Bulk and interfacial interactions between hydroxypropyl-cellulose and bile salts: Impact on the digestion of emulsified lipids

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ABSTRACT

Hydroxypropyl-cellulose (HPC) is a surface-active, non-digestible polysaccharide, commonly used in food emulsions as thickener and/or emulsifier. Due to these dual characteristics, HPC is a potential ingredient to modulate lipid digestion. Since bile salts (BS) are key players during lipid digestion, the aim of this work was to investigate the impact that interactions of HPC with BS has on the digestion of emulsified lipids. We studied the effect of two BS species differing in bile-acid moiety, sodium-taurocholate (NaTC) and sodium-taurodeoxycholate (NaTDC). A Quartz-Crystal-Microbalance (QCM-D) was used to evaluate HPC-BS interfacial interactions during the sequential and simultaneous adsorption of both components at a hydrophobic surface, while micro-Differential-Scanning-Calorimetry was used to examine bulk interactions. In vitro lipid digestion was studied by using a pH-stat method. Results showed that, under fed-state conditions, NaTDC micelles were more effective at displacing a pre-adsorbed HPC layer from the surface than NaTC monomers. Nevertheless, HPC was resistant to complete displacement by both BS. Additionally, HPC was more susceptible to interact with NaTDC in the bulk, compared to NaTC, which made the adsorption more competitive for NaTDC. The reduced amount of free NaTDC in solution could explain the delayed lipolysis shown by HPC-stabilized emulsions when NaTDC was used to simulate duodenal conditions. These findings show that the delay of lipid digestion by HPC is due to the combined effect of HPC-BS interfacial and bulk interactions, with BS-binding in solution mostly contributing to this effect, and the BS molecular and micellar structure playing essential roles on both situations.

1. Introduction

Cellulose derivatives, non-ionic and non-digestible polysaccharides, are commercially relevant ingredients used commonly in processed foods. While they are often used as stabilizers to increase the viscosity of the aqueous phase of oil-water (o/w) emulsions, cellulose derivatives are one of the few high molecular weight polysaccharides with surface-active properties, and therefore they also show good performance as emulsifiers (Dickinson, 2009; Lovegrove et al., 2017; Mezdour, Lepine, Erazo-Majewicz, Ducept, & Michon, 2008; Wuestenberg, 2014). Due to these dual properties in food emulsions, which are a major source of fats in the diet, cellulose derivatives have recently received great interest for their potential to modulate lipid digestion (Bellesi, Martinez, Pizones Ruiz-Henestrosa, & Pilosof, 2016; Borreani et al., 2017; Espinal-Ruiz, Parada-Allonso, Restrepo-Sánchez, Narváez-Cuenca, & McClements, 2014; Pizones Ruiz-Henestrosa, Bellesi, Camino, & Pilosof, 2017; Torcillo-Gómez & Foster, 2014). Controlling the digestion of lipids is thought to be a positive approach to tackle a large number of diseases related to over-consumption of dietary fat (Mei, Lindqvist, Krabisch, Rehfeld, & Erlanson-Getty, 2006).

A major strategy through which cellulose derivatives could modulate lipid digestion involves their interactions with bile salts. Bile salts (BS) are natural, surface-active, small molecules produced in the liver from cholesterol and secreted within the duodenum during digestion. These bio-surfactants play actions that are crucial for the progression of lipid digestion (Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011; Wilde & Chu, 2011). On one hand, BS have the ability to stabilize lipid droplets by rapidly adsorbing to the o/w interface and displacing previously adsorbed material from the interface; this allows for the adsorption of the co-lipase/lipase complex onto the interface, which

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initiates lipolysis (i.e. enzymatic hydrolysis of lipids) (Erlands-
"n-Albertsson, 1983). On the other hand, the ability of BS to self-aggregate into micelles in solution facilitates the solubilization and removal of lipolysis products from the o/w interface, which prevent lipase inhibition thus fostering lipid digestion and absorption (Hofmann & Myeis, 1987). Therefore, interactions of cellulose derivatives with BS could affect interfacial and bulk phenomena taking place during the digestion of lipids. Specifically, cellulose derivatives could limit the access of BS to the lipid surface, either by creating interfacial layers that resist displacement by BS, which will prevent co-lipase/lipase adsorption, or by sequestering BS in the bulk and competing with BS for the surface. However, the specific contribution of the possible interactions between cellulose derivatives and BS towards the progression of lipolysis is not fully understood, and it depends on the type of cellulose.

In most studies, the cellulose derivatives are used, either as thickeners in the continuous phase of the emulsions, or as emulsifiers to form interfacial layers (Bellesi et al., 2016; Borreani et al., 2017; Espinal-Ruiz et al., 2014; Torcello-Gómez & Foster, 2016). Most of these reports have mainly focused either, on competitive adsorption studies, or on sequential adsorption experiments that determine the displacement of pre-adsorbed layers by BS (Bellesi, Ruiz-Henestrosa, Maldonado-Valderrama, Del Castillo Santaella, & Pilosof, 2018; Pizones Ruiz-Henestrosa, Bellesi, Camino, & Pilosof, 2017). However, to our knowledge, there is no fundamental work on the combined study of the effect that bulk and interfacial interactions between cellulose derivatives and BS have on the displacement of mixed layers created at the surface by means of the sequential and the simultaneous adsorption of both surface-active components. The main cellulose derivatives used in food applications are carboxy methylcellulose (CMC), methyl cellulose (MC), hydroxypropylmethyl cellulose (HPMC) and hydroxypropyl cellulose (HPC). Recently, we showed that cellulose derivatives can bind to BS in solution (Torcello-Gómez et al., 2015) and form interfacial networks not easily disrupted by low concentrations of BS (Fernandez-Fraguas, Woodward, Gunning, & Wilde, 2014)(Fernandez-Fraguas et al., in preparation). Those studies have focused on either MC, in which methyl groups are the sole substituent, or HPMC, that contains a predominant proportion of methyl groups and a smaller proportion of hydroxypropyl substituent groups. Limited attention has been paid to HPC, which contains a larger and more polar hydroxypropyl group as sole substituent group (Torcello-Gómez et al., 2015; Torcello-Gómez et al., 2014). Thus, a systematic approach is needed to identify the mechanisms by which HPC can interact with BS in the bulk and at the interface to further understand their potential role to control lipid digestion in complex food emulsions. Additionally, the contrasting properties shown by BS, i.e. interfacial activation of lipase and the simultaneous removal of lipolysis products from the interface, is intriguing. These different functionalities have been attributed to structural BS differences, particularly to the position, number and stereochemistry of the hydroxyl groups in the sterol ring rather than to the conjugated amino acid (Fabois et al., 2019; Parker, Rigby, Ridout, Gunning, & Wilde, 2014).

For these reasons, this study aimed at investigating the potential of HPC to control lipid digestion, either by creating interfacial layers that resist complete displacement by BS, or by sequestering BS in the bulk phase. A second objective was to investigate how minor differences in BS molecular structure impact their interactions with HPC, in these two different situations where the two surface-active components can co-exist during lipid digestion (i.e. at the interface and in the bulk) and how, in turn, this affects the digestion of emulsified lipids.

2. Materials and methods

2.1. Materials

Hydroxypropyl cellulose [HPC, MW ~100 kg/mol, 20 mesh particle size (99% through), viscosity of 5% (w/w) in H2O, ƞ = 75–150 cP, degree of substitution (DS) and molar substitution (MS) hydroxypropyl (HP) (DS (HP) = 2.2, MS (HP) = 4.4)], Bile Salts (BS), sodium taurocholate (NaTDC, ≥97.0% purity; MW = 537.68 g/mol) and sodium taurodeoxycholate (NaTDC, ≥95.0% purity; MW = 521.69 g/mol), and Pancreatin from porcine pancreas (8 x USP specifications, P7545) were purchased from Sigma-Aldrich (St. Louis, MO), stored at room temperature, and used without further purification. Bis-Tris (≥98.0% purity), calcium chloride dihydrate (CaCl2), sodium chloride (NaCl), hydrochloric acid (HCl), sodium hydroxide (NaOH) and Tween® 20 were purchased from Thermo Fisher Scientific (Waltham, MA). The olive oil (Filippo Berio) was purchased from a local grocery store (Kroger) and was purified with activated magnesium silicate (Florisil®). Sigma-Aldrich, <200 mesh).

2.2. Preparation of hydroxypropyl-cellulose (HPC) and bile salts (BS) solutions

HPC stock solution (0.15% (w/v)) was prepared by dispersing the polymer in 2 × 10⁻³ M Bis-Tris buffer pH 7 as previously described (Torcello-Gomez et al., 2015). Briefly, approximately two-thirds of the final volume of Bis-Tris buffer was heated to ~80 °C and the HPC powder was added carefully under stirring. After 5 min, the temperature was lowered to 22 °C and continued to stir at room temperature for at least 2 h. The remaining volume of Bis-Tris buffer was added to the solution to complete the solubilization. These stock solutions were then stored at 4 °C overnight, in order to achieve maximum hydration before use and without stirring, to eliminate air bubbles. BS solutions at different concentrations were prepared by successive dilution from a concentrated 20 mM stock solution prepared in 2 mM Bis-Tris buffer pH 7. These solutions were stored in 4 °C until use. HPC and BS solutions were degassed for 30 min prior to QCM-D and micro-DSC experiments in order to minimize bubbles in the solutions.

2.3. Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D)

The interactions between HPC and BS at the interface were estimated by carrying out the sequential and simultaneous adsorption of HPC and BS at a solid surface. These experiments were performed using a Quartz Crystal Microbalance with Dissipation Monitoring- QCM-D Multi-Channel E4 system (Q-sense AB, Sweden). Two types of QCM-D sensors were used: i) gold, consisting of quartz crystal covered with 5 nm of chromium and 100 nm of gold (QX3 301, Q-Sense), and ii) non-charged polystyrene (PS) sensors, consisting of 40 nm of PS (QXS 305, Q-Sense). Sensors were excited to oscillate by applying an alternating electrical voltage across the surface at their fundamental resonant frequency (4.95 MHz) and at the n = 3–11 odd overtone (15–55 MHz, respectively). Changes in frequency (∆f) and dissipation (∆D) due to HPC and BS adsorption and desorption from the sensors were monitored at each of the five overtone frequencies simultaneously. First, Bis-Tris buffer was introduced into the flow cells at a flow rate of 0.25 mL/min with a cartridge pump (ISMETEC-ISM935) until the system stabilized. Next, sequential and simultaneous adsorption experiments were performed.

2.3.1. Sequential adsorption of HPC and BS

These experiments consisted of continuously flowing a HPC solution in buffer (1 mg/mL) at a rate of 0.25 mL/min for a specific period of time to provide a continuous and saturated layer of cellulose adsorbed onto the solid substrate. This was followed by a 25-min exchange with Bis-Tris buffer to remove any reversible adsorbed mass. Following this, a fixed concentration (2, 5 or 10 mM) of BS (NaTDC or NaTDC) was injected into the flow cell and monitored over 10 min. After each BS injection, buffer was introduced into the system. For comparison, the same experiment was performed without HPC (i.e. BSs were adsorbed at the bare solid surface followed by the buffer rinse step). Three individual experiments were performed for each BS concentration.
2.3.2. Simultaneous adsorption of HPC and BS

Co-adsorption experiments consisted of flowing mixed HPC-BS solutions in Bis-Tris buffer onto the sensor at a flow rate of 0.25 mL/min for a specific period of time. This was followed by a Bis-Tris buffer rinse step for 25 min to remove any loosely adsorbed material from the solid surface. Mixed HPC-BS solutions containing a fixed concentration of HPC (1 mg/mL) and different BS concentrations (2, 5 or 10 mM) were prepared from HPC and BS stock solutions, and left to stand for 1 h at room temperature. Three individual experiments were performed for each BS concentration.

2.3.3. Sensor cleaning

After the completion of the experiment, gold sensors were cleaned by boiling them in a 5:1 vol ratio solution of ultrapure water: 28% (w/w) ammonium hydroxide: 30% (w/w) hydrogen peroxide, respectively, for 45 min. Sensors were rinsed exhaustively with ultrapure water and dried with nitrogen gas prior to being used. Polystyrene sensors were cleaned by immersing them in a 2% (w/v) Hellmanex IIITM solution prepared in ultrapure water, for 1 h, exhaustively rinsed and kept in ultrapure water for at least 2 h. Then, they were rinsed with 95% ethanol and dried with nitrogen gas prior to being used.

2.3.4. Analysis of QCM-D data

Changes in scaled frequency (Δf/n) correspond to changes in the oscillation frequency of the sensor divided by the overtone number. These changes were related to the changes in the hydrated mass adsorbing on the sensor via the Sauerbrey equation (Sauerbrey, 1959) and Voigt model (Voinova, Rodahl, Jonson, & Kasemo, 1999). If the adsorbed layer was evenly distributed, fully elastic, and rigidly attached, the Sauerbrey equation (Equation (1)) was used to calculate the adsorbed mass per unit area in ng cm$^{-2}$ (ΓQCM-D) in the software package QSense Dfind (version 1.1):

$$\Gamma_{QCM-D} = -\frac{C \Delta f}{n}$$

(1)

where $C$ is the crystal specific constant (17.7 ng cm$^{-2}$ Hz$^{-1}$), $n$ is the overtone number ($n = 5$) and $\Delta f$ is the change in frequency (Hz). Changes in dissipation (or damping) are related to the structural properties of the adsorbed layer. The QCM-D E4 system stops the electrical voltage and allows the sensor to freely oscillate until a standstill, while measuring the decay of the resonating sensor. The change in dissipation (ΔD) is given by equation (2) (Höök, Rodahl, Brzezinski, & Kasemo, 1998):

$$\Delta D = \frac{E_{\text{disipated}}}{2\pi E_{\text{stored}}}$$

(2)

where $E_{\text{disipated}}$ is the energy dissipated by the viscous nature of the surrounding medium and $E_{\text{stored}}$ is the energy stored in the sensor after a single oscillation. If the ΔD is greater than 10$^{-6}$ and if the scaled frequencies ($\Delta f/n$) do not overlap, use of the Sauerbrey equation underestimates adsorbed mass/thickness, so adsorption curves for these viscoelastic layers were fit with a Voigt-based viscoelastic model (Voinova et al., 1999). The adsorbed layer was treated as a viscoelastic layer between the quartz crystal and a semi-infinite Newtonian liquid layer. The quartz crystal was assumed to be purely elastic (thickness ($h_j$) = 3 × 10$^{-4}$ m and density ($\rho_j$) = 2650 g L$^{-1}$) and the surrounding buffer solution was assumed to be purely viscous (density ($\rho_f$) = 1000 g L$^{-1}$ and viscosity ($\eta_f$) = 1.00 mPa s$^{-1}$). The adsorption curves for multiple overtones ($n = 5, 7, 9$ and $11$) were used for estimates of thickness, elastic shear modulus, and viscosity of the adsorbed layer in the software package QSense Dfind (version 1.1).

2.3.5. Statistical analysis

All results were reported as average values with standard errors. Data were graphed and statistically analyzed using Prism v.7.04 (GraphPad, la Jolla, CA). Significant differences in adsorbed mass and thickness of the HPC layer were determined by two sample t-test at the two different time points. Significant differences in total adsorbed mass (ng/cm$^2$) and mass desorbed (%) in relation to BS concentration were determined by a one-way ANOVA and Tukey post hoc analysis. Level of significance was set at $p < 0.05$.

2.4. Micro-differential scanning calorimetry (micro-DSC)

The interactions between HPC and BS in the aqueous phase were estimated by studying the thermal properties of HPC (1 mg/mL) in the absence and presence of BS at different concentrations (2, 5 or 10 mM). Micro-DSC experiments were performed in a Multi-Cell Micro-Differential Scanning Calorimeter (MC-DSC, TA Instruments, New Castle, DE, USA) using ampoules made from Hastallloy. Samples (800 ± 5 mg) were placed in the MC-DSC ampoules and sealed. One of the ampoules was filled with the same weight of Bis-Tris buffer, and an empty ampoule was used as the reference. All the ampoules were placed in the MC-DSC cells and equilibrated to 10 °C for at least 60 min before starting scanning. Cells underwent a heating and cooling cycle from 10 °C to 150 °C at a rate of 1 °C/min. This rate was selected as a compromise between the need for proximity to equilibrium (low possible rate) and an acceptable signal/disturbance ratio (not too low rate). Thermograms were recorded and enthalpy values were calculated using the Nano-analyze software (TA Instruments, New Castle, DE, USA).

2.5. In vitro lipid digestion

The digestion protocol used in this study considers the passage of the o/w emulsions through the small intestine phase and incorporates a gastric pH-conditioning step before the duodenal phase to consider the effect of the acidic conditions found in the stomach (Fernandez-Fraguas, Woodward, Gunning, & Wilde, 2014).

2.5.1. Preparation of emulsions

Oil-in-water (o/w) emulsions were prepared by mixing a purified commercial olive oil and emulsifier solutions using an ultrasonic processor (Model 505, 500 W, 20 kHz, Fisher Scientific Inc., Waltham, MA). First, olive oil was purified with activated magnesium silicate (Florisil®, Sigma-Aldrich, <200 mesh), in order to eliminate impurities. A ratio of 2:1 w/v (oil:Florisil) was mixed using a disperser homogenizer (VirTishear, VirTis) while being simultaneously shaken on a shaker (Innova® 2000, New Brunswick ScientificTM) at 80 rpm for 4-6 h. The mixture was centrifuged (EppendorfTM 5804R) at 1340 x g (rcf) for 30 min and the supernatant was filtered under vacuum to remove any remaining resin. The purified olive oil was transferred to round narrow-mouth amber bottles and stored under nitrogen in the dark.

Emulsion premixes containing 1% (w/w) HPC and 20% (w/w) purified olive oil were first vortexed for 30 s before being treated with the ultrasonic processor equipped with a 3.0 mm diameter ultrasound probe at a frequency of 20 kHz and 30% amplitude. While the glass tube with the sample was submerged in an ice bath, a series of ultrasonic pulses were performed to dissipate the heat produced during the sonication and keep the sample temperature below 25 °C. The ultrasonic treatment was performed for a total of 10 min (5 min of pulsing and 5 min of rest periods). The emulsions were then stored for at least 1 h with no light exposure before carrying out any further treatment and/or analysis.

2.5.2. Emulsion droplet size and distribution measurements

The mean droplet size and distribution of the emulsions were determined using a dynamic light scattering instrument (Zetasizer Nano ZS, Malvern Panalytical) at room temperature. The emulsions were diluted at a 1:1000 proportion with a 2 mM Bis-Tris buffer solution before each measurement to avoid multiple scattering effects. In order to determine the particle size distributions, the refractive indices of the oil (olive oil) and aqueous phase used in the calculations were 1.47 and...
1.33, respectively. Each measurement was calculated from the average of 10 readings made for the sample and these experiments were performed in triplicate.

2.5.3. Free fatty acid release during in vitro digestion of emulsions

Static in vitro digestions were carried out using a pH-stat automatic titration double unit equipped with two electrodes and two Dosino 800 dosing units (Metrohm Instruments). A freshly prepared emulsion (1.5 ml) was diluted in 2 mBis-Tris buffer pH 7, containing 0.15 M NaCl, in a jacketed vessel. Once the mixture maintained a constant temperature of 37 °C, samples were exposed to acidic gastric conditions (by adjusting the pH to 2.5) for 1 h. The mixture was then adjusted automatically to pH 7.0 and added with CaCl₂. This was followed by the addition of BS solution and a volume of freshly prepared pancreatin solution (8 x USP) to the mixture. Lipolysis was monitored for 2 h and free fatty acids (FFAs) released during lipolysis were neutralized by addition of 0.1 M NaOH. The volume of NaOH required to maintain neutral pH reflected the extent of lipolysis. The final concentration of BS was 10 mM, which mimics the conditions found in the gastrointestinal tract under fed state conditions (Fernandez-Fraguas, Woodward, Gunning, & Wilde, 2014; McClements & Li, 2010; Minekus et al., 2014).

In order to obtain the most accurate amount of % FFAs released, a back titration was performed by increasing the pH to 9.0 with 0.1M NaOH after the intestinal phase. This step was performed because there was a risk of incomplete ionization of FFAs at a neutral pH environment due to the higher pKₐ value of oleic acid (apparent pKₐ ~7.45-7.78 at 37 °C (Krämer, Jakits-Deiser, & Wunderli-Allenspach, 1997)). Control experiments were performed for every sample tested. The concentration of FFAs released during lipolysis and the back titration were calculated from the volume of NaOH consumed during lipolysis. The percentage of FFAs released was calculated from the number of moles required to neutralize the FFAs divided by the number of moles of FFAs that could be produced from the triglyceride (TG) assuming all were digested. It is assumed that one TG initially releases two FFAs and one 2-monoglyceride (2-MG)(Benito-Gallo et al., 2015). However, it has been reported that the 2-MG isomerizes to 1/3-monoglyceride and subsequently gets lipolyzed to release a third FA and glycerol (Benito-Gallo et al., 2015; Christensen, Schultz, Molland, Kristensen, & Mullertz, 2004). Therefore, 3 FFA molecules are assumed to be produced by 1 TG. The percentage of FFAs released during lipolysis was calculated using the following equation (Equation (3)):

\[
\% \text{ FFA} = 100 \times \left( \frac{V_{\text{NaOH}} \times m_{\text{NaOH}} \times M_{\text{lipid}}}{W_{\text{lipid}} - 3} \right)
\]

(3)

where \(V_{\text{NaOH}}\) is the volume of NaOH required to neutralize the FFAs produced (mL), \(m_{\text{NaOH}}\) is the molarity of the NaOH used (0.1M), \(M_{\text{lipid}}\) is the molecular weight of the oil (assumed to be 800 g/mol (Torcello-Gómez, Maldonado-Valderrama, Martín-Rodríguez, & McClements, 2011) and \(W_{\text{lipid}}\) is the total weight of oil initially present in the vessel. The following kinetic model (Ye et al., 2013) was used to determine the extent and rate of lipolysis:

\[
Y = Y_m \times (1 - e^{-kt})
\]

(4)

Where \(Y_m\) is the maximum percentage of FFA released (extent of lipolysis), \(k\) is the FFA release rate constant (lipolysis rate), and \(t\) is the lipid digestion time (s).

2.5.4. Analysis of lipolysis data

Results are reported as the average and standard error of duplicate measurements made on two freshly prepared emulsion samples. Data were graphed in Excel and statistically analyzed in Prism v. 7.04 (GraphPad, La Jolla, CA). Significant differences in the rate and extent of lipolysis for each BS were determined by two sample t-test (level of significance p < 0.05).

3. Results and discussion

As the aim of this work is to investigate the role of bulk and interfacial interactions of HPC with two BS of different hydrophobicity (NaTC and NaTDC, Fig. 1) on the control of lipid digestion, we will examine possible interactions between HPC and the BS in the continuous aqueous phase by using micro-DSC, while QCM-D will be used to evaluate interactions at the solid-water interface.

3.1. Effect of surface hydrophobicity on the adsorption/desorption behavior of HPC

As a first step, the effect of surface hydrophobicity on the adsorption/desorption behavior of HPC was studied by using two different QCM-D sensors, gold and polystyrene (PS). Clean unmodified gold surfaces are considered hydrophilic with a contact angle to water (θH₂O ~ 0 °) (Smith, 1980), whereas the polystyrene surfaces used in this study are hydrophobic (θH₂O ~ 87.4 °) (Naderi & Claesson, 2006). Fig. 2 shows a QCM-D representative profile of the changes in frequency and dissipation over time after the injection of HPC (1 mg/mL), followed by a buffer rinse step, for gold and PS sensors. Initial exposure of both sensors to the HPC solution resulted in a sharp decrease in frequency (Fig. 2A), indicating a rapid mass deposition of the cellulose molecules onto the gold or the PS surface. This was followed by a slower decrease in frequency until a near stable plateau was reached during the time of the adsorption step, which suggests that HPC was most likely adsorbing onto the already adsorbed layer, rather than onto the sensor itself (Lin, Lopez-Sanchez, Selway, & Gildey, 2018), during the later stage of adsorption. The larger decrease in frequency observed on the PS sensor compared to gold, indicates a larger amount of mass adsorbed onto the hydrophobic surface. Adsorbed mass measured by QCM-D involves the adsorbed polymer (i.e. HPC) as well as the water coupled within the adsorbed layer (Konradi, Textor, & Reimhult, 2012), and therefore it is considered hydrated mass. Table 1 summarizes values of hydrated adsorbed mass and thickness of HPC layers formed onto gold and PS surfaces. Overall, the mean total adsorbed mass and thickness values calculated from the Voigt model were ~2.0 times higher than the Sauerbrey values, indicating that viscoelastic HPC layers were formed on both surfaces (Ash et al., 2013). The drop in frequency was associated with an increase in dissipation (Fig. 2B), further confirming the formation of viscoelastic HPC layers on both sensors. During the buffer rinsing step, a slight increase in frequency was observed, indicating that HPC barely desorbed from the surface. The percentage of mass desorbed from gold (~17%) was higher than from the PS sensor (~9%), which suggests a higher affinity of HPC for the hydrophobic surface. Consequently, the residual mass and the thickness of the HPC layer was significantly larger on the more hydrophobic polystyrene sensor than on gold (Table 1). HPC might adsorb on the solid-water interface in simple train-loop-tail configurations. The model for the adsorption of non-ionic polymers at interfaces is described in terms of trains, loops, and tails, and it proposes that the polymer chains only partly adsorbed, with each polymer molecule attached at one or few points of the surface (trains) forming a succession of loops that end with tails (remaining chain spread out into the water phase) (Charlaganov, Kosovan, & Leerakers, 2009). Therefore, the differences in Δf/n and ΔD, and subsequent adsorbed mass and layer thickness, observed in both surfaces, could be attributed to the existence of specific interactions taking place at each sensor surface. On gold, segments of the HPC polymer chain may interact with the metal through weak Van der Waals forces (Fark, Draper, & Flynn, 2007), leading to physiosorption, and thus to a lower adsorption of HPC onto the surface. On the other hand, the main driving force for adsorption onto the PS sensor might be attributed to stronger hydrophobic attractions between the hydrophilic segments of the polymer backbone and substituted branches, and the hydrophobic surface (Malmsten & Lindman, 1990). As the polymer backbone adsorbs onto the hydrophobic surface, it adopts conformations with higher adsorbed loops and tails than train fractions (Bodratti,
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Sarkar, Alexandridis, 2017) allowing more coupled water mass to accrue on the particle surface (Wind & Killmann, 1998).

By comparing the ratio between $\Delta f/n$ and $\Delta D$ (Fig. 2C) at different stages of the formation of the HPC layer, it is possible to get information about the viscoelastic properties of the adsorbing cellulose layer in relation to the sensor’s induced dissipation per unit mass, and further evaluate differences in interfacial behavior of HPC on both surfaces. Namely, a rigid film would be characterized by a more negative ($\Delta f/n)/\Delta D$ value which indicates that mass addition does not cause a significant dissipation increase. Oppositely, a less negative ($\Delta f/n)/\Delta D$ value would correspond to a softer, more dissipative layer (Ash et al., 2013). Progressive adsorption of HPC on both surfaces produced softer layers having a more viscous structure, as indicated by the less negative $\Delta f/n)/\Delta D$ values.

**Table 1**

<table>
<thead>
<tr>
<th>Sensor Surface</th>
<th>Sauerbrey Model</th>
<th>Voigt Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mass (ng cm$^{-2}$)</td>
<td>Thickness (nm)</td>
</tr>
<tr>
<td>Adsorption step</td>
<td>PS 457 ± 19$^a$</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Gold 226 ± 20$^b$</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Buffer rinse step</td>
<td>PS 416 ± 17$^a$</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Gold 187 ± 18$^b$</td>
<td>1.8 ± 0.2</td>
</tr>
</tbody>
</table>

$^a$Values represent mean ± SEM. Values not sharing a common superscript are statistically significantly different (two sample t-test, p < 0.05).

**Fig. 1.** Bile Salt structures differing by a single hydroxyl group on the sterol ring: (A) NaTC and (B) NaTDC.

**Fig. 2.** Adsorption of HPC onto QCM-D sensors, followed by a buffer rinse step. (A) Frequency ($\Delta f/n$) and (B) dissipation ($\Delta D$) changes over time measured at the 5th overtone (25 MHz) during the adsorption of HPC onto gold or PS-coated sensors. Each experiment was reproduced in triplicate, and a representative profile was selected. Arrows and labels indicate when solutions were introduced in the flow cell after the initial baselines were set. (C) Ratio of ($\Delta f/n)/\Delta D$ on gold and PS-coated sensors during three stages of HPC adsorption/desorption. Values represent mean ± SEM. Values at each stage of HPC adsorption not sharing a common superscript are significantly different (p < 0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
(Δν/ν) / ΔD values observed at the end of the adsorption step compared to the values at 1-min of adsorption. It is likely that initially, when the surface coverage is low and because there is little competition for adsorption sites, the first cellulose molecules might adsorb at the surface forming a higher fraction of trains (Bodratti et al., 2017). As the amount of HPC adsorbed increases, the arrangement of the layer might begin to convert from trains to loops and tails into a brush configuration, retaining more water and forming a more diffuse less elastic layer (Mezdour et al., 2008) (Supplementary information, Fig. S1). In this configuration, only a few hydrophobic segments per chain may have interacted with the hydrophobic surface while the loops and tails in the brush were in the bulk solution, thus forming a more diffuse less elastic layer decreasing surface elasticity (Mezdour, Cuvelier, Cash, & Michon, 2007). The exchange with buffer led to significantly higher negative (Δν/ν) / ΔD values. This increase in film rigidity suggests that the diffuse layer was removed, thereby yielding an elastic HPC layer containing less coupled water. HPC formed more rigid layers on PS than on gold sensors during the entire adsorption period, and after the buffer rinse, probably due to stronger interactions between HPC and PS. By comparing the adsorbed mass detected by QCM-D with mass determined by Surface Plasmon Resonance (SPR) (Supplementary Fig. S2), a technique that is only sensitive to the adsorbing species yielded similar qualitative HPC adsorption/desorption trends. However, the hydrated mass sensed by QCM-D was significantly larger than that detected by SPR, which was expected, because of hydrodynamically coupled water associated with the HPC layer. Namely, we found that ~63% of the hydrated HPC mass on the polystyrene sensor was due to associated water.

Based on these results, it can be concluded that the surface chemistry influences the interfacial behavior of HPC, and consequently the amount and structure of adsorbed HPC onto the solid-water interface. Since HPC had greater affinity for polystyrene and this hydrophobic surface best mimics a lipid droplet surface, the PS sensor was chosen for performing the rest of the adsorption experiments.

### 3.2. Adsorption/desorption behavior of individual BS at a hydrophobic surface

Second, the adsorption/desorption properties of NaTC and NaTDC (Fig. 1) at the bare polystyrene surface were evaluated by monitoring the shift in scaled frequency with respect to time, after individual injections of three fixed BS concentrations (2, 5 or 10 mM) into the buffer subphase. This was followed by a buffer rinse step (Fig. 3A, B & C). These BS concentrations are below, around or above the critical micelle concentration (CMC) of NaTDC (~2–3 mM) (Madenci & Egelhaaf, 2010). Regardless the CMC of NaTC is less well-defined and occur at higher concentrations (~13 mM) (Thongngam & McClements, 2005) (3–18 mM) (Madenci et al., 2010). For both BS, the injection of 2 mM BS led to a decrease in frequency, which reached a near plateau after a few minutes. A more negative frequency was reached for NaTDC compared to NaTC, indicating a higher amount of NaTDC absorbed onto the hydrophobic surface. Above 2 mM, the behavior of the two BS differed: the frequency remained relatively stable for additional injections of NaTDC, whereas the injection of 5 mM NaTC resulted in a decrease in (Δν/ν) to more negative values, which slightly shifted to less negative frequency values at 10 mM (i.e. the taurocholate showed a greater extent of adsorption than the taurodeoxycholate at concentrations above 2 mM). Differences in the kinetics of adsorption between NaTDC and NaTC were also observed: the taurocholate adsorbed more slowly than the taurodeoxycholate, and this was particularly evident at 5 mM. These different behaviors can be associated to the higher concentration that NaTC needs to form micelles (Pabois et al., 2019).

In order to better understand the differences in adsorption behavior between BS, the changes in dissipation are plotted as a function of frequency shift (Fig. 3D, E & F). This plot displays the energy dissipation per coupled unit mass independent of time. The slope of the correlation gives information about the viscoelastic properties of the adsorbed layer, and therefore conformational changes happening during BS adsorption can be inferred (Feiler, Sahlholm, Sandberg, & Caldwell, 2007; Plunkett, Claesson, Erntsson, & Rutland, 2003). A large slope (i.e. high dissipation per frequency change) characterizes a soft viscoelastic
layer, while a small slope (i.e. small dissipation increment per frequency change) describes more rigid layers (Belegrinou et al., 2008). While no significant conformational changes occurred as the taurocholate adsorbed onto the PS surface, two different adsorption processes (i.e. kinetic regimes) were observed for the deoxycholate, which initially formed a rigid layer that changed to a more diffuse and viscous layer at the end of the adsorption process. After the buffer rinse, we can see differences between the desorption behavior of the two BS: NaTDC, which adsorbed more rapidly and to a lesser extent at concentrations above 2 mM, desorbed to a greater extent, whereas for NaTC, which adsorbed more slowly with increasing adsorption, a larger proportion was irreversibly adsorbed at the hydrophobic surface (Fig. 3A, B & C; Supplementary Fig. S3).

Based on the above results, we could conclude that a subtle difference in the BS structure (i.e. one less hydroxyl group at the C7 position in NaTDC), and BS micellar properties, have an impact on the adsorption process of BS at the solid-water interface, with NaTDC being less prone to remain at the surface, despite its greater hydrophobic character. This behavior is consistent with the reported adsorption of taurocholate and taurodeoxycholate BS onto C18-modified silicon oxide surfaces (Parker et al., 2014). The importance of the micellar state in the adsorption of BS has been highlighted by several authors (Subuddhi & Mishra, 2007). Results suggested that BS micellization is a significant factor affecting the interaction of BS with hydrophobic surfaces. Pabois et al., who have also studied the adsorption of NaTC and NaTDC but to the air-water interface and to the surface of DPPC vesicles, found that the adsorption of NaTC at interfaces correlates with a later onset of micellization, while desorption of NaTDC correlates with a lower CMC value (Pabois et al., 2019).

3.3. Interfacial interactions: Sequential adsorption of HPC and BS at the hydrophobic surface

In the next series of experiments, in order to emulate the behavior of HPC-stabilized emulsions after the addition of BS, the sequential adsorption of BS onto the HPC-covered surface was studied by QCM-D. Changes in frequency ($\Delta f/n$) and dissipation ($\Delta D$) were monitored as a function of time during the injections of individual BS at three fixed concentrations (2, 5, and 10 mM) onto the pre-adsorbed HPC layer (adsorbed from a 1 mg/mL solution), followed by a buffer rinse step (Fig. 4). When both BS were injected into the flow cell, an abrupt drop in frequency was observed indicating the initial adsorption of BS onto the HPC layer (Fig. 4A&B) and consequently the formation of a mixed polymer/bio-surfactant layer. The interaction of NaTC and NaTDC with the HPC layer shows an adsorption pattern dependent upon BS concentration. The frequency gradually decreased with the increase in BS concentration; the higher the BS concentration, the lower the frequency plateau is for the mixed adsorption layer, pointing to adsorption layers gradually dominated by more BS molecules. Simultaneously, an initial increase in dissipation was also observed for every concentration and type of BS (Fig. 4C&D), showing that increasing concentrations of both BS progressively formed more viscoelastic HPC-BS mixed layers. However, differences in the frequency and dissipation profile were observed between both BS. Injection of NaTC resulted in a more gradual frequency decrease during the adsorption stage, indicating a slower adsorption of

![Fig. 4](https://example.com/fig4.png)

**Fig. 4.** Evolution of frequency ($\Delta f/n$) and dissipation ($\Delta D$) over time measured at the 5th overtone (25 MHz) during the sequential adsorption of HPC and 2, 5, or 10 mM NaTC (A and C) and NaTDC (B and D), followed by a buffer rinse step. Each experiment was reproduced three times, and a representative profile was selected. Arrows and labels indicate when solutions were introduced in the flow cell after the initial baselines were set.
NaTDC onto the HPC layer compared to NaTDC, except the 2 mM solution. Above a concentration of 2 mM, an increase in frequency was observed during NaTDC loading, which could indicate the desorption of NaTDC from the surface or, instead, the displacement of HPC molecules. No change in dissipation was observed during that period (Fig. 4D), suggesting the absence of any change in viscoelasticity (which may occur with the removal of HPC molecules). However, considering that the adsorption of NaTDC on the bare surface reached a steady state during this time (Fig. 4B&C), it is likely that NaTDC is already penetrating the HPC layer and displacing HPC from the surface during NaTDC loading. Similarly, a previous study showed that NaTDC partially displaced HPMC molecules from the oil-water interface during the adsorption period (Torcello-Gómez et al., 2016). The addition of NaTC, by contrast, did not lead to a frequency change after the initial frequency drop at any of the concentrations studied. This behavior suggests a lower affinity of NaTDC for the hydrophobic surface and/or the HPC layer, compared to NaTC. A recent study has reported that deoxycholate-based BSs have a lower affinity for a lipid-water interface and are more likely to be involved in displacing DPPC molecules from the interface (Pabois et al., 2013), or that NaTDC was starting to remove some HPC from the surface, resulting in polymer chains dangling off the sensor, and thus in an increased dissipation and a more diffuse layer. It has been recently reported that the addition of increasing amounts of NaTDC loosens the packing of a phospholipid (DPPC) monolayer (Pabois et al., 2019). These authors also reported that the major difference between NaTC and NaTDC is the strong desorption of phospholipid molecules from the interface by NaTDC.

To gain more insight into the differences in adsorption behavior between the two BS, the change in dissipation (ΔD) vs frequency change (Δf/n) during the adsorption of BS onto the HPC layer was plotted (Fig. 5). According to this plot, the decrease in frequency is strongly associated with an increase in dissipation for both BS at all three concentrations. However, the slopes of the correlation observed for NaTC and NaTDC indicate differences in the viscoelasticity of the adsorbed layers. Regarding NaTC, for a given concentration, the curve followed a linear relationship throughout the NaTC adsorption period (Fig. 5A), which suggests that no significant conformational changes occurred as NaTC molecules were progressively added to the HPC layer (Amirkhani, Volden, Zhu, Glomm, & Nyström, 2008; Belegrinou et al., 2008; Richert et al., 2004). In addition, the injection of NaTC led to a similar layer viscoelasticity (i.e. identical slopes) regardless of NaTC concentration, which suggests that NaTC adsorbs in the same specific way and remains in the same adsorbed state at 2, 5 and 10 mM NaTC. As mentioned earlier, the CMC of NaTC reported in the literature has a broad range. In a recent study, we determined the CMC of NaTC by pyrene fluorescence and observed no inflexion points in the plot of I₁/I₂ values of the fluorescence spectrum vs BS concentration (Zornjak et al., in preparation). Therefore, we believe that it is likely that, for the BS concentration range used in these experiments, the taurocholate is below their quoted CMC and adsorbs as monomers. With respect to NaTDC, the different slope observed at different concentrations at the end of the adsorption step indicates differences in the final viscoelasticity of the mixed layers, and that the BS does not remain in the same state (Fig. 5B). The larger slope observed at 10 mM, indicates that the HPC layer became much more viscous and diffuse as NaTDC adsorption proceeded. Since the CMC of NaTDC has been determined as being in the range 2–3 mM (Madenci et al., 2010), it is therefore possible to interpret Fig. 5B as showing that NaTDC adsorbs as individual molecules below a concentration of 3 mM and as micelles above this value. The same slope was observed for NaTC and NaTDC at 2 mM, supporting that NaTDC is adsorbing in form of monomers at 2 mM. In addition, for NaTDC 5 and 10 mM, break points appear in the curves, reflecting a transition from a rigid film to a more viscoelastic layer at the end of adsorption. This change in slope and nature of the adsorption process at concentrations above the NaTDC CMC, suggests that either NaTDC micelles become the dominant adsorbing species and adsorb over a saturated monolayer of individual NaTDC molecules (Euston, Baird, Campbell, & Kuhns, 2013), or that NaTDC is starting to remove some HPC from the surface, resulting in polymer chains dangling off the sensor, and thus in an increased dissipation and a more diffuse layer. It has been recently reported that the addition of increasing amounts of NaTDC loosens the packing of a phospholipid (DPPC) monolayer (Pabois et al., 2019). These authors also reported that the major difference between NaTC and NaTDC is the strong desorption of phospholipid molecules from the interface by NaTDC.

The question of whether an increasing number of HPC molecules are displaced from the surface by the BS, still remained and could be better answered by performing a final rinsing step with the pure buffer solution (Fig. 4). It was observed an initial increase in frequency, and a parallel decrease in dissipation, which indicates some desorption of mass (HPC and/or BS) from the surface and that the films became more rigid. Regardless of BS type and concentration, the frequency values obtained after the final buffer rinsing step were higher than the values before BS solutions were first injected into the subphase, and therefore we can assume that both HPC and BS are desorbed from the mixed surface layer. The frequency and dissipation evolution during the final exchange with buffer differs for NaTC and NaTDC, and shows a concentration-dependent desorption pattern. Nonetheless, regardless of BS type, the frequency still did not reach the baseline (i.e. 0 Hz) and there was still a measurable response in dissipation, meaning that a considerable residual (HPC) mass was left in the adsorption layer. Fig. 6 shows the minimum % HPC mass displaced by BS from the PS surface after the final buffer rinse was calculated, assuming that the whole amount of BS that adsorbed onto the HPC layer was desorbed during rinsing. Based on these considerations, we confirmed that NaTDC seems to be more effective at removing HPC from the hydrophobic surface than NaTC. It is important to note that, since the BS did not completely desorb from the bare PS sensor in the absence of HPC (i.e. BS control samples) (Fig. 3), some BS molecules might have remained in the adsorbed HPC layer after the final rinse, and thus, the % of HPC removed during the buffer rinse might have been higher than the values presented in Fig. 6. In any case, HPC was resistant to complete displacement by both BS at concentrations ranging from 2 to 10 mM, at least at the surface level.

![Fig. 5. Change in dissipation (ΔD) versus frequency (Δf/n) measured at the 5th overtone (25 MHz) during the adsorption of 2, 5, and 10 mM NaTC (A) and NaTDC (B) onto the pre-adsorbed HPC layer. Values on the graph indicate the slope of the lines. Hydrated mass increases with the decrease in frequency, therefore adsorbed mass is increasing from right to left.](image-url)
These observations taken together imply that the degree of hydrophobicity in the BS, and consequently the formation of micelles, plays a significant role on the interactions between BS and the pre-adsorbed HPC layer, and on the displacement of HPC from the surface. Specifically, at concentrations above the CMC, NaTDC desorbs from the surface and removes some HPC molecules. On the other hand, NaTC molecules shows a higher probability to interact with the HPC layer in their non-aggregated form.

### 3.4. Bulk interactions between HPC and BS in the aqueous phase

Before considering the simultaneous adsorption of HPC and BS at the hydrophobic surface, the interactions between HPC and BS in the bulk phase will be first evaluated, so that the competitive adsorption between HPC and BS would be easier to interpret. The effect of the BS type on their interaction with HPC in the aqueous phase is characterized by DSC measurements, at a fixed HPC concentration (1 mg/mL) and at BS concentrations ranging from 0 to 10 mM. Micro-DSC thermograms displaying the peaks on heating for HPC in the absence and presence of both types of BS are shown in Fig. 7. In the absence of BS, the thermogram corresponding to HPC shows an endothermic transition peak at 51 °C. In a previous study, we found this peak to arise at 47 °C (Torcello-Gomez et al., 2015), and based on the work of Haque and Morris (1993), we postulated this transition to be a consequence of the association of the hydrophobic segments of hydroxypropyl substituents in HPC as the temperature increased (Haque & Morris, 1993). The difference in temperature is probably due to the different concentration, molecular weight and viscosity of the HPC tested in the studies. The incorporation of increasing concentrations of BS in the mixture, increased the transition temperature and reduced the peak size of the polymer, effect that was clearly perceptible even at the lowest BS concentration tested (2 mM). However, differences are observed between BS. In the case of the more hydrophobic BS (i.e. taurodeoxycholate), the shift to higher temperatures is greater and the decrease in transition enthalpy is more gradual than for the taurocholate BS. It is interesting to point out that for the NaTC mixture at the lowest concentration (2 mM), the decrease in transition enthalpy was greater, compared with NaTDC. The NaTDC mixtures also showed narrower transition peaks as compared with NaTDC, with slight differences among NaTDC concentrations (i.e. the effect of 2, 5 and 10 mM on HPC thermal properties was practically the same). For a given concentration, 2 mM, which is below the CMC of NaTDC and NaTC, the decrease in transition enthalpy was greater for NaTC than for NaTDC. However, at 10 mM, which is above the CMC of NaTDC and likely below the CMC of NaTC, the greatest effect on HPC thermal properties is observed with NaTDC. This suggests that the more hydrophobic BS, NaTDC, seems to bind more efficiently to HPC when it is in its aggregated state, probably because of a more efficient association between NaTDC micelles and HPC hydroxypropyl chains. Previous DSC studies on mixed solutions of triblock copolymers and NaGDC, the glycinic conjugate of a taurodeoxycholate BS, also showed that NaGDC affected the self-assembly of the copolymer P123 by gradually decreasing enthalpy and shifting to higher temperatures (Bayati, Galantini, Knudsen, & Schillen, 2015; Bayati, Anderberg, Pavel, Galantini, & Schillen, 2016). These authors reported that the formation of mixed BS micelles carrying copolymer unimers was responsible of this effect. Therefore, a similar association mechanism between NaTDC and HPC could have occurred. Likewise, mixed aggregates similar to the complexes formed between pluronics and NaTDC micelles (Torcello-Gómez, Maldonado-Valderrama, Jodar-Reyes, & Foster, 2013) could have been formed. We reported similar observations in a previous study testing moderately concentrated BS solutions (all above CMC values of BS) and postulated the formation of cellulose-NaTDC aggregates at any of the concentrations studied (Torcello-Gomez et al., 2015). Our current findings, obtained in a more dilute regime, complement our previous work and show that it is possible to maximize BS-binding efficacy in the bulk phase at minimum HPC concentrations.

From these results, it is clear that the thermal transition of HPC is disrupted by the presence of BS at a physiological range of BS concentrations found in fasted and fed state in the duodenum. Additionally, it seems that the small difference in structure between the taurodeoxycholate and taurocholate BS, which is responsible for the diverse CMC values, plays a role in the interactions between BS and HPC in the aqueous phase. This generalizes and extend the discussion presented in section 3.3 (interfacial interactions of a pre-adsorbed HPC layer with BS). The taurodeoxycholate is more susceptible to interact with HPC at NaTDC concentrations above its CMC, which suggests that each molecule of HPC could sequestrate a larger amount of NaTDC (micelles) than NaTC monomers. Therefore, at bulk concentrations of 5 and 10 mM, a lower amount of free NaTDC (unbound) would be available to adsorb onto the hydrophobic surface as compared to NaTC. The opposite would be true when BS bulk concentrations are 2 mM. This will be discussed in the next section.
3.5. Interfacial interactions: Simultaneous adsorption of HPC and bile salts at the hydrophobic surface

In order to gain insight on how the interactions between HPC and BS in the bulk influence their interfacial properties and adsorption/desorption behavior, the simultaneous adsorption of both surface-active materials onto the hydrophobic solid surface was studied. Adsorption layers were formed onto the PS surface by injecting mixed HPC-BS solutions that contained a fixed amount of HPC (1 mg/mL) and 0, 2, 5, or 10 mM BS, respectively. This adsorption step was followed by an exchange of buffer. Fig. 8 A&B shows a representative profile of the changes in frequency and dissipation monitored as a function of time for the simultaneous adsorption of HPC + BS mixtures. This figure also shows the adsorption profile of control samples (BS and HPC alone) as a reference. As a general trend, a rapid drop in frequency was initially observed, indicating deposition of mass onto the hydrophobic surface. For both BS at every concentration, mixed systems always adsorbed to a larger extent than the individual BS, and to a lower extent than the HPC alone, indicating that HPC was able to adsorb to the hydrophobic surface in the presence of BS. However, the presence of the BS in the bulk, even at the lowest concentration, appeared to affect the HPC adsorption kinetics and prevented the complete adsorption of the polymer. This incomplete adsorption is likely due to the interactions occurring between HPC and BS in the aqueous phase (Fig. 7), which might either, led to the formation of HPC-BS complexes of different surface activity than the original molecules, and/or affected the amount of free BS (unbound) available to reach the surface, and consequently influence how HPC is competing for the surface.

In order to gain more insight about the role and contribution of each component of the mixture to the adsorption process, the change in dissipation (ΔD) was plotted as a function of the scaled frequency shift (Δf/n) (Fig. 9). Some differences between NaTC (Fig. 9A) and NaTDC (Fig. 9B) are observed. As noted in the HPC-NaTC mixed system, at 2 mM, the mixed layer did not change its structure significantly as more mass adsorbed onto the surface, similar to what happened to the pure HPC. Additionally, a strong deviation of the mixed system from the control NaTC 2 mM curve is observed. These results indicate that the adsorption of the mixed system is being driven by the polymer and the adsorbed layer is mainly composed of HPC. The same applied for the HPC-NaTDC 2 mM mixed system. When increasing the NaTC concentration in the mixture (i.e. 5 mM), no significant differences were observed. It is unclear from Figs. 8A and 9A whether the NaTC or HPC controlled the adsorption process in this mixture. Conversely, the adsorption profile of the mixed HPC-NaTDC 5 mM system was similar to that of pure NaTDC 5 mM. The elasticity of this layer seemed to depend on the adsorbed amount: the low dissipation values observed at low amounts indicated a very dense layer, which changed to a more elastic and swollen structure at high adsorbed amounts. Upon further increasing the concentration of BS in the mixture (i.e. 10 mM), the adsorption of both mixed systems seemed to be rather complicated since significant structural differences between the initial and late stages of adsorption were observed. More dramatic conformational changes (i.e. change in slope) and similar to those seen in pure NaTDC 10 mM, were observed as adsorption of HPC-NaTDC mixture proceeded, as compared with HPC alone, which showed a nearly linear adsorption regime. These findings would indicate, that the adsorption of HPC-NaTDC 5 mM and 10 mM systems is driven by the BS. Nevertheless, the contribution of HPC to the mass adsorbed is always greater in these systems (Fig. 8 A&B). These observations taken together would indicate a coexistence of both, HPC and BS, at the hydrophobic surface. We have confirmed
interactions between HPC and NaTDC in the bulk, and thus, it is likely that the adsorption dynamics of this mixture, at 5 and 10 mM, is controlled through the formation of mixed aggregates, and thus the presence of, not only HPC and BS, but also HPC-BS complexes at the surface might be possible. As recognized for mixed polymer-surfactant interfaces (Maldonado-Valderrama et al., 2011), the HPC-BS mixed surface is probably dimensionally heterogeneous having individual domains rich in BS and in HPC, along with domains formed by HPC-BS complexes. Despite a similar behavior was observed for the HPC-NaTC 10 mM system, more significant changes were observed in the NaTDC system. Namely, the layer constantly transformed to a more elastic state as the mass adsorbed increased, probably because HPC, or HPC-NaTDC complexes were starting to be removed and were dangling from the surface, which was noticed as a significant increase in dissipation. The role of BS in the presence of HPC does not seem to be distinct to other BS mixtures at the oil-water interface. According to Torcello-Gómez (Torcello-Gómez et al., 2013) an interfacial complexation between pluronics and NaTDC occurred during their co-adsorption at the oil-water interface. Similarly, it has been previously reported the adsorption of cellulose derivatives-NaTDC complexes at the oil-water interface during their competitive adsorption (Torcello-Gómez et al., 2014), which is in line with our results at the solid-water interface.

During the buffer rinse step, the increase in frequency indicated the removal of loosely adsorbed polymer and/or BS molecules from the surface (Fig. 8 A&B). The percentage of total mass desorbed (relative to the mass adsorbed) from mixed solutions was higher than that corresponding to individual solutions (Fig. 8 C) indicating that BS are responsible for the increased desorption. None of the mixtures was completely washed off from the surfaces, as reflected by the final frequency values, which did not reach 0 Hz. There were, however, small differences between the two BS. While the % of mass desorbed uniformly increased with increasing concentrations of NaTC in the mixed system, no apparent pattern was observed in the NaTDC mixture, probably due to the presence of micelle structures occurring in these adsorbed-rinsed
The greater extent of desorption observed in the HPC-NaTDC system at 2 mM, compared to NaTC, suggests greater ability of NaTDC to compete for the hydrophobic surface, which could be due to i) the presence of a higher amount of free NaTDC molecules in the bulk available to displace the mixed layer, and/or ii) to a less surface-active layer and/or easier to displace than that formed by HPC-NaTC complexes. On one hand, the HPC-NaTDC 2 mM mixture adsorbed to a lesser extent than the corresponding NaTC mixture (Fig. 8 A&B), which could mean that NaTDC-HPC complexes are less surface active than NaTC-HPC complexes. On the other hand, HPC had lower susceptibility to bind NaTDC molecules at 2 mM in the aqueous phase (Fig. 7), which probably led to a higher number of free NaTDC (unbound) molecules. Therefore, both factors seem to be contributing to the higher extent of desorption observed in the HPC-NaTDC 2 mM system. However, the opposite behavior was observed for mixed systems at 10 mM BS, i.e. the HPC-NaTDC system showed a lower extent of desorption than the NaTC mixed system. In this case, since the HPC-NaTDC 10 mM mixture also adsorbed to a lower extent, a lower number of free NaTDC molecules seems to be the main factor responsible for the lower desorption observed. This agrees with the increased susceptibility of HPC to bind NaTDC 10 mM in the bulk (Fig. 7). These results support the idea that the mixed layer structure of BS in the bulk also plays an important role on the competitive adsorption process of HPC and BS and/or the way that polymer-BS complexes adsorb and desorb from the hydrophobic surface.

3.6. Comparison between sequential and simultaneous adsorption of HPC and BS

In order to determine if the route of formation of the mixed HPC-BS interfacial layers plays a role in the properties of the layer and the displacement of HPC, the amount of HPC mass desorbed by NaTC or NaTDC during the final buffer rinse for the sequential and simultaneous adsorption experiments was compared (Fig. 10 A). The desorption profiles have been constructed from the equilibrium mass adsorbed values attained at the end of the adsorption period and after the buffer rinse step at each BS concentration from sequential and simultaneous experiments. The assumption was that the amount of BS desorbed from the bare surface in the absence of HPC (BS controls) was desorbed also in the presence of HPC.

After washing off, the desorption profiles of mixed surface layers formed by the two different routes differ from each other, which translates to diverse composition and properties of the layer. At low concentrations (2 mM), a higher amount of HPC mass desorbed from the surface (i.e. lower amount remained on the surface) when the mixed layer was formed simultaneously compared to sequentially. This suggests that HPC is more effective at hindering displacement by BS monomers by initially forming pre-adsorbed layers than by sequestering BS monomers in the bulk and thereby creating initial mixed layers formed by complexes. While this behavior is noticeable for NaTDC, small differences are observed for NaTC. As discussed before, NaTDC-HPC complexes formed in the bulk during simultaneous adsorption seem to be of low importance to the formation of the layer, probably because of their decreasing hydrophobicity; hence the surface is covered by more strongly adsorbed BS molecules. In contrast, in sequential adsorption, complexes formed at the surface seem to be more stable when BS monomers are involved, and more difficult to desorb into the bulk. This scenario is opposite at higher BS concentrations, i.e. the mass desorbed after rinsing rises earlier in sequential than in simultaneous adsorption experiments. This could be indicative of more surface active HPC-BS complexes created in the bulk that are more strongly adsorbed to the surface than when the HPC layer is first created and BS adsorbed. Therefore, the layer is more easily displaced after sequential adsorption. Again, the difference between the amount of HPC desorbed during the sequential and simultaneous adsorption is more pronounced for NaTDC, compared to NaTC. Since NaTDC is in form of micelles, most hydrophobic domains of the BS are hidden. This could favor association between HPC and NaTDC micelles in a way that leaves hydrophobic regions of HPC exposed conferring a more hydrophobic character to the complexes formed during simultaneous, which easily adsorb to the surface. In addition, this results in a lower amount of freely adsorbed BS molecules at the surface. In sequential adsorption, the limited access to the hydrophobic domains of the pre-adsorbed HPC gives more opportunity for formation of aggregates of lower surface activity that easily desorb.

It should be noted that if the mass desorbed is calculated in percentage relative to the mass adsorbed, the differences in the amount of HPC displaced or total mass desorbed by BS is slightly lower, mainly for NaTC at high concentrations. Nonetheless, the behavior of the surface layers formed sequentially and simultaneously follows a similar profile than that described above, indicating that the way mixed HPC–BS layers are formed, or the order in which these two surface active molecules reach the interface plays a role in the surface composition of the adsorbed species and properties of the layer, and thus in their potential to hinder BS adsorption. Furthermore, this scenario depends on the BS micellar properties.

These findings demonstrate that HPC can compete with two BS of different hydrophobicity for a hydrophobic surface together to the proved resistance of the HPC pre-adsorbed layer to complete displacement by BS (Sequential adsorption experiments). Whether HPC may compete with both BS for the oil–water interface of emulsified lipids, or
whether it may be resistant to displacement by BS under more realistic intestinal conditions, is a question that will be answered in the next section.

3.7. Effect of BS type on the in vitro digestion of HPC-stabilized emulsions

We examined the impact of HPC on the digestion of oil-in-water (o/w) emulsions by using a simplified in vitro digestion model that simulates the acid gastric acid and intestinal conditions (Fernandez-Fraguas, Woodward, Gunning, & Wilde, 2014). Individual BS at 10 mM were employed to simulate intestinal conditions, as this concentration is within the range of physiological BS concentrations found during lipid digestion in the duodenum in the fed state. Fig. 10B shows the lipid digestion profiles (% FFA versus time) of HPC-stabilized o/w emulsions obtained in the presence of NaTC or NaTDC in the intestinal phase using a simple pH-stat method. An initial rapid increase in the release of FFAs from both emulsions was observed during the first 10 min of digestion, followed by a more gradual increase as time progressed until a quasi-steady state was reached. However, the influence of HPC on the lipolysis kinetics of emulsions appeared to be BS dependent. The use of NaTDC as sole BS in the intestinal phase led to slightly slower rate and extent of lipid digestion than when NaTC was used (Table Supplementary information S1), suggesting that NaTDC might be less effective in accessing and disrupting the interface, compared to NaTC. These results contrast with those observed from the sequential adsorption experiments (Section 3.3). To recall, NaTDC at 10 mM was shown to be more effective at disrupting the pre-adsorbed HPC layer and removing some polymer from the hydrophobic surface than NaTC (Fig. 4 and Fig. 6). However, a competitive adsorption is another possible scenario that could explain the delayed lipolysis observed with NaTDC. Since HPC emulsions had relatively large droplets (3.8 μm) (Supplementary Fig. S4), the total interfacial area is relatively small. Thus, it could be assumed that HPC only partially adsorb the o/w interface during emulsification, and a considerable fraction of HPC is present in the bulk aqueous phase available to interact with NaTDC (and/or lipase) and/or to compete with the BS for the interface. On one hand, this would inhibit the formation of the lipase-collipase complex, hindering their adsorption at the lipid droplet surface and thereby diminishing lipase activity. On the other hand, this process would be more competitive for the BS, and consequently the BS would have a reduced impact on lipid digestion. In addition, a lower number of free NaTDC molecules available to disrupt the interface, compared to NaTC, (Fig. 7), responsible for the higher amount of mixed HPC-BS layer (mainly composed by HPC) irreversible adsorbed onto the surface (Fig. 8), could have led to the reduced lipolysis extent. This is in accordance with a previous study that showed that the rate and the extent of lipolysis appeared to be dominated mainly by the presence of BS in the aqueous phase rather than by the BS adsorbed onto the interface (Sarkar, Ye, & Singh, 2016).

Therefore, despite the larger HPC mass displaced by NaTDC (as observed in the sequential experiments, Fig. 4 and Fig. 6), a lower amount of free NaTDC in the bulk (as observed in the simultaneous experiments, Fig. 8) might have hindered the solubilization of products of lipolysis (which generally inhibit the progress of lipolysis) from the interface to the micelles, and hence lipolysis. Furthermore, the presence of a higher amount of NaTC irreversible adsorbed to the surface compared to NaTDC (Fig. 5S) could have led to a longer residence time at the interface than NaTDC, which may have promoted the adsorption of the co-lipase/lipase complex, thus initiating lipolysis more quickly, compared to NaTDC. This suggest the idea that differing adsorption-desorption behaviors of di-hydroxy and tri-hydroxy BS may influence different functions during lipolysis, which have been discussed in depth by Parker and co-authors (Parker et al., 2014).

4. Conclusions

This work has evaluated the impact that bulk and interfacial interactions between a common stabilizer of food emulsions, HPC, and two BS (NaTC and NaTDC) have on the in vitro digestion of lipids, providing qualitative and quantitative information about the sequential and simultaneous adsorption/desorption behavior of these two surface-active materials onto a model hydrophobic surface simulating the surface of a lipid droplet. We have showed for the first time that HPC was able to resist complete displacement by both BS from the surface, as well as to compete with both BS for the surface at a range of BS concentrations relevant to physiological conditions within the duodenum. Subtle differences in BS structure (i.e. the absence of a single hydroxyl group in the sterol ring), which dictate micellization phenomena in the bulk, played a key role on both scenarios. Our findings revealed that a sequentially formed pre-adsorbed HPC layer was more easily accessible and displaced from the solid-water interface by NaTDC micelles than by NaTC monomers. The type of BS also affected interactions with HPC in the bulk, which in turn, had an impact on how HPC competed with BS for the hydrophobic solid surface. While HPC seemed to be more susceptible to interact with the more hydrophilic NaTC when both BS are in its non-aggregated state, each molecule of HPC could bind to more NaTDC molecules than to NaTC, when only NaTDC was in its micellar state. This probably reduced the amount of free NaTDC to concentrations below its CMC, which made the process more competitive for NaTDC and hindered the displacement of the simultaneously adsorbed HPC-NaTDC mixed layer. We also demonstrated that the more hydrophobic NaTDC was shown to be more effective at displacing HPC when the layer was formed sequentially than when it was formed simultaneously. Eventually, all the above phenomena had an impact on the ability of BS to promote lipolysis in the presence of the colipase/lipase complex under fed state conditions. Despite the more hydrophobic NaTDC in its aggregated state was more effective at interacting and displacing the pre-adsorbed HPC interfacial layer than NaTC, a lower amount of free NaTDC molecules available in the bulk to displace HPC from the surface could be responsible for the delayed lipolysis of HPC-stabilized emulsions. As a conclusion, we propose that the ability of HPC to modulate lipid digestion is due to a combination of interfacial and bulk events, with the binding of BS in solution by HPC being the major factor affecting interfacial and displacement behavior of HPC-BS mixed layers, which in turn was dependent on the BS aggregation processes. The contrasting adsorption/desorption behavior exhibited by the two BS supports the idea that different BS may play different roles in lipolysis. Further studies looking at structurally different polysaccharides, and testing higher BS concentrations and different type of BS (i.e. chenodeoxycholate) would provide a deeper insight into the factors that regulate the digestion of lipids.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Jennifer Zornjak: Investigation, Visualization, Validation, Formal analysis. Jianzhao Liu: Validation. Alan Esker: Writing - review & editing, Supervision, Resources. Tian Tian Lin: Investigation. Cristina Fernandez-Fraguas: Conceptualization, Supervision, Project administration, Writing - original draft, Writing - review & editing, Visualization, Resources, Funding acquisition, Validation.

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Appendix A. Supplementary data

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References


