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Lag-burst kinetics of surfactant displacement from the liquid crystal/aqueous interface by bile acids

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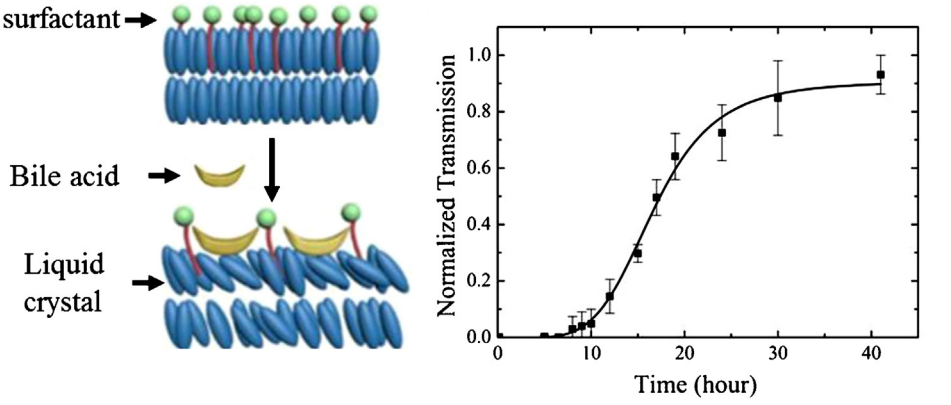
HIGHLIGHTS

- Sodium dodecyl sulfate can be displaced from the liquid crystal/aqueous interface by the competitive adsorption of bile acids.
- The displacement exhibits lag-burst kinetics.
- The lag time and burst rate depends on the number and position of the hydroxyl groups of bile acids.

GRAPHICAL ABSTRACT



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ABSTRACT

Bile acids play an important role in fat digestion by displacing surfactants from the oil–water interface through emulsification. In this paper, we study the time course of the displacement of sodium dodecyl sulfate (SDS) from the liquid crystal (LC)/aqueous interface by four unconjugated bile acids, which differ in the number and position of hydroxyl groups on their steroid backbones. The competitive adsorption of bile acids displaces the SDS from the LC/aqueous interface and consequently triggers a homeotropic-to-tilted anchoring transition of the LC at the interface, which allows the displacement kinetics to be monitored by a polarizing optical microscope. The microscopy image analysis reveals that the displacement exhibits lag-burst kinetics, where a lag phase is followed by a burst phase. We find that the number and position of the hydroxyl groups of bile acids have significant impact on the lag time and burst rate of the displacement kinetics.

1. Introduction

Bile acids are important biological surfactants formed by the enzymatic catabolism of cholesterol in liver [1]. They have a rigid, quasi-planar steroid backbone with hydroxyl groups on the concave α face and methyl groups on the convex β face. The facial amphiphilic nature of bile acids makes them extremely surface active in displacing surfactants from the oil/water interface during fat digestion [2]. The interaction of bile acids with surfactants at the oil/water and air/water interfaces has been studied with several experimental methods, including surface tension [3–5], zeta potential [5], and atomic force microscopy [6]. These studies have suggested that the competitive adsorption of bile acids can disrupt

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the packing of the surfactants and eventually displace them from the interfaces. However, the effect of the nature of bile acids on the displacement kinetic has not been fully understood.

Liquid crystals (LCs) are a sensitive material with long-range orientational order [7]. The orientation of LCs is sensitive to the change of the surface which they are in contact with. This surface-induced local order can be amplified over several tens of micrometers in LC bulk due to the long-range interaction of LCs. The optical amplification of LCs makes them a unique optical probe for imaging the molecular ordering [8,9] and chemical patterns [10–12] of organic layers and sensing the chemical reactions including enzymatic reactions [13,14], DNA hybridization [15,16], ligand-receptor bindings [17,18], and peptide–lipid interactions [19,20] at the LC/aqueous interface.

In a previous publication [21], we showed that the competitive adsorption of cholic acid (CA) could displace surfactants from the LC/aqueous interface and consequently trigger a homeotropic-to-planar anchoring transition of the LC at the interface, which allowed the displacement of the surfactants to be monitored by a polarizing optical microscope. The critical concentration of CA required to displace the surfactants from the LC/aqueous interface was found to be affected by the nature of LCs. There are four unconjugated bile acids in human body. Cholic acid (CA) and chenodeoxycholic acid (CDCA) are primary bile acids, which are directly converted from cholesterol by liver. Deoxycholic acid (DCA) and lithocholic acid (LCA) are secondary bile acids, which are converted from CA and CDCA by bacterial enzymes in colon, respectively [1]. These unconjugated bile acids differ in the number and position of hydroxyl groups on their steroid backbones. In this paper, we study the time course of the displacement of sodium dodecyl sulfate from the LC/aqueous interface by LCA, DCA, CDCA and CA by observing the anchoring transition of the LC at the interface, which is triggered by the displacement. We find that the displacement shows lag-burst kinetics: a lag phase is followed by a burst phase. The lag time and the burst rate are associated with the number and position of the hydroxyl groups of bile acids.

2. Experimental

2.1. Materials

Liquid crystals (LCs) used in our experiments are 4-cyano-4′-pentylbiphenyl (5CB, 98% purity) and 4-(4-pentylcyclohexyl) benzonitrile (5PCH, 99% purity) from Sigma–Aldrich (St. Louis, MO). Sodium dodecyl sulfate (SDS), cholic acid (CA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), and lithocholic acid (LCA) were obtained from Sigma–Aldrich (St. Louis, MO). Cholyl-lysyl-fluorescein (CLF) was purchased from BD Biosciences (Woburn, MA). All chemicals were used without further purification. Water used in our experiments was purified using an Easypure II system (18.2 MΩ cm and pH 5.7). Phosphate buffered saline solution (PBS) with 1.19 mM phosphates, 13.7 mM sodium chloride, 0.27 mM potassium chloride, and pH 7.4 was from Fisher Scientific (Fair Lawn, NJ). Total ionic strength of PBS is 171.88 mM. Polyimide coated glass substrates used for inducing homeotropic anchoring of liquid crystals were purchased from AWAT PPW (Warsaw, Poland). Glass microscopy slides were from Fisher Scientific. Copper TEM grids (18 μm thickness, 285 μm grid spacing, and 55 μm bar width) were obtained from Electron Microscopy Sciences.

2.2. Preparation of liquid crystal films

Copper TEM grids were cleaned with ethanol and then dried. The cleaned TEM grids were placed on a polyimide-coated glass substrate. LCs we used were a mixture of 19 wt% of 5PCH and 81 wt% of 5CB. The mixture was more sensitive in detecting the interaction between SDS and CA, compared to pure 5CB [21]. One microliter of the LC mixture was filled in the pores of the TEM grid supported by the polyimide-coated glass substrate. The excess LC was removed from the grid by dipping the LC filled grid into water, which led to the formation of a thin LC film confined in the pores of the grids. The confined LC film was then immersed in PBS solution containing 50 μM SDS. The adsorption of SDS led to the formation of a SDS-laden LC/aqueous interface.

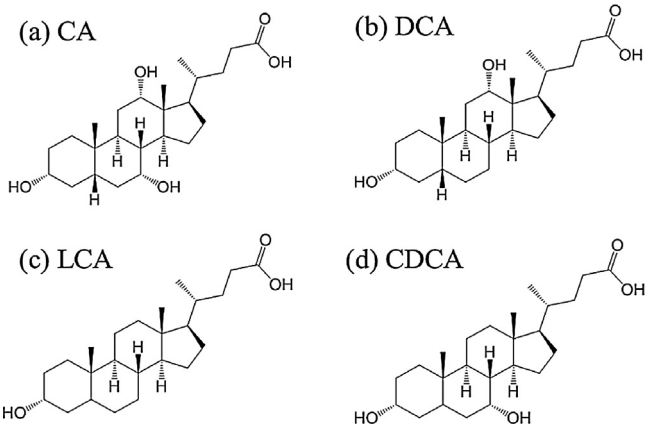

Fig. 1. Chemical structures of CA (a), DCA (b), LCA (c), and CDCA (d).

2.3. Optical observation

The optical texture of the LC films confined in the pores of the TEM grids was examined by using a polarizing optical microscope (BX 40, Olympus) in transmission mode at 25 °C. All optical microscopy images were taken with a digital camera (C2020 Zoom, Olympus) mounted on the polarizing optical microscope and then analyzed with NIH Image J. Fluorescence microscopy images were acquired with a confocal fluorescence microscope (Zeiss TCS SP5MP) with 488 nm excitation from an Ar^+ laser.

3. Results and discussion

Four unconjugated bile acids (CA, CDCA, DCA, and LCA) were chosen for studying the comparative kinetics of the displacement of SDS from the LC/aqueous interface. Their chemical structures are shown in Fig. 1. LCA has only one hydroxyl group at C-3 position. DCA has two hydroxyl groups at C-3 and C-12 positions. CDCA has two hydroxyl groups at C-3 and C-7 positions. CA has three hydroxyl groups at C-3, C-7, and C-12 positions. The hydrophobicity of these bile acids increases with the increase of the number of hydroxyl groups on their steroid backbones. It has been shown that the order of hydrophobicity is LCA > DCA > CDCA > CA [22]. The critical micelle concentration (CMC) of LCA, CDCA, DCA, and CA is 0.9, 9, 10, and 18 mM, respectively [23]. 5PCH and 5CB are widely used cyano-containing LCs. As compared to 5CB, 5PCH has a more flexible and bulky core containing one phenyl ring and one cyclohexane ring. The flexible and bulky 5PCH requires higher anchoring energy to achieve homeotropic anchoring, compared to the rigid 5CB [24]. The adsorption of SDS at the 5PCH/aqueous interface is unable to induce a homeotropic anchoring of the 5PCH at the interface. It has been shown that 5PCH and 5CB can form nematic mixtures over a wide range of mixed ratios [25]. The adsorption of SDS at the 5PCH/5CB mixture-aqueous interface can induce a homeotropic anchoring of the 5PCH/5CB mixtures with the mixed ratio up to 19 wt% of 5PCH. We have shown that the LC mixture of 19 wt% of

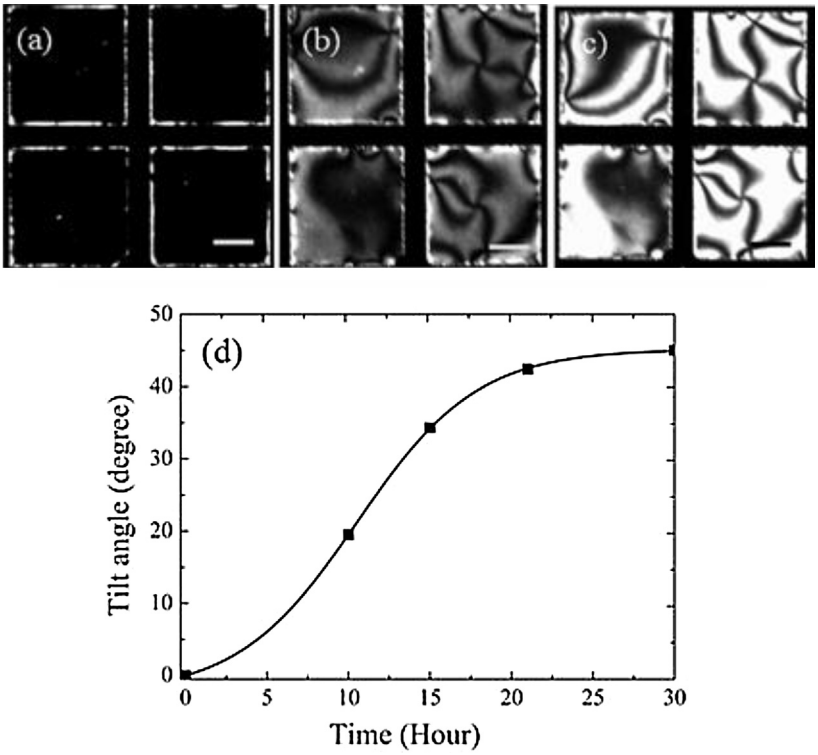

Fig. 2. Polarizing optical microscopy images of the SDS-laden LC/aqueous interface before (a) and after being exposed to 4 μM CA at 25 °C for 9 h (b) and 12 h (c). Scale bar: 97 μm. (d) Tile angle of the LC at the interface as a function of time after the addition of CA.

5PCH and 81 wt% of 5CB is more sensitive in studying the interaction between SDS and CA at the LC/aqueous interface, compared to pure 5CB [21].

In our experiments, the mixture of 19 wt% of 5PCH and 81 wt% of 5CB was prepared at ~70 °C and cooled down to ~25 °C, at which the mixture shows nematic phase. Fig. 2a shows a polarizing optical microscopy image of the LC mixture confined in the square pores of a TEM grid supported by a polyimide-coated glass substrate after being immersed in phosphate buffered saline (PBS) solution with 50 μM SDS at 25 °C. The concentration of SDS is lower than its CMC (~8.2 mM) [26]. The adsorption of SDS at the LC/aqueous interface leads to the formation of a SDS-laden LC/aqueous interface, which induces homeotropic surface anchoring with a dark appearance (Fig. 2a). When 4 μM of CA is added into the PBS phase side of the SDS-laden LC/aqueous interface, the optical appearance of the confined LC film changes from the dark to brush textures emanating from a single defect (Fig. 2b). The appearance of the bright domains reflects a continuous change in the orientation of the LC from homeotropic anchoring at the polyimide-coated glass substrate to tilted anchoring at the SDS-laden LC/aqueous interface. The brightness of the LC film confined in the square pores of the grid increases and eventually reaches a maximum value over time (Fig. 2c). The tilt angle of the LC at the interface was determined from the interference of the LC film after addition of CA. The thickness of the LC film filled in the pores of the TEM grids is ~18 μm. At 25 °C, the extraordinary and ordinary refractive indices are ~1.6 and ~1.5 for 5PCH and ~1.7 and ~1.5 for 5CB, respectively. The extraordinary and ordinary refractive indices of the mixture of 19 wt% of 5PCH and 81 wt% of 5CB LC were calculated to be 1.68 and 1.5, respectively [27]. The effective birefringence of the LC film was estimated from its optical texture and a Michel-Levy chart [28]. Based on the relationship between the effective birefringence of LC film and the tilt angle of the LC at the LC/aqueous interface [29], we estimated the tilt angle of the LC at the interface with respect to the surface normal. Fig. 2d shows the tilt angle change as a function of time after addition of CA. The tilt angle increases over time and reaches to ~45° after 30 h.

The homeotropic-to-tilted anchoring transition of the LC at the interface is a result of the competitive adsorption of CA by displacing the SDS from the interface. Fig. 3a shows a confocal fluorescent microscopy image of the SDS-laden LC/aqueous interface 10 min

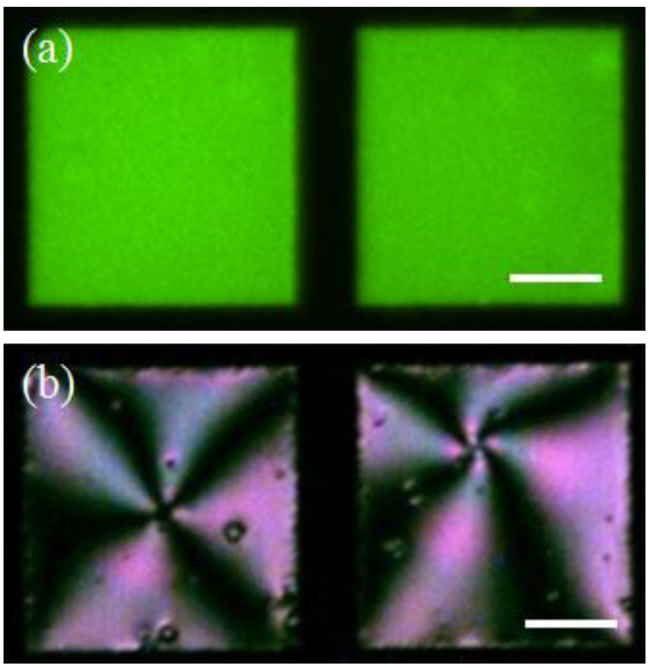

Fig. 3. Fluorescence (a) and polarizing (b) microscopy images of a SDS-laden LC/aqueous interface after being exposed to 0.25 μM CLF solution at 25 °C. Scale bar: 97 μm.

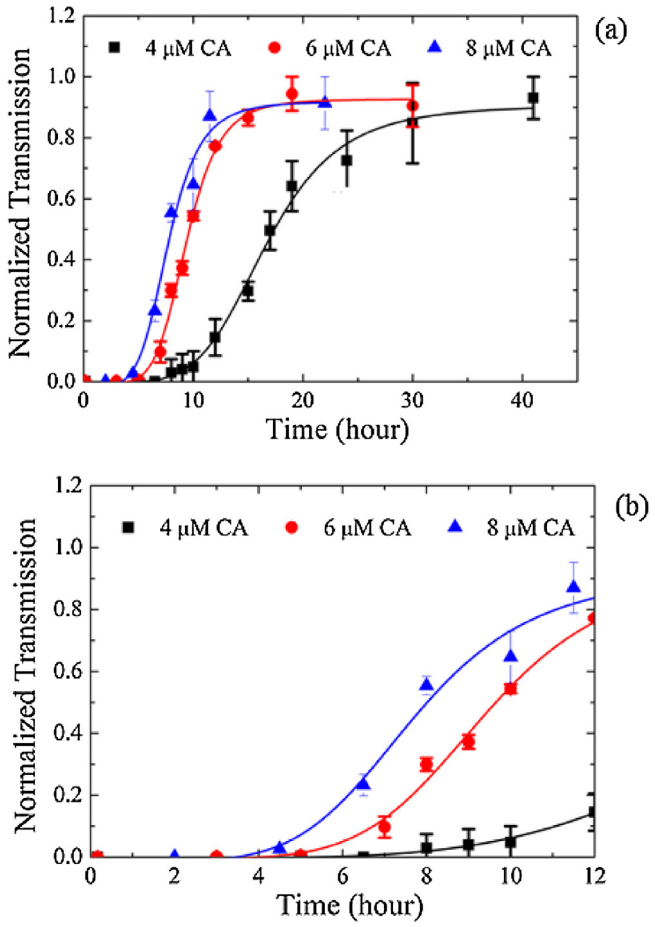

Fig. 4. The normalized transmittance of the LC film confined in a pore of the grid as a function of time after the addition of 4, 6, and 8 μM CA at 25 °C.

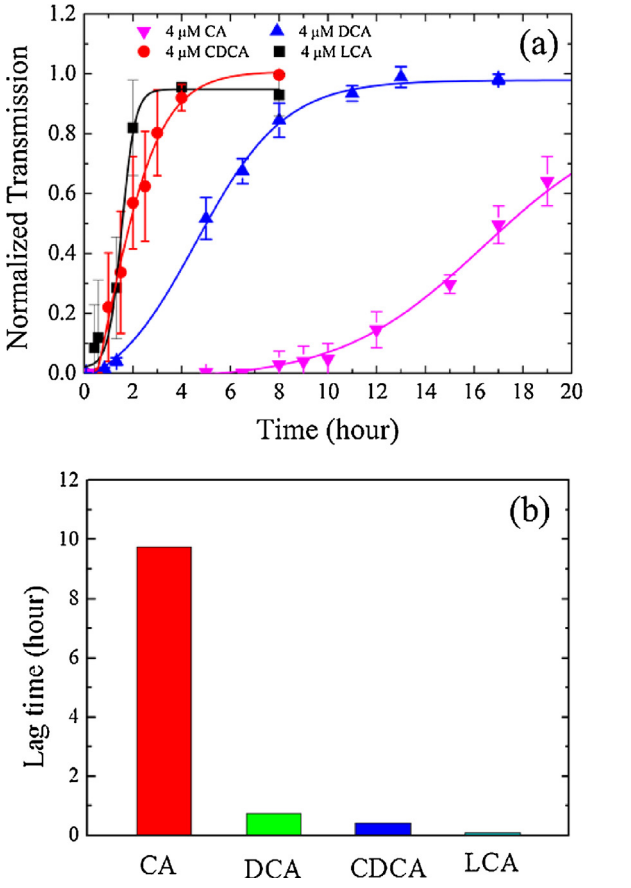

Fig. 5. (a) The normalized transmittance of the LC film confined in a pore of the grid as a function of time after the addition of 4 μM CA, 4 μM DCA, 4 μM CDCA and 4 μM LCA at 25 °C. (b) Lag time of CA, DCA, CDCA and LCA.

after the addition of CLF (a fluorescein-labeled CA) into the aqueous phase side of the LC/aqueous interface. The fluorescence from the interface confirms the adsorption of CLF at the interface. The corresponding polarizing microscopy image shows the bright domain appearance of the LC film after the adsorption of CLF at the interface (Fig. 3b). The displacement of surfactants including SDS from the air/aqueous and oil–water interfaces by bile acids was also reported in the literature [6,30]. The displacement of surfactants from the LC/aqueous interface by the adsorption of bile acids was also reported [31], where the LC/aqueous interface was modified by the adsorption of a fluorescein-labeled surfactant. After addition of bile acids, the significant decrease of the fluorescent intensity from the LC/aqueous interface was observed, suggesting that significant amounts of the fluorescein-labeled surfactant were removed from the interface.

The time-course polarizing microscopy images of the homeotropic-to-tilted anchoring transition were analyzed with NIH Image J, in which the transmission was calculated from the average brightness of the LC film in a square pore of the grid over time and then normalized to the maximum value. We quantify the polarizing microscopy observation by plotting the normalized transmission as a function of time after addition of CA, providing the information of the displacement kinetics of SDS from the LC/aqueous interface by the competitive adsorption of CA. The plots shown in Fig. 4a reveal the lag-burst kinetics: a slow phase (termed as lag phase) followed by a rapid phase (termed as burst phase). The lag-phase should reflect the duration of the adsorption and penetration of CA at the SDS-laden LC/aqueous interface. The burst phase is a measure of the displacement of SDS from the LC/aqueous interface by CA. The slope of the plot of the burst phase represents the displacement rate of SDS from the LC/aqueous interface. It is clear in Fig. 4a that the displacement rate increases with the increase of CA concentrations in the PBS aqueous phase. The lag time decreases from 10 to 4 h when the concentration of CA increases from 4 to 8 μM (Fig. 4b). The increase of CA concentrations in the PBS aqueous phase should increase the availability of CA at the SDS-laden LC/aqueous interface, shorting the lag time and enhancing the displacement rate. The displaced SDS from the LC/aqueous interface may be solubilized by the CA remained in the aqueous phase through emulsification. The critical concentration of CA required to displacing the SDS from the LC/aqueous interface is ~2 μM. There is no the homeotropic-to-planar/tilted anchoring transition of the LC at the interface observed for prolonged time periods (over 40 h) if the concentration of CA is lower than ~2 μM. The sensitivity of this LC-based platform to the concentration of bile acids is slightly higher than that of surface tension and zeta potential measurements (18 μM) [6]. We should also point out that the surfactant used in our experiments is different from that used in the surface tension and zeta potential measurements. In addition, the LC based sensing platform is simple without the need for expensive instruments.

The chemical structure of bile acids also has an import on the lag-burst kinetics of the SDS displacement from the LC/aqueous interface. Fig. 5a shows the normalized transmission of the LC film confined in a square pore of the grid as a function of time after addition of 4 μM CA, 4 μM CDCA, 4 μM DCA, and 4 μM LCA, respectively. The order of the displacement rate (the slope of the plot of the burst phase) of the SDS from the LC/aqueous interface is

LCA > CDCA > DCA > CA. The lag time is ~10 h for CA, ~45 min for DCA, ~25 min for CDCA, and ~5 min for LCA, respectively (Fig. 5b).

The order of hydrophobicity is LCA > DCA > CDCA > CA [22]. It is reasonable for us to expect that more hydrophobic bile acids should be more effective in displacing SDS from the interface. However, we note in Fig. 5a that there is an exchange in the order of the displacement rate between DCA and CDCA with respect to their hydrophobicity. The pK_a is 6.1 for DCA and 6.3 for CDCA, respectively [32]. In the PBS aqueous phase with pH 7.4, both DCA and CDCA are negatively charged. The electrostatic interaction should not be a factor, which causes the exchange of the order of displacement rate between DCA and CDCA. As can be seen in Fig. 1 that CDCA has two hydroxyl groups at C-3 and C-7 positions and DCA has two hydroxyl groups at C-3 and C-12 positions. We infer that the exchange between the C-12 and the C-7 position of the hydroxyl groups between DCA and CDCA may overshadow their hydrophobicity in determining their activities in the displacement of SDS from the LC/aqueous interface. The interaction of SDS with CA, DCA and CDCA was studied by measuring the enthalpy change of the formation of mixed micelles [33]. The enthalpy change was found to be in the same order as the rate of the SDS displacement from the LC/aqueous interface, i.e., CDCA > DCA > CA. Thus, we conclude that CDCA with two hydroxyl groups at C-3 and C-7 positions are more favorable for interacting with SDS, compared to DCA with two hydroxyl groups at C-3 and C-12 positions. The energetic interaction makes CDCA more efficient in displacing the SDS from the LC/aqueous interface.

In summary, we have studied the time course of the displacement of SDS from the LC/aqueous interface by the competitive adsorption of bile acids through the observation of anchoring transition of the LC at the interface. The displacement of SDS from the LC-aqueous interface exhibits lag-burst kinetics. The lag time and the burst rate are found to depend on the number and position of the hydroxyl groups of bile acids.

Acknowledgments

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