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POLYELECTROLYTE AND HYDROGEL STABILIZED LIQUID CRYSTAL DROPLETS FOR THE DETECTION OF BILE ACIDS

by

JINAN DENG

B.S. Beihang University, 2010

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Material Science and Engineering in the College of Engineering and Computer Science at the University of Central Florida Orlando, Florida

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Major Professor: Jiyu Fang
ABSTRACT

Liquid crystal (LC) droplets show great potential as an optical probe for sensor applications due to their large surface areas and stimuli-response director configurations. Bile acids with amphipathic properties, which are formed in liver and secreted into the small intestine, play an important role in the digestion of fats and fat-soluble vitamins. After the digestion process, most of bile acids are recycled back to the liver and ready for the next digestion. Only a few of them are excreted into body fluids. However, there is significant increases in the concentration level of bile acids in body fluids for patients with liver and intestinal diseases, which makes bile acids a biomarker for the early diagnosis of liver and intestinal diseases. Chromatography-mass spectrometry and electrochemical sensors are common methods for the detection of bile acids. However, these detection methods are time consuming, require relatively large sample volumes, and expensive instruments. To date, there is still a demand in the development of simple, low-cost and user-friendly sensing platforms for the rapid detection of bile acids in clinical settings.

In this dissertation, two simple and low-cost LC droplet-based sensing platforms were developed for the rapid and real-time detection of bile acids with a small sample volume. First, a miniaturized LC droplet-based sensor platform was designed and fabricated by the integration of polyelectrolytes/surfactant/sulfate β-cyclodextrin (β-CD) complex-stabilized LC droplets into a microfluidic channel for the selective detection of bile acids in a small amount of solution, in which the β-CD immobilized at the surface of the LC droplets acts as a selective barricade and the director configuration of the LC droplets serves as an optical probe. Second, a flexible LC droplet-based sensor platform was formed by the integration of surfactant-stabilized LC droplets in biopolymer
hydrogel films. The LC droplet-based hydrogel film was cut into small sheets for the real-time detection of bile acids in a small amount of solution, in which the configuration transition of LC droplets induced by the interaction of bile acids with the surfactants absorbing on the surface of LC droplets serves as an optical probe.

Cholic acid (CA) and deoxycholic acid (DCA), which are the most related to the liver and intestinal diseases, were detected in phosphate buffered saline (PBS) solution in the presence of the interference species of uric acid (UA) and ascorbic acid (AA) in this dissertation. These miniaturized LC droplet-based sensor platforms can be used to selectively detect CA and DCA in the presence of UA and AA. The detection limit of these sensor platforms for CA and DCA can be tuned by the number of LC droplets and the nature of surfactants. Furthermore, we find that these sensor platforms are more sensitive for DCA with the shorter response time and lower detection limit over CA due to their difference in hydrophobicity.

These miniaturized 5CB droplet-based sensor platforms are easily handled, allowing the rapid and real-time detection of bile acids in a small sample volume in the presence of interference species, which are highly desirable for the "point-of-care" analysis of bile acids.
This thesis is dedicated to my beloved parents, who have given me endless love, support and encouragement throughout the years.
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CHAPTER 1 INTRODUCTION

1.1 Motivation

Liquid crystal (LC) droplets show great potential as an optical probe for sensor applications due to their large surface areas and stimuli-responsive director configurations. Bile acids with amphipathic properties, which are formed in liver and secreted into the small intestine, play an important role in the digestion of fats and fat-soluble vitamins. After the digestion process, most of bile acids are recycled back to the liver and ready for the next digestion. Only a few of them are excreted into body fluids. However, there is significant increases in the concentration level of bile acids in body fluids for patients with liver and intestinal diseases, which makes bile acids a biomarker for the early diagnosis of liver and intestinal diseases. Chromatography-mass spectrometry and electrochemical sensors are common methods for the detection of bile acids. However, these detection methods are time consuming, require relatively large sample volumes, and expensive instruments. To date, there is still a demand in the development of simple, low-cost and user-friendly sensing platforms for the rapid detection of bile acids in clinical settings.

This dissertation focuses on development of simple liquid crystal droplet-based sensor platforms for the rapid and real-time detection of bile acids with a small sample volume.

1.2 Chapter Outline

In Chapter 2, liquid crystal materials and the applications of liquid crystal materials, especially in sensor applications were introduced. The structures of bile acids and their functions in the digestion process and their relations with liver and intestinal diseases were also introduced.
In Chapter 3, tetradecyl trimethylammonium bromide (C\textsubscript{14}TAB)/β-cyclodextrin (β-CD) host-guest complexes were first presented to functionalize the surface of 4-Cyano-4′-pentylbiphenyl (5CB) liquid crystal droplets as a selective barricade for the detection of a bile acid, cholic acid (CA) in the aqueous solution with pH of 7.4. The detection mechanism of competitive displacement of C\textsubscript{14}TAB from the cavity of β-CD by CA was studied in this chapter.

In order to achieve long-term stability of cyclodextrin based complex-functionalized LC droplets, in Chapter 4, a positively charged polyelectrolyte, Poly (diallyldimethyl ammoniumchloride) (PDADMAC), was employed to stabilize the 5CB droplets by coating the surface of tetradecyl sulfate sodium salt (SC\textsubscript{14}S)/sulfated β-CD complex-modified 5CB droplets through electrostatic interactions. With this PDADMAC/ SC\textsubscript{14}S/sulfated β-CD complex-stabilized 5CB droplets, a miniaturized 5CB droplet-based sensing platform was fabricated by integration of the droplets into a microfluidic channel for the real-time detection of bile acids in phosphate buffered saline (PBS) solution. The concentration dependent response time and droplet density dependent detection limit for the bile acids were investigated.

In Chapter 5, 5CB liquid crystal droplet-embedded hydrogel films were developed for the real-time detection applications. Chitosan (CS) and agarose were chosen as the hydrogel network to stabilize liquid crystal droplets in the hydrogel network. Surfactants with different headgroups and chain length were adopted to functionalize the 5CB droplets in the hydrogel network. The study of interactions between hydrogel networks and surfactants can provide a guide for the design of 5CB droplet-based hydrogel platforms for the detection of bile acids.

In Chapter 6, chitosan was employed to prepare surfactant/5CB droplet-embedded chitosan hydrogel films for the real-time detection of bile acids in the aqueous solution with pH of 7.4. The
configuration transition process of liquid crystal droplets after the addition of bile acids was observed under the polarizing optical microscope in real-time. The response time and detection limit for CA and DCA were discussed. The effects of headgroup and alkyl chain length of surfactants on the detection limit of CA and DCA were studied. The detection limit for DCA and CA in aqueous solution with C_{12}TAB/5CB droplet-embedded CS hydrogel films can be lowered to 0.6μM, and 3μM, respectively.

In Chapter 7, agarose hydrogels were adopted to prepare surfactant/5CB droplet-embedded agarose hydrogel films for the real-time detection of bile acids in PBS solution. The detection limits for DCA and CA are 8 μM and 40 μM, respectively, which are higher than those with liquid crystal-based chitosan hydrogel platforms. However, the detection limits for DCA and CA are still in the acceptable ranges of bile acids for the early diagnosis of liver and intestinal diseases.

Finally, Chapter 8 summarizes this dissertation.
CHAPTER 2 BACKGROUND

2.1 Liquid Crystals

Liquid crystals (LCs) are materials in a state between liquid and solid crystalline. They not only can flow like liquid, but also show some extent of anisotropic feature like crystalline. This unique phase of materials was first identified by Friedrich Reinitzer and Otto Lehman between 1888 and 1889 by studying a cholesterol derivative under a polarizing optical microscope. The properties of liquid crystals were fundamentally understood until 1950s. From then on, liquid crystal science grew rapidly and more and more researchers started to explore the theories and applications of liquid crystals in various fields.

2.1.1 Parameters to Describe Liquid Crystals

The unique state endows LCs with an anisotropic nature, which shows some extent of orientational order and positional order. The positional order describes the extent of translational symmetry showed by a group of molecules. The orientational order describes the extent of alignment of molecules along one direction, which referred as the director of liquid crystals. An order parameter (S) is defined to measure the degree of the order, which gives as follows,

\[ S = \langle 3\cos^2\theta - 1 \rangle /2 \tag{1} \]

Where \( \theta \) is the angle between the long axis of a molecule and the director (Figure 2), the bracket indicates the average value. For a perfect crystal, S=1, whereas, for a completely isotropic state, S=0. The typical value of S for the liquid crystal is between 0.3 to 0.8.\(^1\)
Figure 1. Angle deviation of the long axis of molecule from the director $\hat{n}$

The partially ordered molecules of liquid crystals show a birefringence optical property under the polarizing optical microscope. There are two different indices of refraction for birefringent LC materials. When a light enters the anisotropic LCs, it splits into two components, fast or ordinary ray (o-ray) ray and slow or extra-ordinary ray (e-ray), with different velocities propagating through the LCs. When those two components recombine after going through the LCs, there exists phase difference that has changed the polarization state. The difference between the refractive indexes of the two rays, $\Delta n$, is employed to describe the birefringence of a material. It can be expressed as,

$$\Delta n = n_0 - n_e$$  \hspace{1cm} (2)

Where $n_o$ is the refractive index for o-ray, and $n_e$ is the refractive index for e-ray. Due to the birefringence of LCs, the order change-induced birefringence change within LCs can be characterized under the polarizing optical microscope.

The large anisotropy of electron polarizability of liquid crystal molecules dictates the high birefringence of LCs, which is associated with the long conjugated $\pi$ electron system of bonds.\textsuperscript{2} Thus the aromatic rings in liquid crystal molecules can improve the birefringence of LCs.
2.1.2 Categories of Liquid Crystals

Generally, LC materials are classified into lyotropic LCs and thermotropic LCs, respectively. Lyotropic LCs are concentration dependent. The phase transition of lyotropic LCs is a function of the concentration of LCs in solvent. This kind of materials is common in biological systems, like bio-membranes. Thermotropic LCs are temperature dependent. The phase transition of thermotropic LCs are driven by the thermal process. Thermotropic LC molecules with benzene rings present higher birefringent than lyotropic LCs, which makes thermotropic LCs widely used in various fields.

Based on the different types of ordering in LCs, thermotropic LCs can be divided into four phases, smectic, nematic, cholesteric phases and discotic, respectively. (Figure 2).

Smectic phases (Figure 2a) are a layered structure in which rod-like molecules parallel to each other along the long axes in each layer. Based on the direction of the long axis, the smectic phase can be further divided into three sub-phases, smectic A, smectic C and smectic C* phases. When the long axis in each layer is perpendicular to the plane of the layer, this kind of smectic phase is called smectic A phase. When the long axis of the molecules in each layer forms a tilt angle with respect to the layer plane, we call it smectic C phase. Like the smectic C phase, the smectic C* phase is formed by chiral molecules with a tilt angle to the layer plane. The order parameter of the smectic phase is near to 1.

Nematic liquid crystals (Figure 2b) are made up of rod-like molecules, in which molecules align only along the average direction of the long axes of molecules.

The structure of cholesteric liquid crystals (Figure 2c) is similar to nematic liquid crystals. But it shows chirality. Therefore, the cholesteric phase is usually called chiral nematic phase. The
alignment of molecules in each layer of cholesteric phases is similar to that of molecules in nematic phases. However, the directors of each layer form a helical twist, which leads the chirality of the cholesteric liquid crystals.

Discotic phases (Figure 2d) are made up of disk-like molecules. These disk-like molecules are stacked into columns. The director is perpendicular to the plan of molecules.

Figure 2. Different phases of thermotropic liquid crystal, (a) smectic; (b) nematic; (c) cholesteric; and (d) discotic

Among those phases, nematic LCs possessing only an orientational order but no positional order is the least ordered phase, which makes the ordering of molecules in nematic LCs easier to be disturbed by external perturbations than other phases of LCs. Therefore, nematic liquid crystals are the most widely studied LCs for sensing applications. In this thesis, only nematic liquid crystal materials are discussed.

2.1.3 Free Energy Within Nematic Liquid Crystals

The director of LCs is determined by the free energy of LCs, which can be grouped into four parts, homogeneous, external field, elastic, and surface interaction free energy, which can be expressed as follows,
\[ F = F_{\text{Homogeneous}} + F_{\text{Field}} + F_{\text{Elastic}} + F_{\text{Surface}} \quad (3) \]

Where \( F_{\text{Homogeneous}} \) is the internal free energy of undistorted LCs, which is a function of order parameter \( S \), \( F_{\text{Field}} \) is the free energy imposed by external fields, such as electric and magnetic fields, \( F_{\text{Elastic}} \) is the bulk elastic free energy derived from the distortion of LC, and \( F_{\text{Surface}} \) is the interfacial free energy.

\( F_{\text{Homogeneous}} \) is usually expressed as an expansion in the order parameter \( S \),\(^4\) which is given by

\[ F_{\text{Homogeneous}} = f_0 + \frac{1}{2}a(T - T^*)S - \frac{1}{3}bS^3 + \frac{1}{4}cS^4 \quad (4) \]

Where the terms \( a \), \( b \), and \( c \) are positive and temperature independent; the temperature term \( T^* \) is the supercooling limit of the bulk isotropic phase. These terms can be considered as constants and neglected when the temperature is far from the phase transition temperature of LCs.\(^4\) The free energy related to the external fields such as electric and magnetic fields can be described as

\[ F_{\text{Field}} = -\frac{1}{2}\varepsilon_0\Delta\varepsilon[\vec{E} \cdot \vec{n}]^2 - \frac{1}{2}\mu_0[\vec{B} \cdot \vec{n}]^2 \quad (5) \]

Where \( \varepsilon_0 \) and \( \vec{E} \) are the free space permittivity and field vector for the electric field, \( \Delta\varepsilon \) is the dielectric anisotropy; \( \mu_0 \) and \( \vec{B} \) are the free space permittivity for the magnetic field and the magnetic field vector, respectively; \( \Delta\chi \) is the diamagnetic anisotropy. Due to the long-range order within LCs, the free energy required to induce curvature in the director field can be expressed by three deformations, splay, twist, and bend, respectively, which can be written by

\[ F_{\text{Elastic}} = \frac{1}{2}K_{11}(\vec{V} \cdot \vec{n})^2 + \frac{1}{2}K_{22}(\vec{n} \cdot \nabla \times \vec{n})^2 + \frac{1}{2}K_{33}(\vec{n} \times \nabla \times \vec{n})^2 \quad (6) \]
Where $K_{11}$, $K_{22}$, and $K_{33}$ are splay, twist, and bend elastic constants, respectively. The typical value of $K_{ii}$ for nematic liquid crystal molecules is $\sim 10^{-11}$ N.\(^3\) The interactions of LCs at the interface are usually studied in terms of surface anchoring,\(^4\) namely,

$$F_{\text{surface}} = -g + \frac{1}{2} W_{\theta} \sin^2 (\theta - \theta_0) + \frac{1}{2} W_{\phi} \sin^2 (\phi - \phi_0)$$  \hspace{1cm} (7)

Where $\theta_0$ and $\phi_0$ refer to the preferred polar (out of plane) and azimuthal (in-plane) angle of the director field at the surface; the differences $(\theta - \theta_0)$ and $(\phi - \phi_0)$ show the deviations of the real director field from the preferred angles; $g$ represents the non-directional interactions of LCs at the interface; $W_{\theta}$ and $W_{\phi}$ are the polar and azimuthal anchoring energies, respectively.

The magnitude of the surface free energy aroused by the orientation of LC molecules is comparable to the bulk elastic energies for micrometer-sized LC materials.\(^3,\ 5\) Small perturbations at the liquid crystal interface will alter the surface anchoring of LCs, which will induce the director configuration change of molecules within LCs. This director configuration transitions associated with the surface effects provide LCs a potential for various applications.

Due to the ultra-sensitive and anisotropic properties of LCs, more and more studies are exploring the potential applications of LCs in various areas.

### 2.2 Liquid Crystals for Display Applications

The most widely and mature application of liquid crystals is the liquid crystal display (LCD), which has brought a significant revolution in the field of information displays.
2.2.1 Unpolarized Light Going Through Polarizers

Light is a transverse electromagnetic wave, with its vibrating direction perpendicular to its propagating direction. The vibrating and propagating of a transverse wave form a plane.

An unpolarized light radiates in all directions from the light source. When an unpolarized light goes through a polarizer, only waves fluctuating in one specific planes parallel to the polarizer will transmit through the polarizer, becoming a polarized light. This polarized light will be completely blocked when it tends to further pass through another polarizer which is perpendicular to the first one (Figure 3).

![Diagram of unpolarized light going through polarizers](image)

**Figure 3. Unpolarized light going through crossed polarizers**

2.2.2 Liquid Crystal Displays

With the invention of the twisted nematic (TN) mode by Wolfgang Helfrich and Martine Schadt in 1970 and the full description of the free energy of liquid crystals with the order parameter by Pierre-Gilles de Gennes in 1971, LCD technology has entered into a continuous development.

In a typical TN cell (Figure 4), nematic liquid crystals are confined between two glass electrodes. There are two crossed polarizers on the top and bottom of the cell, respectively. The
surfaces of the glass electrodes contacting with LCs are coated with a layer of polymers which have been rubbed in one direction to introduce a specific orientation of LCs contacting with the electrode surfaces. The director orientation of LCs on one substrate is perpendicular to that of LCs on the other substrate. The director orientations of LCs between the two substrates are controlled by the applied electric field. When there is no applied electric field, the LCs between the two substrates are twisted and can guide and rotate the light to pass through the crossed polarizers. When the applied electric field is on, LCs inside the cell will start to align along the electric filed. When the voltage applied is high enough, the twist of LCs will disappear and will orientate along the electric field. The LCs no longer guide the light through crossed polarizers. No light will pass through the cell. By controlling the voltage applied to the cell, the orientations of LCs within the cell can be finely adjusted, and thus the light going through the cell can be tuned.

Figure 4. Structure of a typical TN cell

For a LCD, each pixel contains three sub-pixels with three color filters, red, blue, and green, respectively. By switching on or off the colored sub-pixels in each pixel very quickly, the LCD can show a lively moving color picture.
Although various designs and improvements have been applied to LCD since its first application in the display field, the basic working mechanism is almost the same.

2.3 Liquid Crystals for Chemical and Biological Sensing Applications

Due to the birefringence and high sensitivity of LCs to tiny surface perturbations or external fields, researchers are seeking new potential of LCs in chemical and biological sensing applications.

LCs as optical sensors are label-free and can amplify the molecular events into the macroscopic level, which makes them well-suited for the simple and rapid detection of analytes and binding events. Furthermore, considering the convenient transportation of analytes in the aqueous phase and the fluidity of the interface, LC/aqueous interfaces are particular interesting for the investigation of biological events. Liquid crystal-based platforms for sensor applications mainly rely on the study of the interactions between liquid crystals and analytes at the LC/aqueous interface at which analytes are applied. The orientational ordering-related surface energy of LCs is on the order of $10^{-2}$-$10^{-3}$ mJ/m$^2$, which endows the LC ordering near the interface extremely sensitive to the perturbations at the interface. In addition, this surface-induced ordering change of LCs can extend up to ~100μm from the interface, which can lead to long-range ordering transition within LCs. Therefore, the interfacial interactions can be easily amplified and transduced by LCs with the polarizing optical microscope.

There are mainly two forms of LCs for sensor applications, LC films and LC emulsions, respectively.
2.3.1 Liquid Crystal Films for Sensing Applications

Various biological systems, such as biological membranes, phospholipids, cholesterols, DNA and the like, exhibit liquid crystalline phases\textsuperscript{11-12} which make LCs advantageous to mimic the biological membranes, at which biomolecular interactions, specific bindings, and enzymatic activities of proteins or molecules usually occur\textsuperscript{13}. In order to mimic biological membranes to investigate the interactions of molecules at the interface, LC planar films are usually adopted as the sensing platform.

2.3.1.1 Preparation of LC Films

![Figure 5](image.png)

**Figure 5.** (a) Schematic illustration of preparation of LC planar film; (b) side view of LC molecules in a single pore of TEM grid supported on the substrate

Figure 5a shows a typical approach to prepare a LC planar film for sensing applications. Liquid crystals are first loaded into the pores of a transmission electron microscopy (TEM) grid by the capillary force. This grid provides a mechanical support for LCs and keeps the LC film stable\textsuperscript{14}. The TEM grid is around 20μm thick with the pore size of around 280μm. Then this LC-loaded TEM grid is put onto a pretreated glass substrate. Octadecyltrichlorosilane(OTS)\textsuperscript{15} or polyimide\textsuperscript{16} are usually adopted to treat the surface of the substrate to induce a homeotropic
anchoring of LCs near the substrate. At last, the LC film supported on the pretreated substrate is immersed into an aqueous solution to form a flat LC/aqueous interface. Figure 5b shows the director of LC molecules in a single pore of the TEM grid. The pretreated substrate induces a homeotropic anchoring of LCs near the substrate, in which the director of LCs is perpendicular to the substrate. However, the LCs on the top tend to align parallel to the interface, which indicates a planar anchoring at the interface.

2.3.1.2 Adsorption of Surfactants at the LC/Aqueous Interface

Researchers found some surfactants\textsuperscript{15, 17} can trigger a perpendicular alignment of LCs at the LC/aqueous interface. Figure 6 shows the optical appearance change of 4-cyano-4'-pentylbiphenyl (5CB) LC film in aqueous solution before and after the addition of sodium dodecyl sulfate (SDS) under a polarizing optical microscope. Without the absorbed SDS, the LC film exhibits a bright appearance under a polarizing optical microscope (Figure 6a), indicating a planar alignment of LCs at the LC/aqueous interface. After the addition of SDS, the LC film starts to show dark domains (Figure 6b), and finally exhibits a completely dark texture after the concentration of SDS added is higher than 1.8mM (Figure 6c), suggesting a homeotropic anchoring of LCs at the LC/aqueous interface.\textsuperscript{16} The mechanism of introducing the surface anchoring change of LCs at the LC/aqueous interface by surfactants is thought to involve the adsorption and penetration of the hydrophobic tails of surfactants into the LC phase and the interactions between the surfactant tails and LCs.\textsuperscript{3, 17-18}
Figure 6. Polarizing optical microscopy images and schematic illustrations of LC films before (a), and after the addition of 1.4mM (b) and 1.8mM (c) of SDS. Scale bar, 97μm

A number of investigations provide insights into the mechanisms of ordering change of LCs near the LC/aqueous interface by studying the interactions of between surfactants and LCs at the LC/aqueous interface. Brake and coworkers studied the effects of the surfactant structure on the ordering of LCs at the LC/aqueous interface. They found the tail configuration and aliphatic chain length of surfactants are important in dictating the homeotropic anchoring of LCs near the LC/aqueous interface. Surfactants adopting a looped conformation at the LC/aqueous interface cannot introduce a homeotropic anchoring of LCs. Surfactants with longer tails are more effective to trigger a homeotropic anchoring of LCs at the interface. Longer aliphatic tail can penetrate deeper into the LC phase, thus can better interact with LC molecules. Lockwood and coworkers further confirmed that it is the ordering of the surfactant tails that plays a role in the perpendicular alignment of LCs at the interface. They used surfactants with branched and linear tails to interact with LCs at the LC/aqueous interface and found surfactants with linear aliphatic tails can induce a homeotropic anchoring of LCs at the interface, which is consistent with the previous results. In contrast, surfactants with branched aliphatic tails cannot trigger a planar-to-homeotropic transition of LCs at all concentrations. They attributed this to the poor packing of
branched tails at the LC/aqueous interface, where the branched aliphatic tails extending into the LC phase tend to disrupt the packing of LC molecules rather than to align them. Fletcher and coworkers compared the anchoring change of LCs at the LC/aqueous interface induced by polymerizations of surfactants happened at the hydrophobic tail and the hydrophilic headgroup, respectively.\textsuperscript{17} They found the homeotropic-to-planar transition of LCs only occurred when the polymerization happened at the hydrophobic tail region. The polymerization of hydrophobic tails has disrupted the packing of surfactants, thus induced a homeotropic-to-planar anchoring transition of LCs. They found the hydrophilic headgroups of surfactants did not affect the anchoring transition of LCs at the interface, which is consistent with other studies\textsuperscript{18-19}. Different headgroups of surfactants dictate the critical concentrations of surfactants required to trigger the planar-to-homeotropic transition of LCs at LC/aqueous interface. The different electrostatic interactions of different headgroups affect the packing density of surfactants at the LC/aqueous interface.\textsuperscript{9,19}

The mechanisms of the surface anchoring change of LCs triggered by surfactants can be applied to other molecules with similar structures.

\textit{2.3.1.3 Study of Biological Activities with LC Films}

With the ultra-high sensitivity to a subtle environmental disturbance, LCs show great potential to be used as a sensing platform to study the variations happened at the LC/aqueous interface.

Biological membranes are important places for most biomolecular events, such as enzymatic process, membrane transportation, cell signaling, receptor interactions and the assemble of proteins and lipids.\textsuperscript{21-22} The biological membrane is a bilayer structure formed by phospholipids in which the hydrophobic tails of lipids in each layer toward the core and the hydrophilic headgroups toward outside. The membranes are flexible permitting the shape changes of cells
during cell growth. Proteins and lipids in membranes are free to move laterally within the bilayer. A lot of studies used self-assembled monolayers of phospholipids at the air/water or oil/water interfaces to mimic the biological membranes to investigate the biomolecular events that occur at the membrane surfaces.\textsuperscript{23}

The lipid with two hydrophobic tails and a hydrophilic headgroup has a similar structure as surfactants. Given the mechanisms of interactions between surfactants and LCs understood previously, combined with the highly fluidic nature of the LC/aqueous interface and biological related mechanical properties of LCs, LC films show great potential to mimic biological membranes.\textsuperscript{10}

Different from surfactants, lipids are not soluble in water, which makes the delivery of lipids to the LC/aqueous interface complex. Brake and coworkers dispersed phospholipids into aqueous solution in the form of small unilamellar vesicles and let these vesicles contact with the LC film.\textsuperscript{13} The fusion of the vesicles with the LC/aqueous interface led to the planar-to-homeotropic anchoring transition of 5CB near the interface and confirmed that a monolayer of phospholipids was formed after the adsorption of the phospholipids at the LC/aqueous interface. They also prepared a mixture of surfactants and phospholipids to transfer phospholipids to the LC/aqueous interface and successfully adjusted the areal density of lipids adsorbed at the interface by controlling the ratio of the surfactant to the phospholipid.\textsuperscript{13} They used fluorescence labeled phospholipids and a photobleaching method to confirm the lateral mobility of phospholipids at the LC/aqueous interface and measured the lateral diffusivity of phospholipids at the LC/aqueous interface as a function of the surface coverage and composition of lipids.\textsuperscript{13}
Enzymes play an important role in metabolic process of living matters. Study the enzymatic activities will help us get insight into various metabolic processes. The formation of phospholipid monolayers at LC/aqueous interface and the lateral mobility of phospholipids at the interface provides a simple way to mimic the biological membranes, where many important enzymatic events happens. Researchers have tried to use this lipid-laden LC film to study the enzymatic activities.

Brake and coworkers used the phospholipid, L-DLPC-laden 5CB film to transduce the enzymatic activity of phospholipase A2 (PLA2). The adsorption of L-DLPC at the LC/aqueous interface induced a dark texture of the LC film under a polarizing light microscope, indicating a homeotropic anchoring of 5CB near the interface (Figure 7a, d). The enzyme, phospholipase PLA2, can selectively hydrolyze the sn-2 ester bond of the glycerol part of L-DLPC in the presence of Ca^{2+}. After the addition PLA2 and Ca^{2+}, the hydrolysis products of L-DLPC cannot firmly adsorb at the LC/aqueous interface and would desorb from the interface (Figure 7e). The 5CB film started to show a bright texture under the polarizing optical microscope due to the desorption of hydrolysis products (Figure 7b-c). Thus, the enzymatic event was transduced and amplified through LC films.

Other enzymatic activities were also reported with LC films. Park and coworkers prepared a mixture of lipids-laden LC film to study the enzymatic activity of protease with oligopeptides. The lipids-laden LC film showed a dark texture under the polarizing optical microscope. The conjugation of oligopeptides to the carboxylic acid terminated-lipid adsorbed at the LC/aqueous interface disrupted the packing of the monolayer of lipids at the LC/aqueous interface and induced a planar anchoring of LCs near the interface, which showed a bright texture under the polarizing optical microscope. After the introduction of a protease, trypsin, which can
selectively cleave the oligopeptide, the texture of the LC film returned to dark, indicating a homeotropic anchoring of LCs near the interface. By monitoring the planar-to-homeotropic anchoring transition of LCs near the LC/aqueous interface, enzymatic activities can be easily monitored in real-time.

**Figure 7.** Polarizing optical microscopy images of enzymatic activity at the 5CB/aqueous interface before (a) and after (b-c) the addition of enzyme PLA2. Schematic illustration of the anchoring transition of 5CB before (a) and after (b) the addition of PLA2 and Ca$^{2+}$. (a-c) Adapted with permission from Ref. [21]. Copyright 2003 American Association for the Advancement of Science.

Besides the enzymatic reactions, the characterizations of protein-protein and ligand-receptor interactions are widely studied for the disease screening. General techniques to study those interactions are either time consuming or high cost. The sensitive nature and fluidity of LCs encouraged researchers to investigate the potential of LCs as a transducer for the study of specific ligand-receptor interactions. Hartono and coworkers employed LC films to study the protein-protein binding through modification the surface of LC films in real-time. They immobilized histidine-tagged ubiquitin at the surface of 1,2-dioleoyl-sn-glycero-3-[N(5-amino-1-carboxypentyl) iminodiacetic acid]-succinyl (DOGS-NTA-Ni)-laden 5CB films through the
coordinate bonding formed between Ni$^{2+}$ and the histidine residue. The concentration of histidine-tagged ubiquitin was kept lower than the critical concentration to keep the homeotropic anchoring of 5CB near the interface. After being exposed to 50ng anti-ubiquitin antibody, the texture of the ubiquitin-laden LC film transformed from black to bright. Thus, the LC film provides a simple and label-free way to study the protein-protein binding interaction by immobilization of proteins at the surface of LC films.

DNA hybridizations were studied with surfactant-laden LC films by Schwartz and coworkers. The adsorption of single-stranded DNA (ssDNA) at the interface of a surfactant-laden LC film induced a tilt anchoring of LCs near the interface, showing a bright texture under the polarizing optical microscope. Exposure of this ssDNA/surfactant-laden LC film to a very small amount of ssDNA complement (as low as 50 fmol) triggered a homeotropic alignment of LCs near the interface through the DNA hybridization. By observing the bright-to-black texture change of LC film under the polarizing optical microscope, the DNA hybridization process can be easily detected.

Besides by modification of the LC/aqueous interface with specific molecules or acceptors, researchers usually prepared LC sensing platforms by doping the LC phase. The doped molecules can adopt different conformations or status to minute environmental variation, leading to the re-orientation of LCs. This doped LC films are also adopted as a transducer and amplifier of biological events or interactions at the LC/aqueous interface by monitoring the re-orientation of LCs-induced texture change of LC films.

Bi and coworkers developed a 5CB LC film doped with 4-(pentyl-biphenyl-4-carboxylic acid (PBA), which is ultra-high sensitive to the local pH change. When the pH increased from
6.9 to 7.0, the 5CB doped PBA LC film showed a bright-to-black texture change (Figure 8). The mechanism of such a small pH change induced a bright-to-black transition is still not unclear. The authors believed that the pH sensitive headgroup and the hydrophobic tails of PBA play an important role dictating the orientations of 5CB. The authors immobilized penicillinase at the surface of a 5CB doped PBA film to monitor the enzymatic reaction of penicillinase and penicillin G, which can release H\(^+\) through hydrolysis of penicillin G by penicillinase.\(^{29}\) The temporal and local pH change by the released H\(^+\) from penicillinase enzymatic reaction in PBS buffer solution was successfully detected and triggered the texture change of LC films. This pH sensitive LC films provide a way to indirectly monitor the enzymatic activities through the detection of the pH change induced by the enzymatic reaction.

![Figure 8. Polarizing optical microscopy images and schematic illustrations of 5CB doped PBA LC film to pH change. Adapted from Ref. [29]. Copyright 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.](image)

A similar platform has been adopted by Jang and coworkers to detect the enzymatic activity of urease.\(^{30}\) The PBA doped 5CB film shows a planar anchoring in aqueous solution (Figure 9a). The ammonia generated from the hydrolysis of urea by urease increased the pH of the solution, which further facilitated the deprotonation of PBA. The deprotonated PAB assembled at
LC/aqueous interface and triggered a planar-to-homeotropic anchoring transition of 5CB (Figure 9b).

Figure 9. Polarizing optical microscopy images and schematic illustrations before (a) and after (a) the addition of urease and urea. Adapted from Ref. [30]. Copyright 2011 KCS Publications.

### 2.3.1.4 Other Detection Applications with LC Films

LC anchoring is so sensitive to environmental variations. A subtle change at the LC/aqueous interface will trigger the anchoring transitions of LCs near the interface, which provide a way for the LC film as the sensing platform for various detection applications. A lot of studies have adopted the LC platforms to detect pH change, glucose, proteins, heavy metal ions, and bile acids.

Kinsinger and coworkers synthesized an amphiphilic polymer to decorate the LC/aqueous interface to reversibly responded to pH change. The tertiary amine groups on the backbone of the polymer can reversibly undergo a protonated/deprotonated process in different pH solution. The pH dependent charge and conformation change of polymer at the LC/aqueous interface induced an ordering change of LCs. By observing the optical appearance change of LCs, they can monitor the pH change. However, this LC platform can only show optical appearance difference at pH 9 and pH 5.
This pH sensitive molecule doped LC films provide a way to design LC platforms to indirectly detect analytes by sensing the localized pH change before and after the reactions. The oxidation of glucose by glucose oxidase (GOx) decreases the local pH through producing gluconic acid, which enables researchers to use pH sensitive LC platforms to detect glucose.\textsuperscript{33-35}

Khan and coworkers used poly(acrylicacid-b-4-cynobiphenyl-4-oxyundecylacrylate) (PAA-b-LCP), a pH sensitive amphiphilic polymer, to dope 5CB LC films to detect glucose.\textsuperscript{33} They immobilized GOx at PAA-b-LCP-laden 5CB/LC interface by conjunction with PAA-b-LCP. By adjusting the salt concentration to control the protonation and deprotonation of PAA-b-LCP, the LC film kept an initial homeotropic anchoring at pH 7. After the addition of glucose, the H\textsuperscript{+} produced from the oxidization of glucose by GOx immobilized at the LC/aqueous interface protonated PAA chains, triggering a homeotropic-to-planar anchoring transition of LC film. By observing the optical appearance change of LC films, the glucose concentration can be detected. They further increased the sensitivity of the above LC platforms by covalently immobilizing both GOx and horseradish peroxidase (HRP) on the LC/aqueous interface.\textsuperscript{34} The H\textsubscript{2}O\textsubscript{2} produced from the oxidation of glucose by GOx was further continuously reduced by HRP\textsuperscript{3+} to produce OH\textsuperscript{-} and consume H\textsuperscript{+} at the same time. The produced OH\textsuperscript{-} facilitated the deprotonation of PAA and triggered the planar-to-homeotropic anchoring change of LC films. The concentration of glucose as low as 0.02mM can be detected with this platform.

Zhong and coworkers exposed 5CB to ultraviolet (UV) to produce 4-cyano-4\textsuperscript{′}-biphenylcarboxylic acid (CBA), which is also pH sensitive.\textsuperscript{35} The protonation and deprotonation of CBA are controlled by the pH. A higher pH increases the density of amphiphilic CB, while a lower pH increases the density of hydrophobic CBA. Based on this, they immobilized GOx on the
TEM grid confining UV-treated 5CB to prepare a pH sensitive LC film to detect glucose. The H\(^+\) released due to the oxidation of glucose facilitated the production of hydrophobic CBA, which triggered the initial black texture of LC films to bright. With this platform, the glucose as low as 1pM can be detected.

Based on the phospholipase induced anchoring transition of LCs at LC/aqueous interface described previously, Hartono and coworkers used a similar platform to detect beta-bungarotoxin, which has a similar structure as phospholipase.\(^{36}\) The phospholipase-like toxin showed a phospholipase like enzymatic activity, which triggered the black-to-bright texture transition of a phospholipid-laden LC film. By monitoring the texture transition of LC films, the toxin as low as 5pg can be detected.

Chen and coworkers synthesized sulfur- and nitrogen-containing ligand 5-(pyridine-4-yl)-2-(5-(pyridin-4-yl) thiophen-2-yl) thiazole (ZT) to dope a 5CB film to specifically detect mercuric ion (Hg\(^{2+}\)).\(^{38}\) The bonding formed between ZT and Hg\(^{2+}\) disrupted the packing of 5CB, which triggered a black-to-bright texture transition of LC films. Hg\(^{2+}\) has a stronger bonding affinity to ZT than other metal ions. Thus, the ZT doped 5CB LC film can specifically detect Hg\(^{2+}\) in the low concentration level.
He and coworkers used surfactant-laden LC films to detect bile acids, which are an important substance for digestion process.\textsuperscript{16,41-42} The amphiphilic bile acids tend to adsorb at the LC/aqueous interface to disrupt the packing of surfactants at the interface, thus induce a black-to-bright optical appearance change of LC films under the polarizing optical microscope (Figure 10). He and coworkers studied the effect of alky chain length of liquid crystals on the detection limit of bile acids and found shorter alky chain length of LCs with lower anchoring energy showed more sensitive to bile acids.\textsuperscript{41} They found that the mixture of 5CB and 4-(4-pentylcyclohexyl) benzonitrile (5PCH) can further increase the sensitivity of LC films for bile acids. They attributed this increased sensitivity to the presence of a bulky cyclohexane ring of 5PCH, which only weakly coupled to neighboring LC molecules compared with 5CB molecules. The detection limit for cholic acid can be sharply decreased from 80 μM for pure 5CB LC films to 1.5 μM by mixing 5CB with 19wt% 5PCH as a LC film.

Figure 10. Polarizing optical microscopy images and schematic illustrations of SDS-laden LC films before (a-b); and after (c-d) the addition of cholic acid. Adapted with permission from Ref. [16]. Copyright 2013 The Royal Society of Chemistry.
2.3.2 Liquid Crystal Droplets for Sensing Applications

Besides the planar LC films described above, researchers usually dispersed LCs in aqueous solution to prepare LC droplets for sensing applications. The adsorption of analytes or interactions happened at the surface of LC droplets trigger the surface anchoring change of LCs inside the droplet and thus induce a configuration transition of LC droplets. This configuration transition of LC droplets can be easily observed under the polarizing optical microscope (Figure 11). When the LC molecules align parallel to the surface of the droplet, it shows bipolar configuration with two point defects at the poles of droplets (Figure 11a). When the LC molecules align perpendicular to the surface of the droplet, it shows radial configuration with a point defect locating at the center of droplet (Figure 11b). By monitoring the configuration transition between then bipolar and then radial, we can easily detect the analytes or study the interactions happened at the surface of LC droplets.

Figure 11. Schematic illustrations and polarizing optical microscopy images of (a) bipolar (a); and (b) radial configurations of LC droplets in LC emulsions

Compared with 2-D planar films, the preparation of LC emulsions does not require a pre-treated solid substrate and TEM grid to support and confine the LCs. Due to the high mobility of LC droplets in LC emulsions, it is simple and easy for sample handling and reagent mixing.⁸
2.3.2.1 Preparation of LC Droplets

There are several ways to prepare LC droplets for sensing applications. Sonication is a
common method to disperse LCs into aqueous solution to prepare LC emulsions. Surfactant
or polyelectrolyte are usually adopted to stabilize LCs in aqueous solution. The surfactant or
polyelectrolyte tend to adsorb at the LC/aqueous interface to stabilize LCs phase as droplets in the
aqueous solution under sonication. The initial director configurations of LC droplets are
determined by the interfacial chemistry of LC droplets, which will be described in details later.

It is generally accepted that the surface anchoring of LC droplets can be affected by the
droplet size. Polyelectrolyte multilayer (PEM) capsules was adopted as a template to prepare
monodispersed LC droplets to eliminate the effect of droplet size on the sensing results. Figure
12a shows the preparation of monodispersed LC droplets with PEM capsules. First, negatively
charged (poly (styrene sulfonate), PSS) and positively charged (poly (allylamine hydrochloride),
PAH) polyelectrolytes are sequentially layer-by-layer deposited on the surface of silica particles
(templates) with fixed size in aqueous solution. Then the silica cores are selectively etched with
hydrofluoric acid, resulting in hollow PEM capsules. After that, liquid crystals are loaded into the
hollow capsules to form monodispersed PEM-stabilized LC droplets. Another method to prepare
monodispersed LC droplets is to use microfluidics. Figure 12b shows the preparation of
monodispersed LC droplets with microfluidic techniques. Amphiphilic polymers are injected from
the two side channels to break the continuous LC phase and stabilize the LCs as droplets by
adsorption at the surface of LC droplets. And then the amphiphilic polymer stabilized-LC droplets
are released from the outlet of the channel into the reservoir. The channel flow rates of the aqueous
continuous and dispersed LC phases need to be carefully manipulated in order to get stable LC
droplets with a desirable size.
Compared with LC planar films, LC droplets prepared with the above methods show a larger specific surface area and high mobility in surrounding aqueous media, which can respond faster to external stimuli. However, sometimes it is a great challenge with respect to the monitoring the configuration transition of individual LC droplets. Pre-treated solid substrates were adopted to fix the LC droplets by Kinsinger and coworkers. The LC emulsions was first prepared by sonication with a suitable polymer as a coating to stabilize LC droplets in aqueous solution. Then LC droplet emulsions were allowed to settle onto the pre-treated substrate for a while and the freely LC droplets were then removed by rinsing the substrate with a buffer solution. The immobilization of LC droplet on pre-treated solid substrates were proved by the author can be through either covalent bonding or electrostatic attractions.
2.3.2.2 Size-Dependent Ordering of LC Droplets

With monodispersed LC droplets prepared with PEM as capsules described above, Gupta and coworkers studied the effect of droplet size on the ordering of LC droplets. They found the director configuration of LC droplets showed a bipolar configuration when the diameter of LC droplets was larger than 3 μm (Figure 13a-c), then changed to pre-radial when the diameter decreased to around 1 μm (Figure 13d-h), and showed a radial configuration when the diameter was further decreased to 0.7 μm (Figure 13i-k). The author concluded that the effect of saddle-splay (K₂₄) on the elastic free energy of LC droplets cannot be ignored when the droplet size became too small. They further used SDS to study the droplet size dependent ordering transition of LC droplets and found the concentrations of SDS required to trigger a bipolar-to-radial configuration transition of LC droplets decreased with decreasing droplet size. This result provides us a way to tune the director configuration of LC droplets to external stimuli by controlling the LC droplet size.

![Figure 13](image)

Figure 13. Polarizing optical microscopy images and schematic illustrations of ordering of LC droplets with different size. Adapted with permission from Ref. [49]. Copyright 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
2.3.2.3 Adsorption of Amphiphiles at the Surface of LC Droplets

The aqueous environment of LC emulsions provides a way to design sensing platforms by transportation of chemical and biological molecules to the LC/aqueous interface to affect the surface anchoring of LC droplets. Motivated by previous studies to investigate the adsorbate-induced anchoring transition of planar LC films with surfactants, simple amphiphiles were adopted to study the ordering transitions of LC droplets triggered by interfacial adsorbates, providing a basis for further detection of chemical and biological analytes and design of complex sensing systems.

Layer-by-layer assembly of polyelectrolytes at the surface of LC droplets provides a way to tune the surface properties of LC surface and thus help us get insight in the surface modification-induced ordering transition of LC droplets. Zou and coworkers alternately coated the surface of 5CB droplets with positively charged Poly (diallyldimethyl ammonium chloride) (PDADMAC) and negatively charged PSS and found the director configuration of 5CB droplets were alternately changed between bipolar and radial when the outmost layer of the polyelectrolyte was changed between PSS and PDADMAC. The polarity of the polyelectrolyte shell is determined by the outer layer of the polyelectrolyte. PDADMAC as the outer layer leads to a polar polyelectrolyte shell, while PSS as the outer layer leads to a nonpolar shell. This different polarity of polyelectrolyte shell caused different director configurations of 5CB droplets. Polar interaction between the cyano groups of 5CB with the polar shell triggered a radial director configuration of 5CB droplets. However, hydrophobic and \( \pi-\pi \) interactions dominated between 5CB and nonpolar and induced a bipolar configuration of 5CB droplets. They found that when the number of adsorbed bilayers of PDADMAC/PSS increased from 1 to 4, the polar force imposed by the shell increased and was more efficient to induce a radial configuration of 5CB droplets. They also
found adjusting the ion strength with salt solution can tune the polar interaction between the polyelectrolyte shell and 5CB cores and thus triggered a radial-to-bipolar configuration transition of (PDADMAC/PSS)-stabilized 5CB droplets.

Given by the tunable properties, polyelectrolyte multilayer (PEM)-coated LC droplets show a potential as a chemical and biological sensor. Tjipto and coworkers prepared layers of PSS/PAH-stabilized LC droplets and exposed to SDS to get insight in the interactions between polyelectrolyte and analytes. It took 5 min for SDS to penetrate the seven layers of PSS/PAH coating to interact with 5CB cores to trigger a bipolar-to-radial configuration transition of (PSS/PAH)$_7$-coated 5CB droplets, while the time required for SDS to trigger a bipolar-to-radial configuration transition of naked 5CB droplets is just several seconds.

Gupta and coworkers studied the configuration transition of monodispersed (PSS/PAH)-coated 5CB droplets by exposing the droplets to increasing concentrations of SDS (Figure 14). Without SDS, the (PSS/PAH)-coated 5CB droplets showed a bipolar configuration with two point defects on the poles of the droplet. With increasing the concentration of SDS, the two point defects disappeared and a loop at the equator showed up. Then the loop moved toward one of the poles of the 5CB droplet. When the concentration of SDS increased to 0.6 mM, the loop shrank into a point defect near the surface of the 5CB droplet. By further increasing the concentration of SDS, the point defect finally moved toward the center of the 5CB droplet, indicating a radial configuration.
Figure 14. Configuration transitions of (PSS/PAH)-coated 5CB droplets to increasing concentrations of SDS. Adapted with permission from Ref. [48]. Copyright 2009 American Chemical Society.

2.3.2.4 Detection of Chemical and Biological Analytes with LC Droplets

Based on the mechanisms of interactions happened at the surface of LC droplets understood with polyelectrolytes and/or surfactants, researchers applied LC droplets to detect various chemical and biological analytes, such as endotoxin\(^{56-57}\), enzymes and proteins\(^{48,58-60}\), glucose\(^{61}\), and urea\(^{62}\).

Lin and coworkers found the bipolar-to-radial configuration transition of LC droplets was triggered by 1pg/mL of endotoxin, which is six orders of magnitude lower than adsorbate-induced configuration transition at the surface of LC droplets (Figure 15)\(^{56}\). They attributed this ultra-high sensitivity of LC droplets to this six-tail lipid to the diffusion of endotoxin to the point defect at the center of LC droplets.

By increasing the salt concentration to form electric double layer, Bera and coworkers prepared PDADMAC or Poly(ethylenimine) (PEI)-coated 5CB droplets with a radial configuration and exposed to bovine serum albumin (BSA), an abundant protein in nature.\(^{58}\) The adsorption of BSA at the surface of 5CB droplets through electrostatic attraction between the negatively charged...
BSA and the positively charged polyelectrolyte coating disrupted the electric double layer formed near the LC/aqueous interface, and thus induced a radial-to-bipolar configuration transition. They also studied the effect of molecular weight of polyelectrolyte on the detection efficiency of 5CB droplets and found 5CB droplets coated with higher molecular weight of polyelectrolyte showed more efficient to response to BSA. Higher molecular weight of polyelectrolyte with higher charge density can attracted more BSA to adsorb on the surface of 5CB droplets to trigger the radial-to-bipolar configuration transition of 5CB droplets.

**Figure 15.** (a) Chemical structure of endotoxin; bright field (b,e) and polarizing optical microscopy images (e,f), schematic illustrations (d,g) of LC droplets before (b-c) and after (e-g) the addition of edotoxin. Adapted with permission from Ref. [56]. Copyright 2011 American Association for the Advancement of Science.

Yang and coworkers demonstrated that LC droplets can be adopted to specifically detect antibodies by immobilization of proteins at the surface of LC droplets. The immobilization of immunoglobulin G (IgG) at the surface of 5CB droplets in the presence of Tween 20 induced a radial configuration of PEI-coated 5CB droplets, in which the Tween 20 penetrated through the PEI coating to induce a perpendicular alignment of 5CB to the surface of the droplets (Figure 16a-d). After the addition of anti-IgG (AlIgG), the immune-complex formed between AlIgG and IgG at the surface of LC droplets displaced some Tween 20, causing a radial-to-bipolar configuration transition.
transition (Figure 16e). By monitoring the configuration transition of LC droplets, the AlgG can be easily detected.

![Figure 16. Schematic illustrations of configurations of PEI-coated LC droplet before (a) and after (b) the addition of tween 20, (c) immobilization of IgG on the surface of PEI-coated LC droplet, (d) in the presence of Tween 20 after immobilization of IgG, and (e) after the addition of AlgG. Adapted from Ref. [59]. Copyright 2011 American Chemical Society.](image)

Motived by the planar LC films responding to enzymatic activity \(^{21}\), Gupta and coworkers demonstrated LC droplets decorated with phospholipid, L-DLPC, can also be adopted to report the enzymatic activity of phospholipase A\(_2\). \(^{48}\) The radial L-DLPC-coated 5CB droplets were transformed to bipolar ones after the enzyme PLA\(_2\) cleaved one hydrophobic tail of L-DLPC, which led to the desorption of hydrolysis products of L-DLPC.

### 2.3.2.5 Detection of Viruses, Bacteria, and Cells with LC Droplets

Detections of cancer cells, viruses and bacteria plays a fundamental role in areas including clinical diagnosis, food, environment. Common methods to detect those cells and microorganisms are either time consuming or high cost.
Previous studies proved that the lipid can induce a homeotropic anchoring of LCs. The cell-wall or envelop of Gram-ve bacteria with outer membrane and enveloped viruses have lipids, while Gram +ve bacteria and non-enveloped viruses do not. Based on this, Sivakumar and coworkers used LC droplets to distinguished different types of bacteria and viruses. When exposed naked LC droplets to bacteria or viruses in PBS solution, they found *Escherichia coli* (*E. coli*) with outer membrane and the enveloped virus *A/NMW/Tokyo/67* triggered a radial configuration of LC droplets, while the Gram -ve bacteria *B. subtilis* and *M. luteus* and non-enveloped virus *M13 helper phage* did not (Figure 17). They attributed this difference to the transportation of lipids from the cell wall or the envelop of *E. coli* and *A/NMW/Tokyo/67* to the surface of naked LC droplets. They found no configuration transition happened when exposed PEM-coated LC droplets to those bacteria and viruses.
Yoon and coworkers synthesized folic acid conjugated block copolymers (PS-b-PAA-FA) to modify the surface of LC droplets to specifically detect KB cancer cells, which has a folate receptor on the cell membrane. The adsorption of PS-b-PAA-FA at the surface of LC droplets in the presence of SDS show a radial configuration (Figure 18). The specific adsorption of KB cancer cells at the surface of LC droplets through the folate receptor-folic acid ligand interaction disrupted the packing of SDS, and thus triggered a radial-to-bipolar configuration transition. They also exposed the LC droplets to other normal cells, fibroblasts and osteoblasts, but no configuration transition was observed, which proved that PS-b-PAA-FA/SDS-coated LC droplets can selectively detect KB cancer cells. A similar detection platform using ligand-receptor interactions with LC droplets to selectively detect HepG2 cells was reported by Choi and coworkers. They synthesized β-galactose-conjugated poly(styrene-b-acrylic acid) block copolymer (PS-b-PA-G) to
modify the surface of 5CB droplets in the presence of SDS, inducing a radial configuration of LC droplets. After being exposed to HepG2 cells, the LC droplets showed a radial-to-bipolar configuration transition due to the ligand-receptor interactions between the HepG2 cells and β-galactose-containing ligands.

### 2.3.2.6 Other Sensing Applications with LC Droplets

Due to the easy modification of the surface of LC droplets, LC droplets are also employed to detect pH $^{51, 66}$ and heavy metal ions $^{67}$.

By decorating the surface of LC droplets with a pH sensitive polymer, PAA-b-LCP, Khan and coworkers reported the configuration transition of LC droplets from pH 12 to 2. $^{51}$ At pH 12, PAA chain adopted an expanded conformation, which allowed an oblate conformation for the LCP chain in LC phase, inducing a radial configuration of LC droplets (Figure 19a). At pH 2, a compact conformation was preferred by PAA chain, which allow the LCP block to extend into the LC cores to induce a bipolar configuration of 5CB droplets (Figure 19b).

![Figure 19. Schematic illustrations of PAA-b-LCP-coated LC droplets at (a) pH 12 and (b) pH 2. Adapted with permission from Ref. [51]. Copyright 2011 of The Royal Society of Chemistry.](image)

Based on similar mechanisms, Kwon and coworkers decorated the outer surface of nematic liquid crystal (NLC) double emulsion droplets (DED) with PAA-b-LCP to respond to different pH
solutions. PAA-b-LCP chains adopted a compact and an extend conformations, respectively, below and above its pKa, at the outer surface of NLC-DED. This conformation change induced a configuration transitions of NLC-DED, which can be observed under the polarizing optical microscope. However, the preparation method is so complex and this platform can only show an obvious configuration change around the pKa of PAA-b-LCP, which does not show any improvement compared with the previous pH sensing platform prepared with PAA-b-LCP-coated 5CB droplets.

Figure 20. Schematic illustrations (a-b) and polarizing optical microscopy images (c-d) of stearic acid-coated LC droplets on OTS substrate before (a,c) and after (b,d) exposed to heavy metal ions. Adapted from Ref. [67]. Copyright 2014 Elsevier B.V.

Han and coworkers prepared stearic acid-coated LC droplets on an n-octyltrichlorosilane (OTC)-treated substrate to detect heavy metal ions through electrostatic interactions. The negatively charged stearic acid coated-LC droplet shows a dark crossed texture (Figure 20a), due to the stearic acid induced a homeotropic alignment of LCs. The adsorption of heavy metal ions to the deprotonated carboxylate groups of stearic acid at the surface of LC droplets through
electrostatic attraction interrupted the packing of stearic acid at the surface of LC droplets, triggering a bright appearance of LC droplets (Figure 20b).

2.4 Bile Acids

Bile acids, as the major components of bile, are synthesized in the liver through catabolism of cholesterol and stored in the gallbladder between meals. Figure 21 shows the amphipathic structure of the bile acid with one hydrophobic face and one hydrophilic face. Those bile acids can be conjugated at C24 site with either taurine or glycine to form conjugated bile acids.

Bile acids are formed in liver and play an important role in the digestion process. When digestion process happens, the bile acids in the liver and gallbladder are secreted into the duodenum to promote the digestion and absorption of fats and fat-soluble vitamins. The amphipathic bile salts play an important role in the digestion and absorption of fats and fat-soluble vitamins through the formation of emulsifications with fats and fat-soluble vitamins. The primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), can be dehydroxylated by the bacteria in the intestine to form deoxycholic acid (DCA) and lithocholic acid (LCA), respectively (Table 1). The deconjugation of conjugated primary bile acids also occurs there by the enzymatic action of bacteria. Then the bile acids are reabsorbed into the portal vein and transported back to liver through enterohepatic circulation, where the deconjugated bile acids are reconjugated with either taurine or glycine. After the digestion process, most of the bile acids are recycled back to the liver and ready for the next digestion. Only less than 5% of the bile acids are excreted in the body fluids. However, the concentrations of bile acids will dramatically increase in serum, liver, gallbladder, urine and feces for patients with liver or intestinal diseases.
concentration of bile acids in the biological fluid of healthy individuals is less than 11 μM, while it can increase up to 100 μM for patients with liver and intestinal diseases. Therefore, bile acids have a long history of being used as a biomarker for the early diagnosis of liver and intestinal diseases.

![Hydrophobic face and Hydrophilic face]

**Figure 21. Amphiphilic structures of bile acids**

<table>
<thead>
<tr>
<th>Table 1. Types of bile acids</th>
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<tr>
<td>Cholic acid</td>
</tr>
<tr>
<td>Chenodeoxycholic</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
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<tr>
<td>Lithocholic acid</td>
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</tbody>
</table>

Current detection methods for bile acids include the use of complex instruments, redox cycling, or optical approaches by fluorescent labeling. Chromatography-mass spectrometry is a common method to detect bile acids with high sensitivities. Chromatograph is
used as a separator to separate bile acids from sample matrix based on their different affinities for
the stationary phase from other molecules. Gas chromatograph or liquid chromatograph are usually
used. Then the separated bile acids along with other molecules being sent into mass spectrometer
separately to allow mass spectrometer to further identify the structures of the bile acids. The
method requires tedious sample preparation approaches and expensive instruments. Another
common method to detect bile acids is electrochemical sensors. However, this detection method
is also time consuming, require relatively large sample volumes, and expensive instruments.

The routine clinical diagnosis of total bile acids is through enzymatic assay through
reversible oxidation and reduction of the C-3 site of bile acids by 3α-hydroxysteroid
dehydrogenase (3α-HSD). Figure 22a shows the principle of this enzyme cycling method. Bile
acids with 3α-hydroxysteroid are reversibly oxidized and reduced by 3α-HSD in the presence of
thio-NAD\(^+\) and NADH. The concentration of bile acid is indirectly determined by measuring the
product of thio-NADH through the ultraviolet. However, it cannot directly determine the
concentration levels of bile acids and the cost of the enzymes in this method is very high.

Recently researchers developed a simple optical probe for the rapid detection of bile acids.
Since both β-cyclodextrins (β-CDs) and bile acids are basically optical inert, Liu and coworkers
synthesized β-CDs bearing fluorophores as fluorescence probes for the detection of bile acids
through the competition of the bile acids with the fluorophores for the hydrophobic cavity of the
β-CDs. After the addition of bile acids, the fluorophore was pushed towards the more
hydrophobic region of the cavity by competitive inclusion of bile acids molecules and showed a
fluorescence intensity enhancement (Figure 22b). The different bonding stabilities of bile acids
with modified PM-β-CD will result in a molecular selectivity. Though this method provides a way
to directly observe the selective interaction process by naked eyes, the synthesis of modified β-CD will be time-consuming. Therefore, a simple and rapid method for the detection of bile acids with a sufficient sensitivity is desired.

Figure 22. Schematic illustrations of common detection of methods of bile acids: (a) redox cycling; and (b) fluorescent labeling. Adapted with permission from Ref [76]. Copyright 2007 American Chemical Society.

2.5 Conclusion

Liquid crystal materials with high birefringence and ultra-sensitivity to external perturbations at the LC/aqueous interface show great potential as a simple and label-free platform to transduce and amplify various interfacial interactions. The planar LC films is a desirable simple platform to monitor a variety of biological events by mimicking the bio-membranes. Compared with planar LC films, LC droplets have a high mobility in the aqueous environment and have a larger specific surface area. The ordering of LCs within LC droplets can be manipulated by adsorption or immobilization of various molecules at the surface of LC droplets to fulfill sensing goals. Considering the versatility, easy handling and manipulation, large specific surface area, low cost, simple and label-free natures, LC droplets show tremendous potential for sensing applications. Therefore, in this dissertation we aim to develop liquid crystal droplet-based sensing platforms for
the rapid and real-time detection of bile acids through stabilization of liquid crystal droplets with polyelectrolytes and hydrogels.
3.1 Introduction

Supramolecular chemistry has attracted an enormous attention since Donald J. Cram, Jean Marie Lehn and Charles J Pedersen were honored with the Nobel Prize in Chemistry 1987 for their discoveries of host-guest supramolecular systems. Supramolecular structures are formed by association of two or more molecules through noncovalent interactions. These noncovalent interactions include hydrogen bonding, van der Waals force, hydrophobic or hydrophilic, electrostatic and π-π interactions. The weakness of noncovalent interactions endows the supramolecular materials with a lot of unique properties, such as easy fabrication, reversibility, and stimuli-responsibility.

Among various supramolecular systems, host-guest interaction-based supramolecular materials has aroused a tremendous interest for their distinct properties in studying the selective interaction between host and guest molecules. Host-guest supramolecular systems are formed by inclusion of guest molecules into the cavities of host molecules through noncovalent interactions. The common host molecules are cyclodextrins (CDs), and cucurbit[n]urils (CBs). Cyclodextrins are the most popular host molecules because they are commercially available, biocompatible, and inexpensive and can be easily modified.

CDs are truncated cone-like oligosaccharides linked by α-1, 4 glycosidic bond with a hydrophilic outer surface and a hydrophobic cavity. The secondary hydroxyl groups locate at the wide opening of the cavity, whereas, the primary hydroxyl groups locate at the narrow one, which give the cyclodextrins a hydrophilic exterior (Figure 23). The hydrophobic steroid skeleton and
the glycosidic oxygen contribute to the hydrophobic interior of CDs (Figure 23). There are three natural CDs, α-, β-, and γ-CD, comprised six, seven, and eight glucopyranose units, respectively. The size of CDs depends on the number of glucose units (Table 2), which enables CDs to encapsulate various guest molecules with different sizes, such as small organic molecules, surfactants, and proteins. The different binding constants of CDs with guest molecules provide an opportunity to study the competitive interactions between different guest molecules.

Figure 23. Schematic representation of chemical structure (a), 3D structure (b), and longitudinal section of CD.

Table 2. Dimensions of natural cyclodextrins [81]

<table>
<thead>
<tr>
<th></th>
<th>α-CD</th>
<th>β-CD</th>
<th>γ-CD</th>
</tr>
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<tbody>
<tr>
<td>No. of glucose units / nm</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Internal diameter / nm</td>
<td>0.47-0.53</td>
<td>0.60-0.65</td>
<td>0.75-0.83</td>
</tr>
<tr>
<td>Height of cavity / nm</td>
<td>0.79</td>
<td>0.79</td>
<td>0.79</td>
</tr>
<tr>
<td>External diameter / nm</td>
<td>1.46</td>
<td>1.54</td>
<td>1.75</td>
</tr>
<tr>
<td>Approx. volume of cavity / nm³</td>
<td>0.174</td>
<td>0.262</td>
<td>0.427</td>
</tr>
</tbody>
</table>
Bile acids in the small intestine are thought to be an important competitor in the displacement. The thermodynamics and structures of the host-guest interactions between bile acids and CDs and their derivatives have been deeply studied. Liu and coworkers synthesized β-CDs bearing fluorophores as a fluorescence probe for the detection of bile acids through the competition of the bile acids with the fluorophores for the hydrophobic cavity of the β-CDs. 76

In this chapter, we prepared β-CD/tetradecyl trimethylammonium bromide (C_{14}TAB) complex-coated 4-cyano-4'-pentylbiphenyl (5CB) lipid crystal droplets for the selective detection of cholic acid (CA), in which the β-CD/C_{n}TAB complex adsorbed at the surface of the 5CB droplets is used as a selective barricade and the director configuration transition of the 5CB inside the droplets serves as an optical probe. we functionalized the 5CB liquid crystal droplets with β-CD/C_{n}TAB inclusion complexes at the aqueous/liquid crystal interface to detect bile acids by the competitive host-guest interaction induced a radial-to-bipolar configuration transformation of LC droplets.

3.2 Experimental

3.2.1 Materials

4-cyano-4'-pentylbiphenyl (5CB), cholic acid (CA), β-cyclodextrin (β-CD), tetradecyl trimethylammonium bromide (C_{14}TAB), uric acid (UA), and ascorbic acid (AA) were obtained from Sigma-Aldrich (St. Louis, MO). Synthetic urine concentrate was purchased from RICCA Chemical Company (Arlington, TX); Cholyl-lysyl-fluorescein (CLF) was obtained from BD
Biosciences (Woburn, MA). All chemicals were used without further purification. Water used in experiments was purified with Easypure II system (18.2 MΩ cm and pH 5.7).

![Chemical structures of 5CB, CnTAB, β-CD, CA and CLF](image)

**Figure 24. Chemical structures of 5CB, CnTAB, β-CD, CA and CLF**

### 3.2.2 Preparation of β-CD/C₁₄TAB Complex-Modified Liquid Crystal Droplets

The β-CD/C₁₄TAB complex was formed by mixing C₁₄TAB and β-CD in deionized water at the ratio of 1:1 with a bath sonicator (Bradson 2510) for 5min at room temperature. The as-received mixture was magnetically stirred overnight.

The adsorption of β-CD/ C₁₄TAB complexes at 5CB/water interface was carried out by mixing 3 μL 5CB in 3 ml complex solution with a tip sonicator (FB505, Fisher Scientific, Pittsburgh, PA) at the amplitude of 20% for 20s at room temperature.

### 3.2.3 Characterization

The formation of CₙTAB/β-CD complexes in D₂O was characterized by ¹H NMR spectroscopy (Bruker AvanceIII 400 spectrometer, Bruker, Billerica, MA) at room temperature.
Surface tension measurements were carried out with a NIMA Wilhelmy plate. A polarizing optical microscope (BX 40, Olympus) was used to observe the director configuration of CₙTAB/β-CD complex-coated 5CB droplets. The droplet density of CₙTAB/β-CD complex-coated 5CB droplets was analyzed with an optical microscope, in which a drop (2 μL) of the droplet solution was placed between two cover glass slides and a large number of optical microscopy images were captured to represent the whole sample area. The fluorescence microscopy images were acquired with a confocal fluorescence microscope (Nikon Eclipse Ti, Nikon Instrument) with 488 nm excitation. ζ-potential measurements were carried with a Zetasizer Nano ZS90 (Malvern Instruments Inc.).

3.3 Results and Discussion

It has been shown that β-CD can include alkyl trimethylammonium bromide (CₙTAB) to form 1:1 complexes, in which the head group of the CₙTAB locates outside the secondary face of the β-CD and the hydrophobic chain of the CₙTAB partially protrudes from the primary face of the β-CD. In our experiments, the inclusion complex of β-CD and CₙTAB was formed at 1:1 molar ratio in deionized water. The concentration of CₙTAB (n=12, 14, and 16) is lower than their critical micelle concentrations, which are 15 mM for C₁₂TAB, 3.9 mM for C₁₄TAB, and 0.9 mM for C₁₆TAB, respectively. The formation of β-CD/C₁₄TAB inclusion complexes is evident from ¹H NMR spectra (Figure 25), which show that the hydrogen resonances of the H-3 and H-5 protons located inside the cavity of the β-CD shift upfield upon the inclusion with C₁₄TAB.
Figure 25. $^1$H NMR spectra of 1.6 mM β-CD without (a) and with (b) of 1.6mM C$_{14}$TAB in D$_2$O

We find that β-CD/C$_n$TAB inclusion complexes can be used to stabilize 5CB droplets in deionized water when $n$ is larger than 12. Figure 26a is a polarizing optical microscopy image of β-CD/C$_{14}$TAB complex-coated 5CB droplets. All the droplets show a radial director configuration. Since the height of β-CD is 7.8 Å, the protruded hydrocarbon chain of the C$_{14}$TAB from the β-CD cavity is approximately 13 Å. The protruded hydrocarbon chain of the C$_{14}$TAB is expected to enter the 5CB droplets to induce the radial configuration, in which the β-CD is oriented at the 5CB/water interface with its primary face toward the 5CB phase and its secondary face toward the water phase (Figure 27). The adsorption of β-CD/C$_{14}$TAB complexes at the 5CB/water interface is evident from the positive zeta potential of the droplets (+17.6 mV). The concentration of β-CD/C$_{14}$TAB complex-coated 5CB droplets was estimated to be ~ $1.2 \times 10^6$ droplets per μL. The radial director configuration is also observed for β-CD/C$_{16}$TAB complex-coated 5CB droplets in deionized water. The minimum concentration required to stabilize 5CB droplets in deionized water is 0.6 mM for β-CD/C$_{16}$TAB complexes and 1.6 mM for β-CD/C$_{14}$TAB complexes, respectively. The β-CD/C$_n$TAB complex-coated 5CB droplets are stable at room temperature for weeks.
Figure 26. Polarizing optical microscopy images of C\textsubscript{14}TAB/\beta\text{-}CD complex-coated 5CB droplets at pH 7.4 before (a) and after (b) the addition of 20\(\mu\)M CA

Figure 27. Schematic illustrations of CA-induced radial-to-bipolar configuration transition of C\textsubscript{14}TAB/\beta\text{-}CD complex-coated 5CB droplets via competitive host-guest recognitions

Cholic acid (CA) is one of primary bile acids. It comprises 31\% of the total bile acids produced in liver.\textsuperscript{68} The concentration level of CA was used as an effective biomarker for diagnosing liver disease and evaluating the effectiveness of treatments. In our experiments, CA was dissolved in NaOH aqueous solution with pH 9.0 at concentrations lower than its concentration (18mM).\textsuperscript{97} 50 \(\mu\)L of CA solution was then added into 200 \(\mu\)L of \(\beta\text{-}CD/C\textsubscript{14}TAB\) complex-coated 5CB droplet solution. The pH value of the mixed solution was adjusted to 7.4. We find that the 5CB droplets stabilized by 1.6 mM \(\beta\text{-}CD/C\textsubscript{14}TAB\) complexes undergo a radial-to-bipolar transition if the CA concentration is higher than 20 \(\mu\)M in the mixed solution (Figure 26b). It has shown that CA can
deeply enter the cavity of β-CD from its primary face to drive other hydrophobic guests out of the cavity due to its high bending constant for β-CD. Thus, we infer that the radial-to-bipolar transition of the 5CB inside the β-CD/C₁₄TAB complex-coated droplets may be a result of the displacement of the C₁₄TAB from the cavity of the β-CD by the competitive CA (Figure 27). To verify the hypothesis, we carried out controlled experiments. The surface tension of 3mL C₁₄TAB/β-CD complex-coated 5CB droplet solution is 51.8 mN/m, which is reduced to 45.8 mN/m after the addition of 20 μM CA (Figure 28a). The surface tension is 52.6 mN/m for 20 μM CA solution and 54.8 mN/m for 1.6 mM β-CD/C₁₄TAB complex solution, respectively (Figure 28a). Therefore, the reduction of the surface tension after the addition of CA is a result of the displacement of the C₁₄TAB from the cavity of the β-CD into the aqueous solution rather than the removal of the β-CD/C₁₄TAB complexes from the surface of the 5CB droplets. It has been shown that the surface tension of C₁₄TAB linearly decreases with the increase of its concentrations in aqueous solution. Based on the reduced surface tension, we estimated that 0.44 mM C₁₄TAB was released into aqueous solution, suggesting that 27.5% C₁₄TAB was displaced from the cavity of the β-CD by CA. Figure 28b is a polarizing optical image of C₁₄TAB/β-CD complex-coated 5CB droplets in aqueous solution after the addition of 1.5 μM choly-lysyl-fluorescein (CLF, a fluorescent CA, Figure 24), which shows that all the 5CB droplets transit into a bipolar configuration. The corresponding confocal fluorescent microscopy image shows a strong fluorescence from the surface of the 5CB droplets (Figure 26c), suggesting that the adsorption of CLF at the surface of the 5CB droplets. Based on the results of the controlled experiments, we conclude that the radial-to-bipolar configuration transition of the 5CB inside the droplets is a result of the displacement of the C₁₄TAB from the cavity of the β-CD by the competitive CA.
Figure 28. (a) Surface tension of C_{14}TAB/β-CD complex coated-5CB droplet solution, C_{14}TAB/β-CD complex coated-5CB droplet + 20 µM CA solution, 20 µM CA solution, and 1.6mM C_{14}TAB/β-CD complex solution. (b) Polarizing optical (b) and fluorescent (c) microscopy images of C_{14}TAB/β-CD complex coated-5CB droplets in NaOH aqueous solution after addition of CLF.

The minimum concentration (detection limit) of CA with ionic strength of 0.08mM at pH 7.4 required to triggering the radial-to-bipolar configuration transition is 20 µM for C_{14}TAB/β-CD complex-coated 5CB droplets and 30 µM for C_{16}TAB/β-CD complex-coated 5CB droplets, respectively. This is because the binding constant of C_{n}TAB/β-CD complexes increases with n.\(^{100}\) The detection of CA in urine often suffers from the potential interference of uric acid and urea. However, we observe no radial-to-bipolar configuration transition for the C_{14}TAB/β-CD complex-coated 5CB droplets exposed in 1mM uric acid and 3.16 mM urea solution at pH 7.4, which suggests that they are unable to displace the C_{14}TAB from the cavity of β-CD adsorbed at the surface of the 5CB droplets. In addition, the detection limit of C_{14}TAB/β-CD complex-coated 5CB droplets for CA in aqueous solution remains unchanged in the presence of 1mM uric acid and 3 mM urea (Figure 29). Furthermore, we carried out the detection of CA in diluted synthetic urine (a dilute factor of 0.01) with 3.16 mM urea, 42.6 µM MgSO\(_4\), 90.1 µM CaCl\(_2\), and 1.36 mM NaCl with C_{14}TAB/β-CD complex-coated 5CB droplets. The detection limit for CA in the diluted synthetic urine is ~ 60 µM (Figure 29), which is three times higher than that of NaOH aqueous solution with an ionic concentration of 0.08 mM. Since the interference of uric acid and urea for
the CA detection is negligible, the increased detection limit for CA in the diluted synthetic urine is likely due to the effect of ion concentrations. It has been shown that 5CB favors for a homeotropic anchoring at the LC-aqueous interface in the aqueous solution with high ionic strength due to the formation of an electrical double layer, 101 which strengthens the radial configuration of the C$_{14}$TAB/β-CD complex-coated 5CB droplets. To verify the effect of ion concentrations, we carried out the CA detection in aqueous solution containing 42.6 µM MgSO$_4$, 90.1 µM CaCl$_2$, and 1.36 mM NaCl and found that the detection limit of C$_{14}$TAB/β-CD complex-coated 5CB droplets for CA is ~ 60 µM.

![Figure 29. Detection limit of C$_{14}$TAB/β-CD complex coated-5CB droplets for CA in different solutions](image)

3.4 Conclusion

In this chapter we showed that the radial-to-bipolar configuration transition of C$_{14}$TAB/β-CD complex-coated 5CB droplets can be used as a simple and rapid optical probe for the selective detection of CA in the presence of uric acid and urea. The detection limit for CA is 20
μM in NaOH aqueous solution and 60 μM in diluted synthetic urine, respectively. The increased detection limit for CA in the diluted synthetic urine is a result of the effect of ion concentrations.
4.1 Introduction

In last chapter, we showed that LC droplets dispersed in aqueous solution could be functionalized by the adsorption of β-CD/surfactant complexes at the LC/aqueous interface, in which the β-CD adsorbed at the LC/aqueous interface is expected to be oriented with its primary face toward the LC phase and its secondary face toward the water phase. The protruded hydrocarbon chain of the surfactant from the β-CD cavity enters the LC droplets to induce a radial configuration of the LC inside the droplets. In order to achieve long-term stability, rapid detection, high selectivity, and small sample volume are critical for developing the application of LC droplet-based biosensors in clinical settings.

The miniaturized sensor systems offer several advantages over conventionally benchtop testing, including small sample volumes, rapid analysis, portability, and low cost. Recently, efforts have been made in integrating grid supported LC films and inkjet printed LC dotes on the internal surface of microfluidic channels for the detection of biological species. In this chapter, we have fabricated a miniaturized LC droplet-based sensor platform, in which LC droplets dispersed in aqueous solution are stabilized by the adsorption of PDADMAC/C14TAB/sulfate β-CD complexes at the LC/aqueous interface, followed by integrating them into a microfluidic channel. The miniaturized LC droplet-based sensor platform allows the real-time and selective detection of bile acids in a small sample volume with the interference specie of ascorbic acid (AA) and uric acid (UA), in which the sulfated β-CD immobilized at the surface of the LC droplets acts as a
selective barricade and the director configuration transition of the LC inside the droplets serves as an optical probe.

4.2 Experimental

4.2.1 Materials

Poly(diallyldimethylammonium chloride) (PDADMAC, $M_w = 150$ kDa), tetradecyl sulfate sodium salt (SC$_{14}$S, 99% purity), $\beta$-cyclodextrin-sulfated sodium salt (sulfated $\beta$-CD), 4-cyano-4'-pentylbiphenyl (5CB, 98% purity), cholic acid (CA, $\geq$ 98% purity), deoxycholic acid (DCA, $\geq$ 98% purity), uric acid (UA, 98% purity), and ascorbic acid (AA, 98% purity) were obtained from Sigma-Aldrich (St. Louis, MO). Phosphate buffered saline (PBS, 10×, pH 7.4) solution was purchased from Fisher Scientific. All chemicals were used without further purification. Water used in experiments was purified with Easypure II system (18.2 MΩ cm and pH 5.7).

4.2.2. Preparation of Polyelectrolyte/Sulfated $\beta$-CD/SC$_{14}$S Complex-Coated 5CB Droplets

Sulfated $\beta$-CD/SC$_{14}$S complexes were formed by mixing 0.2 mM SC$_{14}$S and 0.2 mM sulfated $\beta$-CD in deionized water with magnetic stirring overnight. SC$_{14}$S/sulfated $\beta$-CD complex-coated 5CB droplets were formed by mixing 10 μL 5CB and 10 mL SC$_{14}$S/sulfated $\beta$-CD complex solution with a tip sonicator (FB505, Fisher Scientific, Pittsburgh, PA) for 10s at room temperature. After the formation of SC$_{14}$S/sulfated $\beta$-CD complex-coated 5CB droplets, 10 mg/mL PDADMAC was added. After 1-hour incubation, the resultant PDADMAC/SC$_{14}$S/sulfated $\beta$-CD complex-coated 5CB droplets were washed with deionized water by centrifugation to remove excess...
PDADMAC, SC$_{14}$S, sulfated β-CD and SC$_{14}$S/sulfated β-CD complex in solution and re-suspended in aqueous solution with pH 7.4 and phosphate-buffered saline (PBS) buffer solution.

4.2.3 Fabrication of Microfluidic Channels

The microfluidic channel used in this work was created by the photolithography and wet etching techniques on a soda lime glass substrate, which was coated with chromium and photoresist (Nanofilm, Westlake Village, CA). The soda lime glass substrate was exposed to light underneath a photomask of microchannel design using a 100 W, 365 nm ultraviolet flood lamp (SB-100PC; Spectroline, Westbury, NY). Immersion of the exposed substrate in 0.1 N NaOH followed by chromium etchant (Transene Company, Inc. Danvers, MA) removed the photoresist and the underlying chrome layer. A microchannel (~ 300 µm wide, ~ 66 µm deep, and ~ 4 cm long) was formed on the developed substrate by wet etching using buffered oxide etchant 6:1 (Transene Company, Inc. Danvers, MA). The sealing of the microfluidic channel was achieved by thermally bonding the patterned substrate and a blank substrate with two channel-access holes drilled by a Craftsman rotary power tool from (Sears, Inc.) equipped with a diamond coated drill bit (Crystalite Lapidary and Glass Products, Lewis Center, Ohio).

4.2.4 Characterization

The formation of SC$_{14}$S/sulfated β-CD complexes was characterized by $^1$H NMR spectroscopy (Bruker Avance III 400 spectrometer, Bruker, Billerica, MA) in D$_2$O at room temperature. Surface tension measurements were carried out with a NIMA Wilhelmy plate at 25°C. The density of PDADMAC/SC$_{14}$S/sulfated β-CD complex-coated 5CB droplets in bulk aqueous
solution was estimated with an optical microscope, in which a drop (2 μL) of PDADMAC/SC$_{14}$S/sulfated β-CD complex-coated 5CB droplets was placed between two cover glass slides and a large number of optical microscopy images were captured to represent the whole sample area. The number of the droplets confined by the two cover glass slides was carefully counted from the optical microscopy images and then used to calculate their density in the bulk solution. The final concentration of PDADMAC/SC$_{14}$S/sulfated β-CD complex-coated 5CB droplets used for the detection of CA and DCA was adjusted. Zeta potential measurements were carried out using Zetasizer Nano ZS90 (Malvern Instruments Inc.) at room temperature. For the detection of CA and DCA, 1.5 μL PDADMAC/SC$_{14}$S/sulfated β-CD complex-coated 5CB droplet solution was loaded into the microfluidic channel with 300 μm width and 66 μm depth from its one open end and CA and DCA solutions with varied concentrations were injected from its other open end. A polarizing optical microscope (BX 40, Olympus) was used in transmission mode to characterize the director configuration of 5CB droplets.

4.3 Results and Discussion

It has been shown that β-CD can partially include surfactants to form 1:1 supramolecular complexes, in which the head group of the surfactant locates outside the secondary face of the β-CD and the hydrocarbon chain of the surfactant partially protrudes from the primary face of the β-CD. In last chapter, it has been showed that the partial inclusion complex of β-CD and C$_{14}$TAB could adsorb at the 5CB/aqueous interface to form radial 5CB droplets. However, C$_{14}$TAB/β-CD complex-coated 5CB droplets become unstable after being washed with water. There is no improvement in the stability of the 5CB droplets after the coating of an anionic
polyelectrolyte, poly(sodium 4-styrenesulfonate) (PSS). The radial-to-bipolar configuration transition of PSS/C\textsubscript{14}TAB/\(\beta\)-CD complex-coated 5CB droplets still occurs after being washed with water through centrifugation, likely due to the weak electrostatic interaction between the positively charged C\textsubscript{14}TAB/\(\beta\)-CD complex-coated 5CB droplets and the negatively charged PSS.

Therefore, we formed the partial inclusion complexes of tetradecyl sulfate sodium salt (SC\textsubscript{14}S) with sulfated \(\beta\)-CD by mixing 0.2 mM SC\textsubscript{14}S and 0.2 mM sulfated \(\beta\)-CD in deionized water and used them to functionalize 5CB droplets in aqueous solution. The formation of SC\textsubscript{14}S/sulfated \(\beta\)-CD inclusion complexes is confirmed by \(^1\)H NMR spectra (Figure 30). Four kinds of protons, i.e., a-methylene, b-methylene, ten bulk methylene, and the terminal methyl protons near the head group of SC\textsubscript{14}S, are clearly identified in the \(^1\)H NMR spectra shown in Figure 30a. In the presence of sulfated \(\beta\)-CD, all the proton signals of SC\textsubscript{14}S shift downfield (Figure 30b), suggesting the inclusion with sulfated \(\beta\)-CD. Since the height of \(\beta\)-CD is 7.8 Å, we expect that the protruded hydrocarbon chain of the SC\textsubscript{14}S from the \(\beta\)-CD cavity is ∼13 Å, which enters the 5CB droplets to induce the radial configuration of the 5CB inside the droplets, in which the sulfated \(\beta\)-CD adsorbed at the 5CB/water with its primary face toward the 5CB phase and its secondary face toward the water phase (Figure 31a). Based on the binding constant of SC\textsubscript{14}S and \(\beta\)-CD reported in the literature,\(^{102}\) we estimated that there is 0.14 mM SC\textsubscript{14}S/sulfated \(\beta\)-CD complex formed. Thus, the surface of 5CB droplets should be largely covered by negatively charged SC\textsubscript{14}S/sulfated \(\beta\)-CD complexes. The zeta potential of SC\textsubscript{14}S/sulfated \(\beta\)-CD complex-coated 5CB droplets is -28 mV. Figure 31b is a polarizing optical microscopy image of SC\textsubscript{14}S/sulfated \(\beta\)-CD complex-coated 5CB droplets in D.I. water, showing that all the droplets have a radial director configuration. The SC\textsubscript{14}S/sulfated \(\beta\)-CD complex-coated 5CB droplets become unstable and transit into a bipolar
configuration after being washed with water through centrifugation (Figure 31c). The radial-to-bipolar configuration transition is due to the weak interaction of SC$_{14}$S/sulfated β-CD complexes with 5CB droplets, which is disrupted by centrifugation.

Figure 30. $^1$H NMR spectra of SC$_{14}$S with (a) and without (b) sulfated β-CD in D$_2$O. The chemical structure of SC$_{14}$S is inset.

Figure 31. (a) Schematic illustration of the formation of SC$_{14}$S/sulfated β-CD complex-coated 5CB droplets. Polarizing optical microscopy images of SC$_{14}$S/sulfated β-CD complex-coated 5CB droplets before (b) and after (c) being washed with water through centrifugation.

The advantage of using charged β-CD is that the SC$_{14}$S/sulfated β-CD complex-coated 5CB droplets can be stabilized by the adsorption of the cationic polyelectrolyte, polydiallyldimethylammonium chloride (PDADMAC) through electrostatic interaction.
PDADMAC is a strong polyelectrolyte, the charge density and the structural conformation of do
not change in aqueous solutions with different pH values. The adsorption of PDADMAC on the
surface of SC\textsubscript{14}S/sulfated β-CD complex-coated 5CB droplets is evident from the zeta potential
transition from −28 mV to +31 mV of the droplets. The radial director configuration of the
SC\textsubscript{14}S/sulfated β-CD complex-coated 5CB droplets remains unchanged after the adsorption of
PDADMAC (Figure 3\textsuperscript{2a}). The diameter of PDADMAC/SC\textsubscript{14}S/sulfated β-CD complex-coated
5CB droplets is 0.91 ± 0.20 μm. We find that PDADMAC/SC\textsubscript{14}S/sulfated β-CD complex-coated
5CB droplets are stable in aqueous solution over time. Also, there is no director configuration
transition observed after the droplets are washed with water through centrifugation. After the
removal of excess PDADMAC, SC\textsubscript{14}S, sulfated β-CD, and SC\textsubscript{14}S/sulfated β-CD complexes from
the solution through centrifugation, the purified PDADMAC/SC\textsubscript{14}S/sulfated β-CD complex-
coated 5CB droplets were suspended in aqueous solution with pH 7.4 and phosphate-buffered
saline (PBS).

It has been shown that polyelectrolyte layers coated on 5CB droplets are permeable for
small molecules.\textsuperscript{43,55} We exploited the application of PDADMAC/SC\textsubscript{14}S/sulfated β-CD complex-
coated 5CB droplets for the detection of DCA in aqueous solution with pH 7.4. As can be seen in
Figure 3\textsuperscript{2b}, the addition of 140 μM DCA triggers the radial-to-bipolar configuration transition of
PDADMAC/SC\textsubscript{14}S/sulfated β-CD complex-coated 5CB droplets. In this case, the negatively
charged sulfated β-CD is expected to be immobilized at the 5CB/aqueous interface by positively
charged PDADMA (Figure 3\textsuperscript{2c}). Thus, it is reasonable for us to infer that DCA, which penetrates
through the PADAMAC coating, displaces the SC\textsubscript{14}S from the cavity of the sulfated β-CD via
competitive host-guest recognition, consequently triggering the radial-to-bipolar configuration
transition of the 5CB droplets (Figure 32c). To verify this hypothesis, we measured the surface tension of solution of sulfated β-CD, SC\textsubscript{14}S, SC\textsubscript{14}S/sulfated β-CD complex, and PDADMAC/sulfated β-CD complex-coated 5CB droplets. The surface tension is 65.5 ± 0.60 mN/m for 0.2 mM sulfated β-CD solution, 46.9 ± 0.06 mN/m for 0.2 mM SC\textsubscript{14}S solution, 54.6 ± 0.13 mN/m for 0.2 mM SC\textsubscript{14}S/sulfated β-CD complex solution, 60.2 ± 0.32 mN/m for 0.14 mM DCA solution, and 62.3 ± 0.25 mN/m for PDADMAC/sulfated β-CD complex-coated 5CB droplet solution, respectively (Figure 33a). After the addition of 140 μM DCA in PDADMAC/sulfated β-CD complex-coated 5CB droplet solution, the surface tension dropped from 62.3 ± 0.25 mN/m to 47.6 ± 0.20 mN/m, which is lower than the surface tension of 0.14 mM DCA solution, 0.2 mM sulfated β-CD solution and 0.2 mM SC\textsubscript{14}S/sulfated β-CD complex solution. Thus, we conclude that the reduction of the surface tension is a result of the displacement of SC\textsubscript{14}S from the surface of the 5CB droplets by DCA. Since the displacement is based on the 1:1 competitive host-guest recognition, the amount of released SC\textsubscript{14}S from the cavity of the sulfated β-CD by DCA should be not higher than 0.14 mM. The surface tension of SC\textsubscript{14}S solution linearly decreases with the increase of SC\textsubscript{14}S concentrations in the range from 0.0 mM to 0.2 mM (Figure 33b). According to the reduced surface tension after the addition of 0.14 mM DCA, we estimate that 0.12 mM SC\textsubscript{14}S is released by DCA from the surface of the 5CB droplets.
Figure 32. Polarizing optical microscopy images of PDADMAC/SC_{14}S/sulfated β-CD complex-coated 5CB droplets before (a) and after (b) being exposed to 140μM DCA. (c) Schematic illustrations of the adsorption of PDADMAC on SC_{14}S/sulfated β-CD complex-coated 5CB droplets and the DCA induced radial-to-bipolar configuration transition of the 5CB inside the droplets.

Figure 33. (a) Surface tensions of sulfated β-CD (0.2mM), SC_{14}S (0.2mM), sulfated β-CD/SC_{14}S complex (0.2mM), DCA (140 μM) and PDADMAC/SC_{14}S/sulfated β-CD complex-coated 5CB droplet emulsion before and after the addition of 140 μM DCA; (b) surface tensions of SC_{14}S aqueous solution.

To form a miniaturized droplet-based sensor platform for the rapid and real-time detection of bile acids in small sample volumes, we loaded PDADMAC/SC_{14}S/sulfated β-CD complex-
coated 5CB droplets dispersed in PBS solution into a microfluidic channel from one of its inputs. The microfluidic channel with the 66 µm depth is able to prevent the 5CB droplets in the channel for diffusing out of the focus plane during optical microscopy observations (Figure 34a). Figure 34b shows the polarizing microscopy image of the PDADMAC/SC14S/sulfated β-CD complex-coated 5CB droplet solution with the droplet density of $2.5 \times 10^4/\mu\text{L}$ in the microfluidic channel. The droplet density was estimated from a large number of polarizing optical microscopy images captured to represent the whole channel. As can be seen in Figure 34b, all the droplets in the channels show a radial configuration, suggesting that the loading process does not disrupt the director configuration of the droplets. 1 µL of DCA solution was pipetted into the channel from its other input after the loading of PDADMAC/SC14S/sulfated β-CD complex-coated 5CB droplets.

![Figure 34](image.jpg)

**Figure 34.** (a) Schematic illustrations of the miniaturized 5CB droplet-based sensor platform. (b) Polarizing optical microscopy image of PDADMAC/SC14S/sulfated β-CD complex-coated 5CB droplets in a microfluidic channel.
Figures 35a-35d are the time-course polarizing microscopy images of PDADMAC/SC₁₄S/sulfated β-CD complex-coated 5CB droplets loaded in the channel after the addition of 20 μM DCA. These images were taken in the middle section of the channel. During the real-time optical observation, we note that the 5CB droplets slowly diffuse in and out the optical field. All the 5CB droplets in the channel show initially a radial configuration (Figure 35a) and become bipolar over time (Figure 35d).

Figure 35 Time-course polarizing microscopy images of PDADMAC/SC₁₄S/sulfated β-CD complex-coated 5CB droplets in a microfluidic channel taken at 0s (a), 72s (b), 104s (c), and 106s (d) after the addition of 20 μM DCA at pH 7.4.

We measured the response time of the miniaturized sensor platform with the droplet density of 2.5 × 10⁴/μL, defined as the time required for all the 5CB droplets in the channel to become bipolar, for DCA and CA. It is found to linearly decreases from 1.6 min to 0.6 min when the concentration of DCA increases from 20 μM to 80 μM (Figure 36a). The concentration dependence of the response time is also observed for CA. We find that it linearly decreases from 2.5 min to 1.1 min when the concentration of CA increases from 60 μM to 120 μM (Figure 36b). For the same
concentration (80 μM), the response of the miniaturized 5CB droplet-based sensor platform for DCA (0.6 min) is faster than for CA (2 min).

![Figure 36. Response time of PDADMAC/SC₁₄S/sulfated β-CD complex-coated 5CB droplets with the droplet density of 2.5 × 10⁴/μL in a microfluidic channel for CA (a) and DCA (b) as a function of their concentrations in PBS solution with and without 500 μM UA and 500 μM AA.](image)

Furthermore, we measured the detection limit of the miniaturized sensor platform, which is defined as the minimal concentration of bile acids required to triggering the radial-to-bipolar configuration transition of all the PDADMAC/SC₁₄S/sulfated β-CD complex-coated 5CB droplets, as a function of droplet densities in the microfluidic channel. The detection limit is found to decrease and from 40 μM to 2 μM for DCA and from 80 μM to 20 μM for CA by decreasing the droplet density from 7.0×10⁴/μL to 2.5×10³/μL, respectively (Figure 37). These results indicate that the miniaturized 5CB droplet-based sensor platform is more sensitive for DCA over CA. The pKₐ is 6.2 for DCA and 5.2 for CA, respectively. In PBS solution with pH 7.4, both DCA and CA are negatively charged. The critical micelle concentration (CMC) is 10 mM for DCA and 13 mM for CA, respectively. The concentration of CA and DCA used in our experiments is lower than their CMCs. Although DCA and CA share a common steroid backbone, the number of the hydroxyl groups at their steroid backbone is different. It has been shown that the binding constant
of CA and DCA for β-CD is ~ 2.2×10^3 M^{-1} and ~ 3.5×10^4 M^{-1}, respectively. Thus, DCA should be more effective to displace the SC_{14}S from the cavity of the sulfated β-CD immobilized at the 5CB/aqueous interface, giving the lower detection limit and the faster response time, compared to CA. The detection limit of the miniaturized 5CB droplet-based sensor platform for CA and DCA is higher than that of chromatography-mass spectrometry methods, but comparable with that of electrochemical sensors. The advantage of the miniaturized 5CB droplet-based sensor platform is simple and fast without needing expensive instruments and large sample volume.

Figure 37. Detection limit of PDADMAC/SC_{14}S/sulfated β-CD complex-coated 5CB droplets for CA and DCA in PBS solution as a function of droplet densities in microfluidic channel with/without 500 μM and 500 μM AA.

In biological fluids, ascorbic acid (AA) and uric acid (UA), which coexist with bile acids in much high concentrations, are major interference species for the detection of bile acids. However, we find that there is no radial-to-bipolar configuration transition for PDADMAC/SC_{14}S/sulfated β-CD complex-coated 5CB droplets in the channel after the addition
of 500 μM AA and 500 μM UA, respectively (Figure 38). This suggests that AA and UA are unable to remove the SC14S from the cavity of the sulfated β-CD immobilized at the 5CB/aqueous interface due to their low binding constants with β-CD ($2 \times 10^{-3}$ M$^{-1}$) and without necessary amphiphilic nature toward to the 5CB/aqueous interface. Finally, we measured the response time for CA and DCA in the presence of 500 μM UA and 500 μM AA in PBS solution, respectively. In our experiments, bile acid solution with UA and AA was pipetted into the microfluidic channel hosting PDADMAC/SC14S/sulfated β-CD-5CB droplets with the density of $2.5 \times 10^{4}$/μL. There is no significantly difference observed in the response time with/without UA and AA (Figure 36). Also the detection limit for CA and DCA remains unchanged in the presence of 500 μM UA and 500 μM AA (Figure 37), suggesting that the detection for bile acids is specific in the presence of UA and AA.

Figure 38. Polarizing optical microscopy images of PDADMAC/SC14S/sulfated β-CD complex-coated 5CB droplets with the droplet density of $2.5 \times 10^{4}$/μL in a microfluidic channel after the addition of 500 μM AA (a) and 500 μM UA (b) for 60 min.

4.4 Conclusion

We fabricate a miniaturized 5CB droplet-based sensor platform, in which 5CB droplets are stabilized by the adsorption of PDADMAC/SC14S/sulfated β-CD complexes, followed by
integrating the stabilized 5CB droplets into a microfluidic channel. The miniaturized sensor platform allows the real-time and selective detection of CA and DCA in a small amount of solution with the interference species of AA and UA by simply observing the director configuration transition of the 5CB droplets with the naked eye under a polarizing optical microscope. We show that the miniaturized 5CB droplet-based sensor platform shows the shorter response time and the lower detection limit for DCA bearing two hydroxyl groups, compared to CA with three hydroxyl groups. Furthermore, the detection limit for DCA and CA can be altered by the density of the 5CB droplets integrated in microfluidic channels. Unlike stabilized 5CB emulsions, the miniaturized 5CB droplet-based sensor platform is easily handled, allowing the rapid and real-time detection of bile acids (< 2 min) in a small sample volume (1μL), which are highly desirable for the "point-of-care" analysis of bile acids.
CHAPTER 5 LIQUID CRYSTAL DROPLET-EMBEDDED HYDROGELS FOR REAL-TIME DETECTION APPLICATIONS

5.1. Introduction

Recently, polymer-stabilized LC droplets in aqueous solution have been developed as a simple and label-free optical probe for the detection of chemical and biological species.\textsuperscript{43, 51, 54-55, 58-59, 61, 63} For example, Abbott and coworkers reported the use of poly(methacrylic acid)/poly(N-vinylpyrrolidone) multilayer-stabilized LC droplets for the detection of lipid-enveloped viruses, in which the lipid transferred from the viruses to the polyelectrolyte multilayer-stabilized LC droplets induced the bipolar-to-radial configuration transition of the LC droplets.\textsuperscript{63} Yang and coworkers designed an immune sensor based on polyethylene imine/surfactant complex-coated LC droplets, in which the formation of anti-IgG/IgG complexes at the surface of the LC droplets disrupted the packing of the surfactant and consequently induced the radial-to-bipolar transition of the LC droplets.\textsuperscript{59} Fang and coworkers reported the formation of LC droplets in aqueous solution by the adsorption of chitosan (CS) at the LC-aqueous interface, followed by the penetration of surfactants.\textsuperscript{108} The CS/surfactant complex-coated LC droplets could be used for the detection of bile acids, a biomarker for the clinical diagnosis of liver and intestinal diseases. Although polymer-stabilized LC droplets provide a simple and label-free optical probe, the response time of LC-based sensing platform is largely unknown because the mobility of the LC droplets in aqueous solution limits their applications for the real-time detection of chemical and biological species.

Hydrogels are a 3-D cross-linked network structure formed by hydrophilic polymers. They are able to adsorb a significant amount of water and highly permeable for biomolecules. Due to
their viscoelastic properties, hydrogels can be shaped or casted into different sizes and shapes. These unique features make hydrogels highly desirable for biomedical applications.\textsuperscript{109-110}

Chitosan, a linear polysaccharide, consists of randomly distributed units of $\beta$-1,4 linked D-glucosamine and N-acetyl-D-glucosamine (Figure 39). The biocompatible nature of chitosan makes it widely used in tissue engineering and drug delivery applications.\textsuperscript{111} The interaction of lipids and bile acids with CS has been proposed to play an important role for reducing cholesterol in the small intestine.\textsuperscript{112-113} Recent studies have shown that CS hydrogels can be simply and rapidly formed from CS aqueous solution by the complexation of transition metal ions and CS chains,\textsuperscript{110} which provides us an opportunity to integrate LC droplets into CS hydrogels.

Agarose is also a linear polysaccharide as chitosan, composed of alternating units of $\beta$-1,3-linked-D-galactose and $\alpha$-1,4-linked 3,6-anhydro-L-galactose (Figure 39). It can be only dissolved in the water at high temperature and can form a hydrogel through intermolecular hydrogen bonding upon cooling to around 35$^\circ$C.\textsuperscript{114} No other reagent is required for the hydrogel formation and the agarose hydrogel matrix is neutral.

In this chapter, we develop a new sensing platform of LC droplets by immobilizing LC droplets in the chitosan and agarose hydrogel networks to form a hydrogel film. With those hydrogel films, surfactants with different headgroups and chain length were adopted to functionalize LC droplets in the hydrogel network and the configuration transition of LC droplets after the addition of surfactants was observed in real-time under the polarizing optical microscope. Compared with previous sensing platforms, this LC droplet-embedded hydrogel system is freestanding, simple, fast-fabricated, portable, and allows real-time observation.
5.2. Experimental

5.2.1 Materials

Chitosan (CS, low molecular weight), agarose, silver nitrate (AgNO₃), agarose, acetic acid, 4-cyano-4'-pentylbiphenyl (5CB, 98% purity), sodium alkyl sulfate (SCₙS, n=8, 12, and 14, 99% purity), and alkyl trimethylammonium bromide (CₙTAB, n=8, 12, and 14, 99% purity) were obtained from Sigma-Aldrich (St. Louis, MO). All chemicals were used without further purification. Water used in our experiments was purified with Easypure II system (18.2 MΩ cm and pH 5.7).

![Chemical structures of CₙTAB, SCₙS, agarose and chitosan](image)

**Figure 39. Chemical structures of CₙTAB, SCₙS, agarose and chitosan**

5.2.2 Preparation of CS-Stabilized 5CB Emulsions

2wt% CS solution was prepared by dissolving 2g CS powder in 100mL 0.5% acetic acid aqueous solution at pH 5.43 under magnetic stirring overnight. CS-stabilized 5CB droplet emulsion was prepared by adding 10μL 5CB into 10mL of as-prepared 2wt% CS aqueous solution, followed by the tip sonication (FB505, Fisher Scientific, Pittsburg, PA) at the amplitude of 20% for 10s at room temperature.
5.2.3 Preparation of Agarose-Stabilized 5CB Emulsions

The agarose-stabilized 5CB emulsion was prepared as follows: first, 0.1 g agarose powder was dissolved in 10 mL water at 70 °C with magnetic stirring until the solution became transparent to get 1wt% agarose aqueous solution. Then, 1 μL 5CB was quickly mixed with 1 mL of the as-prepared 1wt% agarose solution with tip sonication (FB505, Fisher Scientific, Pittsburg, PA) at the amplitude of 20% for 10s at the room temperature to form agarose-stabilized 5CB droplet emulsion.

5.2.4 Preparation of 5CB Droplet-Embedded CS and Agarose Hydrogel Films

The 5CB droplet-embedded CS hydrogel film was formed by casting the CS-stabilized 5CB emulsion on a glass substrate, followed by immersing the emulsion casted glass substrate into 0.3M AgNO₃ aqueous solution for 10s. The thickness of the resultant 5CB-embedded CS hydrogel film was ~0.2 mm.

The 5CB droplet-embedded agarose hydrogel film was formed by quickly casting the agarose-stabilized 5CB emulsion on a glass substrate on a hot plate at 38 °C and then cooled it at 4°C for 10 min to let it form 5CB droplet-embedded agarose hydrogel films. The thickness of the 5CB droplet-embedded agarose hydrogel film was ~0.2mm.

5.2.5 Characterization

The configuration of 5CB droplets was characterized with a polarizing optical microscope (Olympus BX40) in transmission mode. The structure of CS hydrogel films placed on carbon coated grids was characterized with a transmission electron microscope (JEOL TEM-1011, 100
kV). Tensile testing of CS hydrogel sheets was carried out with a micro-tensile tester (Tytron 250, MTS Systems Corporation) at room temperature with a loading cell of 5N and a loading speed is 0.1 mm/min. The size of sample for tensile testing is 10mm × 2mm.

The viscoelastic properties of CS and agarose hydrogels were measured with a rotational rheometer (TA Instruments, AR2000ex). The hydrogel sample was loaded on a 40mm parallel plate with 1mm gap at 25°C. An amplitude sweep was first carried out to determine the linear viscoelastic region of the hydrogel sample. 1% strain was chosen for frequency sweep. Then the frequency sweeps were measured from 0.1 to 100 rad/s with 1% strain with oscillation mode.

5.2.6 Detection of Surfactants with 5CB Droplet-Embedded Hydrogel Sheets

The 5CB droplet embedded-CS and agarose hydrogel films were cut into 10mm×2mm×0.2mm sheet and was then placed onto the 10mm diameter glass bottom of a culture dish (MatTeck Corporation, MA). The configuration transitions of 5CB droplets embedded in the hydrogel sheets were in-situ monitored under a polarizing optical microscope after the addition of surfactant aqueous solution. The response time was recorded when the configuration of 5CB droplets in the focus plane showed a bipolar-to-radial configuration transition.

5.3 Results and Discussion

The CS-stabilized 5CB droplet emulsion shows a bipolar configuration under the polarizing optical microscope (Figure 40a), suggesting a parallel surface anchoring of 5CB within LC droplets (Figure 41b), which is consistent with our previous results. The adsorption of CS at the LC/aqueous interface can stabilize 5CB droplets in aqueous solution. Recent studies have
shown that transition metal ions can fast cross-link the chitosan chains through coordinate bonding with the amino and hydroxyl groups on the chitosan,\textsuperscript{110} which provides a way to integrate 5CB droplets into CS hydrogels. The bipolar configuration was preserved after the gelation of the CS-stabilized 5CB droplet emulsion by Ag\textsuperscript{+} (Figure 40b), which indicates the fast gelation process does not disrupt the ordering of 5CB molecules within the droplets.

![Polarizing optical microscopy images of CS-stabilized 5CB droplet emulsion before (a) and after (b) gelation with Ag\textsuperscript{+}](image1)

**Figure 40.** Polarizing optical microscopy images of CS-stabilized 5CB droplet emulsion before (a) and after (b) gelation with Ag\textsuperscript{+}

![Chemical structures of 5CB and CS and schematic illustrations of the formation of 5CB droplet-embedded CS hydrogel films by the Ag\textsuperscript{+} ion-triggered fast gelation of CS-5CB emulsion](image2)

**Figure 41.** (a) Chemical structures of 5CB and CS and (b-d) schematic illustrations of the formation of process of 5CB droplet-embedded CS hydrogel films by the Ag\textsuperscript{+} ion-triggered fast gelation of CS-5CB emulsion.
The CS hydrogel film (Figure 42a) formed in less than 1 minute after immersing the CS-stabilized 5CB emulsion casted on a glass substrate into 0.3M AgNO₃ aqueous solution for 10 s. Transmission electron microscopy measurements show that the CS hydrogel film consists of the network of CS nanofibers with an average diameter of 23.7 ± 6.4 nm (Figure 42b), which are cross-linked by Ag⁺ ions through complexing with the -NH₂ and -OH groups on CS chains (Figure 41d). The mechanical properties of the 5CB droplet-embedded hydrogel sheets were studied with tensile testing which was conducted in room temperature at the constant loading speed of 0.1 mm/min. Figure 42c shows a representative strength-strain curve of the 5CB droplet-embedded CS hydrogel sheets. The tensile strength of the 5CB droplet-embedded hydrogel sheets is 125 kPa with the ultimate strain of ~ 95%. The extensibility of CS hydrogel sheets is higher than that of collagen hydrogels (~80%). The Young’s modulus of CS hydrogel sheets is ~ 63.92 kPa, which is also higher than that collagen hydrogels (20kPa). Figure 42d shows the viscoelastic behaviors of CS hydrogels coordinated with Ag⁺. The CS hydrogel shows a much larger G’ than G”, suggesting the hydrogel is not changed during the sweep range of frequency. The good properties of 5CB droplet-embedded CS hydrogels make them a suitable sensing platform for detection applications.

The mobility of 5CB droplets are confined within the cavities of the CS hydrogel network, providing us a way to in-situ monitor the configuration transitions of 5CB droplets induced by the interactions at the LC/aqueous interface. Figure 43 shows the time-course polarizing optical microscopy images of the 5CB droplet-embedded CS hydrogel sheet after the addition of 20 μM SC₁₄S. Initially, the 5CB droplet showed a bipolar configuration with two defect points at the poles of the droplet (Figure 43a). Then the two defect points were disappeared and a defect point showed
up staying away from the center of the droplet (Figure 43b), leading a escaped-radial director configuration. Then this defect gradually moved toward the center the 5CBd droplet (Figure 43b-g). Finally, it formed a radial configuration with a defect point at the center of the 5CB droplet. Previous studies have shown that the interaction between the hydrophobic tails and the liquid crystal molecules is important in dictating the homeotropic anchoring of liquid crystals at the LC/aqueous interface. Fang and coworkers have also shown it is the hydrophobic tails of surfactants penetrating the chitosan coating to interact with the 5CB cores that induced a bipolar-to-radial configuration transition of CS-stabilized 5CB emulsion. Therefore, we can conclude that the bipolar-to-radial configuration transition is the result of the adsorption of SC\textsubscript{14}S at the surface of CS- stabilized 5CB droplets, where the hydrophobic tails of SC\textsubscript{14}S penetrate the CS network and extends into the 5CB droplets to induce the re-orientation of 5CB molecules inside the droplets.

![Figure 42](image)

**Figure 42.** (a) Photography, and (b) TEM images of 5CB droplet embedded CS hydrogel film; (c) strength-strain curve, and (d) rheological properties of 5CB droplet-embedded CS hydrogel films. The photography image of the hydrogel sheets cut from the hydrogel films was inset in (c). The scale bar shown in the inset image is 300 mm.
Figure 43. Polarizing optical microscopy images of 5CB-embedded CS hydrogel sheets taken at 0 s (a), 8 s (b), 31 s (c), 36 s (d), 60 s (e), 67 s (f), 72 s (g), and 74 s (h) after the addition of 20 μM of SC14S.

We find the response time increases from 54 s to 2.11 min when the concentration of SC14S decreases from 40 μM to 8 μM (Figure 44a). It has been shown that a critical density of surfactant adsorbed at the LC/aqueous interface is required to trigger the bipolar-to-radial configuration transition of liquid crystals. The higher concentration of SC14S, the more SC14S molecules will approach the surface of CS-stabilized 5CB droplets in a short time, which will trigger the bipolar-to-radial configuration transition faster. For different hydrophobic tail length of SCnS, the response time of the 5CB droplet-embedded CS hydrogel also show a concentration dependence (Figure 44a). The concentration required to induce a bipolar-to-radial configuration transition for SCnS increases from 4 μM for n=14 to 18 μM for n=12 (Figure 44c). The chain length of surfactants is thought to affect the anchoring between 5CB cores and surfactants. Surfactants with longer hydrophobic chains can extend deeper into the liquid crystal phase, thus have a stronger interaction with liquid crystal molecules to re-orientate liquid crystal molecules. Therefore, only 4 μM SC14S can trigger a bipolar-to-radial configuration transition of 5CB droplets within CS hydrogels, while
the concentration of SC<sub>12</sub>S required to induce the bipolar-to-radial configuration transition is 18 μM.

![Figure 44](image)

**Figure 44. Concentration dependent response time of 5CB droplets in 5CB-embedded CS hydrogel to (a) SC<sub>n</sub>S; and (b) C<sub>n</sub>TAB; (c) detection limit of 5CB droplets in 5CB-embedded CS hydrogel as a function of chain length**

Previous studies have shown that surfactant headgroups have few effect on the anchoring transition of liquid crystals, they only determine the adsorption density of surfactants at the LC/aqueous interface via headgroup-headgroup repulsion. Figure 44b shows the response time of 5CB droplet-embedded CS hydrogel sheets to C<sub>n</sub>TAB. The chain length dependence and concentration dependent response time are also observed for 5CB droplet-embedded CS hydrogel sheets exposed to C<sub>n</sub>TAB (Figure 44b). However, the concentration of C<sub>n</sub>TAB required to trigger a bipolar-to-radial configuration transition of 5CB droplets embedded in the CS hydrogel sheet is higher than that of SC<sub>n</sub>S. And the response time for C<sub>n</sub>TAB is also longer than that for SC<sub>n</sub>S at the same concentration level. The pKa of CS is around 6.5. The pH for chitosan solution adopted to prepare CS hydrogels is 5.43, at which most of the amino groups on the CS chain are positive charged even though the coordinate bonding formed between Ag<sup>+</sup> and amino groups can screen some positive charged amino groups. Thus, we hypothesize that the electrostatic repulsion between positively charged CS network and the positively charged headgroups of C<sub>n</sub>TAB prevents C<sub>n</sub>TAB from approaching toward the surface of 5CB droplets. In contrast, the electrostatic attraction
between positively charged CS network and negatively charged SC\textsubscript{n}S facilitates SC\textsubscript{n}S toward the surface of 5CB droplets. Therefore, the 5CB droplet-embedded CS hydrogels show less efficient responding to C\textsubscript{n}TAB than SC\textsubscript{n}S. This response difference was also reported by Fang and coworkers\textsuperscript{118}, in which higher concentrations of C\textsubscript{n}TAB than SC\textsubscript{n}S were required to trigger the bipolar-to-radial configuration transition of CS-stabilized 5CB droplet emulsions.

To eliminate the influence of electrostatic interactions on the response of 5CB droplet-embedded CS hydrogel sheets to the surfactants. We integrated 5CB droplets into agarose hydrogel sheets. The preparation of 5CB droplet-embedded agarose hydrogel is as follows: 0.1 g agarose powder was dissolved in 10 mL water at 70 °C with magnetic stirring until the solution became transparent to get 1% agarose aqueous solution; then 1 μL 5CB was quickly mixed with 1 mL of the as-prepared 1% agarose solution with tip sonication (FB505, Fisher Scientific, Pittsburg, PA) at the amplitude of 20\% for 10s to form agarose-stabilized 5CB droplet emulsion at room temperature; at last the agarose-stabilized 5CB emulsion was quickly casted on the glass substrate at a hotplate with the temperature of 38 °C and cool it at 4°C for 10 min to let it form 5CB droplet-embedded agarose hydrogel films. The 5CB droplet-embedded agarose hydrogel film was cut into small sheets with same size as 5CB droplet-embedded CS hydrogel sheets to put it onto the bottom of culture dishes for experiment.

The 5CB droplets in the agarose hydrogel show a bipolar configuration (Figure 45a). Figure 45b shows the rheological properties of 1wt\% agarose hydrogel. The G’ is larger than G” in the all sweep range of frequency, indicating the structure of agarose hydrogel is not disrupted at that frequency range. The good properties of agarose hydrogels make them a good matrix to confine 5CB droplets for sensing applications.
Figure 45. (a) Polarizing optical microscopy image of 5CB droplet-embedded in agarose hydrogel (b) Rheological properties of 1wt% agarose hydrogel; concentration dependent response time of 5CB droplet-embedded agarose hydrogel to (c) SC<sub>14</sub>S; and (d) C<sub>14</sub>TAB

Without electrostatic attraction, much higher concentration (180 μM) of SC<sub>14</sub>S is required to trigger a bipolar-to-radial configuration transition of 5CB droplets inside the agarose hydrogel (Figure 45c), which is 22.5 times higher than that of CS hydrogels. However, the concentration of C<sub>14</sub>TAB required for a bipolar-to-radial configuration transition of 5CB droplets shows a dramatic decrease (Figure 45d), from 40 μM for CS hydrogel sheets to 4 μM for agarose hydrogel sheets. Interestingly, on the contrary, the response of 5CB droplet-embedded agarose hydrogel sheets to C<sub>14</sub>TAB is much more efficient than to SC<sub>14</sub>S. It has been shown that C<sub>n</sub>TAB is more surface active than SC<sub>n</sub>C, suggesting C<sub>n</sub>TAB prefers to the interface than SC<sub>n</sub>S. Given these results, we can also conclude that the electrostatic interaction plays an important role.
role dictating the adsorption of surfactant at the surface of 5CB droplets, which provides a guide to choose hydrogel matrix for the detection of bile acids.

5.4 Conclusion

In this chapter, a method to immobilize 5CB liquid crystal droplets in chitosan and agarose hydrogel films was presented. Both CS and agarose hydrogel films can serve as a portable sensing platform for real-time detection applications by confining the 5CB droplets within the hydrogel network. Surfactants with different headgroups and different length of hydrogel tails were adopted to functionalize 5CB droplets in the hydrogel network. Surfactants with a longer hydrophobic tail can trigger a bipolar-to-radial configuration transition of 5CB droplets at a lower concentration. The high charge density of the hydrogel network is found to play an important role in detection applications, which provides a guide for us to design 5CB droplet-embedded hydrogel platforms for the detection of bile acids.
6.1 Introduction

Based on the results in last chapter, in this chapter, we developed a 5CB droplet-embedded CS hydrogel film by the Ag⁺ ion-triggered gelation of CS/surfactant complex-stabilized 5CB emulsions casted on substrates. The formation process of 5CB droplet-embedded CS hydrogel is shown in Figure 46. The sheets cut from the 5CB droplet-embedded CS hydrogel films are used as a portable and label-free sensing platform for the real-time detection of bile acids in a small amount of solution by simply observing the director configuration transition of the embedded 5CB droplets induced by the competitive adsorption of the bile acid penetrated through in the CS matrix.

6.2 Experimental

6.2.1 Materials

Chitosan (CS, low molecular weight), silver nitrate, 4-cyano-4'-pentylbiphenyl (5CB, 98% purity), cholic acid (CA, ≥ 98% purity), deoxycholic acid (DCA, ≥ 98% purity), tetradecyl sulfate sodium salt (SC_{14}S), alkyl trimethylammonium bromide (C_{n}TAB, n=12, and 14, 99% purity), and sodium hydroxyl (NaOH) were obtained from Sigma-Aldrich (St. Louis, MO). All chemicals were used without further purification. Water used in our experiments was purified with Easypure II system (18.2 MΩ cm and pH 5.7).
6.2.2 Preparation of CS/Surfactant-Stabilized 5CB Droplets Emulsions

2wt% CS solution was prepared by dissolving CS in 0.5% acetic acid solution under magnetic stirring. CS-stabilized 5CB droplet emulsion was prepared by adding 10 μL 5CB in 10 mL of 2 wt% CS aqueous solution, followed by the tip sonication (FB505, Fisher Scientific, Pittsburg, PA) at the amplitude of 20% for 10s at room temperature. The CS-stabilized 5CB droplets showed a bipolar configuration that transit to a radial configuration after the addition of specific concentration of surfactants in CS-5CB emulsions.

6.2.3 Preparation of 5CB Droplet-Embedded CS Hydrogel Films

The formation process of 5CB droplet-embedded CS hydrogel films is as follows: First, the as-received emulsion was casted on a glass substrate. The 5CB droplet-embedded CS hydrogel film was formed by immersing the emulsion casted glass substrate in 0.3M AgNO₃ solution for 10s. The thickness of the resultant CS hydrogel film was measured to be ~ 0.2 mm.

6.2.4 Characterization

The director configuration of 5CB droplets was analyzed with a polarizing optical microscope (Olympus BX40) in transmission mode.

6.2.5 Detection of Bile Acids with 5CB Droplet-Embedded CS Hydrogel Sheets

The 5CB-embedded CS hydrogel film was cut into a small sheet (10 mm length × 2 mm width × 0.2mm thickness), that was then placed on the bottom of a small glass dish (MatTek Corporation, MA, USA) with 90 μL aqueous solution at pH 7.4. The director configuration of
the 5CB droplets embedded in CS hydrogel sheets was in situ monitored with a polarizing optical microscope in transmission mode after the addition of CA and DCA, respectively.

Figure 46. (a) Chemical structures of 5CB and CS. (b-e) Schematic illustrations of the formation process of 5CB droplet-embedded CS hydrogel films by the Ag⁺ ion-triggered fast gelation of CS/SC₁₄S stabilized 5CB emulsion.

6.3 Results and Discussion

Figure 47a shows the polarizing optical microscopy image of CS-stabilized 5CB droplet emulsion, which was formed by adding 10 μL 5CB in 10 mL of 2 wt% CS aqueous solution, followed by sonication. The adsorption of CS at the 5CB-aqueous interface stabilize the 5CB droplets in aqueous solution. The CS-stabilized 5CB droplets show a bipolar configuration, suggesting a parallel surface anchoring of the 5CB inside the droplets (Figure 46b). After the addition of 100 μM SC₁₄S, the director configuration of the CS-stabilized 5CB droplets becomes radial (Figure 47b), suggesting a perpendicular surface anchoring of the 5CB inside the droplets.
(Figure 46c). The bipolar-to-radial configuration transition is a result of the adsorption SC\textsubscript{14}S at the surface of CS-stabilized 5CB droplets, in which the negatively charged head group of the SC\textsubscript{14}S located in the positively charged CS coating by electrostatic attraction, while the hydrophobic tail of the SC\textsubscript{14}S extended into the 5CB droplets to induce the radial configuration. In a previous publication, we showed that 5CB droplets could be stabilized in 0.1wt% CS aqueous solution.\textsuperscript{108} Therefore, there is a large amount of excess CS in the aqueous phase of the CS/SC\textsubscript{14}S complex-stabilized 5CB emulsion formed in 2wt% CS aqueous solution. To gel the CS/SC\textsubscript{14}S complex-stabilized 5CB emulsion, the viscous emulsion was casted on a glass substrate for form an emulsion film, followed by being immersed in 0.3M AgNO\textsubscript{3} solution for 10s, in which Ag\textsuperscript{+} ions complex with the \textsuperscript{-NH\textsubscript{2}} and \textsuperscript{-OH} groups on the CS presented in the aqueous phase and at the surface of the 5CB droplets, leading to the formation of a 5CB droplet-embedded CS hydrogel film (Figure 46d).

![Figure 47](image)

**Figure 47.** Polarizing optical microscopy images of CS-stabilized 5CB droplets dispersed in aqueous solution at pH 4.05 before (a) and after (b) addition of 100 µM SC\textsubscript{14}S; (c) Polarizing optical microscopy image of SC\textsubscript{14}S/5CB droplet-embedded CS hydrogel film.

The 5CB droplet-embedded hydrogel film can be peeled off from the glass substrate. The embedded 5CB droplets remain a radial director configuration in the CS hydrogel film (Figure
The good mechanical properties of 5CB droplet-embedded hydrogel sheets make them a suitable sensing platform for the real-time detection of bile acids, in which the director configuration transition of the embedded 5CB droplets induced by the competitive adsorption of the bile acid penetrated through in the CS matrix. The advantages of using the 5CB droplet-embedded hydrogel sheets is that only a small amount of bile acid solution is required.

In our experiments, a 5CB droplet-embedded hydrogel sheet was placed on the bottom of a small glass dish with 90 μL aqueous solution at pH 7.4. Figures 48a-48f show the time-course polarizing microscopy images of the 5CB droplets embedded in the hydrogel sheet after the addition of 200 μM DCA in aqueous solution. Initially, the embedded 5CB droplets show a radial director configuration with a single defect point at the center of the droplets (Figure 48a). The defect point shifts away from the center over time (Figures 48b-48d), leading to a pre-radial director configuration. Finally, the director configuration of the embedded 5CB droplets turns into bipolar with two defect points at the poles of the droplets (Figures 48e-48f). The radial-to-bipolar transition of the embedded 5CB droplets is a result of the competitive adsorption of the DCA at the surface of the droplets (Figure 49). It is known that bile acids are facial amphiphilic molecules that are extremely surface active. They are able to displace other surface active molecules from the oil-water interface during fat digestion through the competitive adsorption. The surface anchoring energy of the 5CB inside the droplets embedded in the CS hydrogel films depends on the packing and density of the SC$_{14}$S at the surface of the droplets. The observed radial-to-bipolar
transition of the embedded 5CB droplets after the addition of DCA (Figure 48) indicates that the CS hydrogel matrix is permeable for DCA. The competitive adsorption of the penetrated DCA disrupts/displaces the SC14S from the surface of the embedded 5CB droplets, inducing the radial-to-bipolar configuration transition.

We find that the response time of the 5CB droplet-embedded hydrogel sheet for DCA is 30s when the concentration of DCA in aqueous solution is in the range from 400 μM to 100 μM, followed by the sharp increase from 30s to 6.2 min when the concentration of DCA is reduced from 100 μM to 10 μM (Figure 48g). There is no configuration transition of the embedded 5CB droplets observed for prolonged time periods if the concentration of DCA is lower than 10 μM (defined as a detection limit). The concentration dependence of the response time of the 5CB droplet-embedded hydrogel sheet is also observed for CA (Figure 48g). However, the response time for CA is much longer than that for DCA. For example, the response time of the 5CB droplet-embedded hydrogel sheet is 30s for DCA and 4 min for CA at the same concentration of 100 μM. In addition, the detection limit of the 5CB droplet-embedded hydrogel sheet for CA is 40 μM, which is four times higher than that for DCA (Figure 48h). These results suggest that 5CB droplet-embedded hydrogel sheets are more sensitive for DCA. The pKₐ is 6.2 for DCA and 5.2 for CA, respectively. 69 At pH 7.4, both DCA and CA are negatively charged. The critical micelle concentration (CMC) is 10 mM for DCA and 13 mM for CA, respectively. 103 The concentration of CA and DCA used in our experiments is lower than their CMCs. Although DCA and CA share a common steroid backbone, the number of the hydroxyl groups at their steroid backbone is different (see the inset in Figure 48g). It has been shown that the hydrophobicity of DCA bearing two hydroxyl groups is higher than that of CA bearing three hydroxyl groups. 103 The more
hydrophobic DCA should be more effective in disrupting/displacing the SC$_{14}$S from the surface of the embedded 5CB droplets. This may explain why the 5CB droplet-embedded hydrogel sheets show shorter response time and lower detection limit for DCA, compared to CA.

Figure 48. (a-f) Polarizing optical microscopy images of 5CB droplet-embedded CHI hydrogel sheets taken at 0s (a), 8s (b), 12s (c), 17s (d), 20s (e), and 23s (f) after the addition of 200 μM DCA. (g) The response time of the SC$_{14}$S/ CB droplet-embedded CS hydrogel sheets as a function of DCA and CA concentrations, respectively. (h) The detection limit of the the SC$_{14}$S/ CB droplet-embedded CS hydrogel sheets for DCA and CA. The chemical structures of DCA and CA were inset in 3g.
Figure 49. Schematic illustrations of the competitive adsorption of bile acid-induced radial-to-bipolar configuration transition of SC₁₄S/5CB droplet-embedded CS hydrogel films.

From last chapter, it is known that electrostatic interactions between the hydrogel matrix and the analyte play an important role in the detection results. The pKa of CA and DCA are around 5.2 and 6.2, respectively. At pH 7.4, most of CA and DCA molecules are negatively charged. We expect that CₙTAB/5CB droplet-embedded CS hydrogels are more sensitive for CA and DCA than SCₙS/5CB droplet-embedded CS hydrogels due to the electrostatic attraction between the positively charged headgroups of CₙTAB and negatively charged bile acids. Thus, 100μM C₁₄TAB were used to prepared C₁₄TAB/5CB droplet-embedded CS hydrogel films follow the same procedures as the preparation of SC₁₄S/5CB droplet-embedded CS hydrogel films.

The concentration dependence of response time to DCA and CA was also observed for CₙTAB/5CB droplet-embedded CS hydrogel sheets to DCA at pH 7.4 (Figure 50a). The response time of C₁₄TAB/5CB droplet-embedded CS hydrogel sheets for DCA increased from 16.2 s to 1.7 min when the concentration of DCA decreased from 10 μM to 2 μM (Figure 50a). The response time C₁₄TAB/5CB droplet-embedded CS hydrogel sheets for CA increased from 25.5 s to 2.1 min when the concentration of CA decreased from 50 μM to 10μM (Figure 50b). Compared with the
SC$_{14}$S/5CB droplet-embedded CS hydrogel sheets, C$_{14}$TAB/5CB droplet-embedded CS hydrogel sheets showed more sensitive to both DCA and CA. The detection limit of C$_{14}$TAB/5CB droplet-embedded CS hydrogel sheets for DCA and CA are 2 μM and 10 μM, respectively (Figure 51), while the detection limit of SC$_{14}$S/5CB droplet-embedded CS hydrogel sheets for DCA and CA are 10 μM and 40 μM, respectively (Figure 48h). The response of C$_{14}$TAB/5CB droplet-embedded CS hydrogel sheets for DCA and CA is also faster than that of SC$_{14}$S/5CB droplet-embedded CS hydrogel sheets. For example, for 10μM DCA, the response time of the C$_{14}$TAB/5CB droplet-embedded CS hydrogel sheet is 16.2s, while the response time of the SC$_{14}$S/5CB droplet-embedded CS hydrogel sheet is 6.2 min. Thus, it is reasonable for us to infer that the positively charged C$_{14}$TAB facilitates the approach of negatively charged DCA and CA toward the surface of 5CB droplets through electrostatic attractions, which makes C$_{14}$TAB/5CB droplet-embedded CS hydrogel sheets more sensitive than SC$_{14}$S/5CB droplet-embedded CS hydrogel sheets for DCA and CA.

![Figure 50. The response time of C$_n$TAB/5CB-embedded 5CB droplet-CS hydrogel sheets as a function of (a) DCA, and (b)CA concentrations.](image-url)
It is also known from last chapter that surfactants with shorter alkyl chain length are less efficient to induce a radial configuration of 5CB droplets within the CS hydrogel network. Thus, we expect that surfactants with shorter alkyl chain length may be easily disrupted by bile acids to trigger a radial-to-bipolar configuration of 5CB droplets within the CS hydrogel network. 1.5 mM C\textsubscript{12}TAB was used to stabilize a radial configuration of the CS-5CB droplet emulsion and the as-received emulsion was further formed C\textsubscript{12}TAB/5CB droplet-embedded CS hydrogel films. The concentration dependence of the response time of C\textsubscript{12}TAB/5CB droplet-embedded CS hydrogel sheets was also observed for DCA and CA (Figure 50). We found the sensitivity of C\textsubscript{12}TAB/5CB droplet-embedded CS hydrogel sheets for DCA and CA was further improved compared with C\textsubscript{14}TAB/5CB droplet-embedded CS hydrogel sheets. The detection limit of C\textsubscript{12}TAB/5CB droplet-embedded CS hydrogel sheets for DCA and CA at pH 7.4 is 0.6 μM and 3 μM, respectively (Figure 51).
Ascorbic acid (AA) and uric acid (UA), which coexist with bile acids in biological fluids, are major interference species for the detection of bile acids. However, we find that 5CB droplet-embedded hydrogel sheets are insensitive to both AA and UA. There is no configuration transition of the embedded 5CB droplets observed after the addition of 1mM AA or 1mM UA in aqueous solution (Figure 52). The selectivity of 5CB droplet-embedded hydrogel sheets for bile acids over AA and UA should associate with their different amphiphilic nature. Due to lacking amphiphilic nature, AA and UA are unable to disrupt the SC<sub>14</sub>S packing at the surface of the embedded 5CB droplets.

![Figure 52. Polarizing optical microscopy images of 5CB droplet-embedded CS hydrogel sheets after the addition of 1mM AA (a) and 1mM UA (b).](image)

**6.4 Conclusion**

In this chapter, we presented the formation of surfactant/5CB droplet-embedded CS hydrogel films by the Ag<sup>+</sup> ion-triggered fast gelation of the CS/surfactant stabilized 5CB emulsion casted on substrates. The sheets cut from the 5CB droplet-embedded hydrogel films can serve as a portable and label-free sensing platform for the detection of bile acids without needing complex detection systems for signal transitions. Unlike 5CB droplet emulsions, the 5CB droplet-embedded hydrogel sheets are easily handed and more stable, allowing the real-time and selective detection
of bile acids. We find that the response time and detection limit of 5CB droplet-embedded hydrogel sheets for bile acids relate to the hydrophobicity of bile acids and the headgroups and alkyl chain length of surfactant. C_{12}TAB with positively charged headgroups and short alkyl chain length makes the C_{12}TAB/5CB droplet-embedded CS hydrogel for the detection of bile acids with a high sensitivity and a fast response. The low cost, portability and simplicity of LC droplet-embedded hydrogel films are highly desirable for the "point-of-care" analysis of bile acids.
CHAPTER 7 LIQUID CRYSTAL DROPLET-EMBEDDED AGAROSE HYDROGELS FOR THE DETECTION OF BILE ACIDS

7.1 Introduction

From last chapter, the portable and label-free 5CB droplet-embedded CS hydrogel platforms show great potential in the real-time detection of bile acids. However, we find the stability of 5CB droplet-embedded CS hydrogels becomes an issue when applied the 5CB droplet-embedded CS hydrogels into PBS solution, the properties and ion concentrations of which match those of the human body fluids. The PBS solution includes a large of amount of phosphates and chloride salts, which can form sediments with silver ions. The silver ion is the linker to non-covalently cross-link the chitosan chains through the coordination bonding. The formation of sediments of silver ions with phosphates and chloride salts in PBS solution can lead to the dissociation of the CS hydrogels.

Agarose, a natural polysaccharide as chitosan, consists of alternating units of β-1,3-linked-D-galactose and α-1,4-linked 3,6-anhydro-L-galactose (Figure 39). Agarose can only be dissolved in aqueous solution at high temperature. The agarose solution can form hydrogel through intermolecular hydrogen bonding upon cooling the agarose solution below 35 °C. The proposed hydrogel structure is the aggregation of double helices by entanglements of anhydro bridges on agarose polymer chains.

To apply 5CB droplet-embedded hydrogel platforms into PBS solution for the detection of bile acids. In this chapter, agarose was adopted as the hydrogel matrix to prepare 5CB droplet-embedded agarose hydrogels for the detection of bile acids in PBS solution.
7.2. Experimental

7.2.1 Materials

4-cyano-4'-pentylbiphenyl (5CB, 98% purity), agarose, alkyl trimethylammonium bromide (CₙTAB, n=8, 12, and 14, 99% purity), and PBS solution (pH 7.4) were obtained from Sigma-Aldrich (St. Louis, MO). All chemicals were used without further purification. Water used in our experiments was purified with Easypure II system (18.2 MΩ cm and pH 5.7).

7.2.2 Preparation of CₙTAB/5CB-Embedded Agarose Hydrogel

0.1 g agarose powder was dissolved in 10 mL water at 70 °C with magnetic stirring until the solution became transparent to form 1wt% agarose aqueous solution. Then 1 μL 5CB was quickly mixed with 1 mL of the as-prepared 1wt% agarose solution with tip sonication (FB505, Fisher Scientific, Pittsburg, PA) at the amplitude of 20% for 10s at room temperature to form agarose-stabilized 5CB droplet emulsions. The agarose-stabilized 5CB emulsion was quickly casted on the glass substrate on a hot plate at 38 °C and cool it at 4°C for 10 min to let it form 5CB droplet-embedded agarose hydrogel film.

The CₙTAB/5CB-embedded agarose hydrogel was formed by immersing 5CB-embedded agarose hydrogel into specific concentrations of CₙTAB aqueous solution to make sure all the 5CB droplets within the agarose hydrogel network are radial configurations.
7.2.3 Characterization

The configuration of 5CB droplets was characterized with a polarizing optical microscope (Olympus BX40) in transmission mode. The C_{14}TAB/5CB droplet embedded-agarose hydrogel film was cut into a 10mm×2mm×0.2mm sheet and was then placed onto the 10mm diameter glass bottom of a culture dish (MatTeck Corporation, MA). The configuration of 5CB droplets embedded in the agarose hydrogel sheet was in-situ monitored under a polarizing optical microscope after the addition of bile acids in PBS solution. The response time was recorded when the configuration of 5CB droplets in the focus plane showed a radial-to-bipolar configuration transition after the addition of bile acids.

7.3 Results and Discussion

The 5CB droplet-embedded agarose hydrogel film was formed by quickly casting the agarose stabilized 5CB droplet emulsion on a glass substrate on a hot plate at 38 °C and cooling it 4 °C for 10 min. It can be easily peeled off from the glass substrate (Figure 54a). The good mechanical properties of 1wt% agarose hydrogel showed in Chapter 5 (Figure 45b) indicates agarose hydrogels are a suitable platform for detection applications.

The 5CB droplets in agarose hydrogel showed a bipolar configuration (Figure 54b), indicating a parallel alignment of 5CB molecules to the surface of droplets (Figure 53b). After the addition of 50 μM C_{14}TAB, all the 5CB droplets embedded in agarose hydrogel sheets showed a radial configuration (Figure 54c). The hydrophobic tails of C_{14}TAB penetrated the agarose network and extended into the liquid crystal droplets to induce a perpendicular alignment of 5CB molecules within the droplets (Figure 53e).
Figure 53. (a) Chemical structure of 5CB and agarose; (b-e) schematic illustrations of the formation of CₙTAB/5CB droplet-embedded agarose hydrogels

Figure 54. Polarizing optical microscopy images of 5CB-embedded agarose hydrogel before (a) and after (b) the addition of 50 μM C₁₄TAB

For the detection of bile acids, a 5CB droplet-embedded agarose hydrogel sheet was placed on the bottom of a small glass dish with 90 μL PBS solution containing a specific concentration of bile acids. Figure 55 shows the time-course polarizing optical microscopy images of C₁₄TAB/5CB droplet-embedded agarose hydrogel sheets after the addition of 100 μM DCA. The 5CB droplets in the agarose hydrogel network showed a radial configuration with a single defect point at the center of the droplets (Figure 55a). Then the defect point started to shift away from the
center (Figure 55b-c), indicating a pre-radial configuration. Finally, the configuration of the 5CB droplets turned to bipolar with two defect points at the poles of the droplets (Figure 55d). The facial amphiphilic nature of bile acids enables them to displace other surface active molecules from the oil/aqueous interface through competitive adsorption. Thus we can conclude that the radial-to-bipolar configuration transition of 5CB droplets within the agarose network is the result of the competitive adsorption of DCA at the surface of the 5CB droplets by penetrating the agarose hydrogel network, which has disrupted the packing of C_{14}TAB at the surface of 5CB droplets (Figure 56).

Figure 55. Polarizing optical microscopy images of C_{14}TAB/5CB droplet-embedded agarose hydrogel sheets taken at 0 s (a), 11 s (a), 14 s (c), and 22 s (d) after the addition of 100 μM DCA.
Figure 56. Schematic illustrations of the competitive adsorption of bile acid-induced a radial-to-bipolar configuration transition of 5CB droplets within the agarose network.

The response time of C\textsubscript{14}TAB/5CB droplet-embedded hydrogel sheets for DCA increases from 22.6 s to 1.72 min, when the concentration of DCA decreases from 100 μM to 20 μM (Figure 57a). The concentration dependence of response time is also observed for CA. The response time of C\textsubscript{14}TAB/5CB droplet-embedded hydrogel sheets for CA increases from 36.6 s to 2.55 min when the concentration of CA decreases from 220 μm to 140 μM (Figure 57b). The detection limit of C\textsubscript{14}TAB/5CB droplet-embedded hydrogel sheets for DCA and CA is 20 μM and 140 μM, respectively (Figure 58b). The pH of PBS solution is 7.4. Both DCA and CA are negatively charged. The critical micelle concentration (CMC) is 10 mM for DCA and 13 mM for CA, respectively.\textsuperscript{103} The concentration of CA and DCA used in the experiments is lower than their CMCs. Although DCA and CA share a common steroid backbone, the number of the hydroxyl groups on their steroid backbone is different. DCA bearing two hydroxyl groups shows a higher hydrophobicity than that of CA bearing three hydroxyl groups.\textsuperscript{103} The more hydrophobic DCA should be more effective in disrupting/displacing the C\textsubscript{14}TAB from the surface of the embedded
5CB droplets. This can explain why the 5CB droplet-embedded hydrogel sheets show lower detection limit for DCA, compared to CA.

![Figure 57](image1.png)

Figure 57. Response time of C14TAB/5CB droplet-embedded agarose hydrogel sheets for (a) DCA, and (b) CA as a function of their concentrations in PBS solution with and without 500 μM UA and 500 μM AA.

![Figure 58](image2.png)

Figure 58. (a) Response time of C14TAB/5CB droplet-embedded agarose hydrogel sheets for DCA and CA at pH 7.4 aqueous solution; (b) Detection limit of C14TAB/5CB droplet-embedded agarose and C14TAB/5CB droplet-embedded chitosan sheets for DCA and CA.

The detection limit of C14TAB/5CB droplet-embedded agarose hydrogel sheets for both DCA and CA are higher than that of C14TAB/5CB droplet-embedded chitosan hydrogel sheets. This is because the cationic nature and high charge density of chitosan hydrogel matrix can attract
the negatively charged bile acids through electrostatic interactions, while the neutral network of agarose hydrogel cannot. In addition, the large amount of salt ions in PBS solution can form electrical double-layer at the 5CB/aqueous interface, which can induce a homeotropic anchoring of 5CB. Then, the detection for DCA and CA was performed in pH 7.4 aqueous solution, in order to eliminate the effect of salt ions on the detection results. Figure 58a shows the response time of C_{14}TAB/5CB droplet-embedded agarose hydrogel sheets for DCA and CA. The detection limit and response time of C_{14}TAB/5CB droplet-embedded agarose hydrogel sheets for both DCA and CA were lowered. However, the detection limit of C_{14}TAB/5CB droplet-embedded agarose hydrogel sheets for DCA and CA is still higher than that of C_{14}TAB/5CB droplet-embedded CS hydrogel sheets. This indicates that the cationic nature and high charge density of CS hydrogel network plays a significant role to improve the sensitivity of 5CB droplet-embedded CS hydrogel platforms for DCA and CA.

![Figure 59. Polarizing optical microscopy images of C_{14}TAB/5CB droplet-embedded agarose hydrogel sheets after the addition of (a) 500 μM UA and (b) 500 μM AA for 1 hour](image)

In biological fluids, ascorbic acid (AA) and uric acid (UA), which coexist with bile acids in much high concentrations, are major interference species for the detection of bile acids. However, we find that the C_{14}TAB/5CB droplet-embedded agarose hydrogel sheets are insensitive
to 500 μM AA and 500 μM UA, respectively (Figure 59). This suggests that AA and UA are unable to disrupt the packing of C14TAB at the surface of 5CB droplets. Then the response time of C14TAB/5CB droplet-embedded agarose hydrogel sheets for DCA and CA were measured in the presence of 500 μM UA and 500 μM AA in PBS solution, respectively. No significant difference was observed in the response time for DCA and CA with and without UA and AA (Figure 57). The detection limit for DCA and CA is not changed with and without UA and AA (Figure 60c).

We further used 1 mM C12TAB to prepare C12TAB/5CB droplet-embedded agarose hydrogel sheets for the detection of DCA and CA in PBS solutions. The concentration dependence of response time for DCA and CA is also observed for C12TAB/5CB droplet-embedded agarose hydrogel sheets. The response time of C12TAB/5CB droplet-embedded agarose hydrogel sheets for DCA increases from 24.6 s to 2.5 min when the concentration of DCA decreases from 60 μM to 8 μM (Figure 60a). The response time of C12TAB/5CB droplet-embedded agarose hydrogel sheets for CA increases from 22 s to 2.3 min when the concentration of CA decreases from 120 μM to 40 μM (Figure 60b). We also found the response time and detection limit of C12TAB/5CB droplet-embedded agarose hydrogel sheets for both DCA and CA with and without UA and AA are not changed (Figure 60), indicating the presence of UA and AA does not have an interference on the detection results. Compared with C14TAB/5CB droplet-embedded agarose hydrogel sheets, the sensitivity of C12TAB/5CB droplet-embedded agarose hydrogel sheets for both DCA and CA shows an improvement, decreasing from 20 μM to 8 μM for DCA, and decreasing from 140 μM to 40 μM for CA, respectively (Figure 60c).
Figure 60. Response time of C_{12}TAB/5CB droplet-embedded agarose hydrogel sheets for (a) DCA, and (b) CA as a function of their concentrations in PBS solution with and without 500 μM UA and 500 μM A; (c) detection limit of C_{n}TAB/5CB droplet-embedded agarose hydrogel sheets for DCA and CA.

Compared with the C_{12}TAB/5CB droplet-embedded CS hydrogel sheet, the C_{12}TAB/5CB droplet-embedded agarose hydrogel sheet is less efficient to detect DCA and CA. However, the concentration of bile acids in biological fluid of healthy individuals is around 10 μM. DCA is found to be more hepatotoxic than CA. Thus, the C_{n}TAB/5CB droplet-embedded agarose hydrogel sheets can be a candidate for the detection of bile acids.

7.4 Conclusion

In this chapter, the preparation of C_{n}TAB/5CB droplet-embedded agarose hydrogel sheets for the real-time detection of bile acids in PBS solution was presented. The C_{n}TAB/5CB droplet-embedded agarose hydrogel platforms showed more sensitive to DCA than to CA due to more hydrophobicity of DCA than that of CA. The sensitivity of C_{n}TAB/5CB droplet-embedded agarose hydrogel sheets to DCA and CA can be improved with shorter alkyl chain of C_{n}TAB. The detection limit of C_{12}TAB/5CB droplet-embedded agarose hydrogel for DCA and CA is 8 μM, and 40 μM, respectively.
CHAPTER 8 CONCLUSION

Bile acids with amphipathic structures, which are formed in liver and secreted into the small intestine, play an important role in the digestion of fats and fat-soluble vitamins. After the digestion process, most of bile acids are recycled back to the liver and ready for the next digestion. Only a few of them are excreted into body fluids. However, there is significant increases in the concentration level of bile acids in body fluids for patients with liver and intestinal diseases, which makes bile acids a biomarker for the early diagnosis of liver and intestinal diseases. Chromatography-mass spectrometry and electrochemical sensors are common methods for the detection of bile acids. However, these detection methods are time consuming, require relatively large sample volumes, and expensive instruments. To date, there is still a demand in the development of simple, and low-cost platforms for the rapid detection of bile acids.

Chapter 1 and 2 provided the basic concepts of liquid crystal materials and the current applications of liquid crystal-based sensing platforms. The structures of bile acids, their functions in the digestion process and their applications as a biomarker for early diagnosis of liver and intestinal diseases were also introduced the first two chapters.

In Chapter 3, a C₁₄TAB/β-CD host-guest complex was adopted to functionalize the surface of 5CB droplets as a selective barricade for the detection of CA in pH 7.4 aqueous solution. In order to achieve a long-term stability, in chapter 4, a polyelectrolyte, PDADMAC, was employed to stabilized SC₁₄S/sulfated β-CD-5CB droplets by coating the surface of SC₁₄S/sulfated β-CD-5CB droplets. This PDADMAC/SC₁₄S/sulfated β-CD-stabilized 5CB droplets were then integrated into a microfluidic channel for the real-time detection of bile acids. With this miniaturized platform, a small amount of solution was required for the real-time detection of CA.
and DCA in the presence of the major interference species of AA and UA in PBS solution. The
droplet density dependent detection limit and concentration dependent response time for DCA and
CA were studied with this miniaturized platform.

In Chapter 5, CS and agarose hydrogels were employed to stabilize and confine 5CB
droplets in the hydrogel network to prepare 5CB droplet-embedded hydrogels. Surfactant with
different headgroups and different alkyl chain length were adopted to functionalize the 5CB
droplets immobilized in the hydrogel networks. The interactions between the hydrogel matrix and
surfactants with different head groups were studied.

In Chapter 6, CS was employed to prepare surfactant/5CB droplet-embedded CS hydrogel
sheets for the real-time detection of bile acids. The positively charged C\textsubscript{n}TAB combined with
cationic CS hydrogel network had a huge electrostatic attraction for negatively charged bile acids,
which can improve the sensitivity of 5CB droplet-embedded CS hydrogel for bile acids.
C\textsubscript{n}TAB/5CB droplet-embedded CS hydrogel sheets with a shorter hydrophobic chain of C\textsubscript{n}TAB
showed a further improvement in sensitivity for DCA and CA. The detection limit of C\textsubscript{12}TAB/5CB
droplet-embedded CS hydrogel sheets for DCA and CA can be lowered to 0.6 \mu M and 3 \mu M,
respectively.

In Chapter 7, agarose was adopted to prepare C\textsubscript{n}TAB/5CB droplet-embedded agarose
hydrogel sheets for the real-time detection of DCA and CA in PBS solution in the presence of
interference species of UA and AA.
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