodríguez et al., 2011). As this knowledge improves, rational strategies can be developed to improve the biological impact of foods and pharmaceuticals.

2. Materials and methods

2.1. Materials

As non-ionic surfactant we used poloxamer Pluronic F68 from Sigma-Aldrich. It is a triblock copolymer based on poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) structure which is also typically expressed as $PEO_{75}PPO_{30}PEO_{75}$ (MW = 8350 g/mol). The central block has a hydrophobic character and hence adsorbs onto the oil–water interface, whereas the two chains of poly(ethylene oxide) remain in the aqueous phase. As ionic surfactant we used Epikuron 145 V (around 800 g/mol), a deoiled, wax-like phosphatidylcholine (PC) enriched soybean lecithin (min. 45% PC) from Cargill Ibérica S. L. This phospholipid presents negative charge at pH 7. Sodium taurodeoxycholate (NaTDC, 97% purity) was purchased from Sigma-Aldrich. This bile salt is negatively charged and its molecular weight is 521.7 g/mol. Highly refined olive oil was also purchased from Sigma-Aldrich, and purified with activated magnesium silicate (Florisil, Fluka) to eliminate free fatty acids and surface active impurities. The oil was kept under mild agitation with the resins for 3 h and centrifuged at 12 000 rpm for 30 min in a bench centrifuge. It was then filtered and stored away from light.

The Pluronic and NaTDC solutions were prepared in a 1.13 mM phosphate buffer (pH 7), whereas Epikuron samples were dissolved in purified olive oil under stirring and gentle warming to guarantee complete dissolution. All chemicals used were of analytical grade. Milli-Q purified water 0.054 μS was used for buffer preparation and all other purposes. All glassware was washed with 10% Micro-90 cleaning solution and exhaustively rinsed with tap water, ethanol, distilled and ultrapure water in this sequence. Only freshly prepared solutions were made by successive dilution from a concentrated stock and used for each experiment.

2.2. Interfacial tension set-up and subphase exchange technique

The interfacial tension measurements were carried out in a Pendant Drop Film Balance equipped with subphase exchange technique, which has been fully assembled and developed at the University of Granada. This device is described in detail elsewhere (Cabrerizo-Vílchez, Wege, Holgado-Terriza, & Neumann, 1999). A solution droplet is formed at the tip of a coaxial double capillary, connected independently to a double microinjector. The whole set-up is computer controlled through the software DINATEN© developed by Dr. Holgado Terriza. DINATEN© fits the experimental drop profiles, extracted from digital drop micrographs, to the Young–Laplace equation of capillarity by using Axisymmetric Drop Shape Analysis (ADSA), providing as outputs the drop volume V, the interfacial tension γ, and the interfacial area A. The adsorption process is recorded at constant interfacial area ($30\ mm^2$) through a modulated fuzzy logic PID algorithm (proportional, integral, and derivative control) by changing the volume (Wege, Holgado-Terriza, & Cabrerizo-Vílchez, 2002). The drop is immersed in a glass cuvette (Hellma), which contains the oil phase and is kept in a thermostatized cell. The interfacial tension of the clean interface was measured before every experiment to ensure the absence of surface-active contaminants obtaining values of (29.5 ± 0.5) mN/m at 20 °C. All the experiments were performed at room temperature and their reproducibility was verified from the standard deviation of at least three replicate measurements.

The experiments were designed following this sequence. First, the interfacial tension was recorded for the pure systems at the bare oil–water interface at constant interfacial area in order to pre-form the emulsifier interfacial layer (12 mM Pluronic, 1 mM Epikuron). Next, the subphase was exchanged by means of the coaxial double capillary, which enables to substitute the surfactant bulk solution by that of BS, hence mimicking the passage through the intestine. The subphase is exchanged by extracting the surfactant solution through the outer capillary, and injecting simultaneously the BS through the inner one. Hence, the effect of bile salt on a previously covered interface was monitored by recording the change in interfacial tension and dilatational elastic modulus as detailed below.

2.3. Interfacial dilatational rheology

The dilatational rheology of the different adsorbed layers was measured with the same pendant drop device. An oscillatory perturbation was applied to the interface by injecting and extracting volume to the drop. The system records the response of the interfacial tension to the area deformation, and the dilatational modulus (E) of the interfacial layer can be calculated from this response by following

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