Developing Surface Engineered Liquid Crystal Droplets For Sensing Applications

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DEVELOPING SURFACE ENGINEERED LIQUID CRYSTAL DROPLETS FOR SENSING APPLICATIONS

by

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

Spring Term
2012

Major Professor: Jiyu Fang
ABSTRACT

Diagnosis plays a very crucial role in medicine and health care, which makes biosensors extremely important in modern technological context. Till date, various types of biosensors have been developed that are capable of detecting a wide range of biologically important species with great sensitivity and selectivity. However, most of these sensing units require highly sophisticated instrumentation and often lack the desired portability. Liquid crystal (LC) droplets, on the other hand, are a new type of functional material that are finding increasing research attention as a new sensing unit due to their tunable optical property, high surface area, portability and cost-effectiveness.

In this dissertation, functionalized LC droplets for biosensing at aqueous-LC interface are highlighted. Chemically functionalized LC droplets dispersed in aqueous solution were prepared by the self-assembly of amphiphilic molecules at the aqueous/LC interface. These functionalized LC droplets showed a well-defined director of configuration and a specific optical pattern when observed with a polarizing light microscope. It was discovered that the interaction of chemically functionalized LC droplets with an analyte triggers transition of the director of configuration of the LC within the droplets, providing a simple and unique optical sign for the detection of the analyte. Moreover, the director of configuration transition happened in a concentration dependent manner, allowing both qualitative and quantitative detection of the analyte. The sensitivity of chemically functionalized LC droplets depends not only on the nature of amphiphilic molecules but also the size and number of the droplets.
The dissertation essentially deals with the application of these chemically functionalized LC droplets in detecting several biologically important species. It was observed that the adsorption of charged macromolecules (dendrimers, proteins, and viruses) on polyelectrolyte functionalized LC droplets triggered a bipolar-to-radial configuration transition based on the polar verses non-polar interaction. By using a simple optical microscope, microgram per milliliter concentrations of bovine serum albumin, cowpea mosaic virus, and tobacco mosaic virus could be detected in aqueous solution. The detection limit of Mastoparan X polypeptide decorated LC droplets in detecting *E. coli* could reach to approximately 10 bacteria per milliliter. In this case, the high affinity of the polypeptide towards the bacterial causes the former to detach from the LC droplets, triggering the director of configuration transition of the LC inside the droplets. Finally, surfactant decorated LC droplets were used to detect lithocholic acid (LCA), a toxic bile acid used as a specific biomarker for colon cancers. In this case, the director of configuration transition of the LC inside the droplets is a result of the replacement of the surfactant from the aqueous/LC interface by LCA. The microgram per milliliter concentration of LCA, a clinically significant concentration, could be easily detected by changing the length of surfactants.

These studies highlight the novel use of surface functionalized LC droplets to detect biologically important species. Due to their tunable optical property, coupled with high surface area and portability, surface functionalized LC droplets have great potentials in the design of next generation biosensors.
Dedicated to my parents, for their faith and love that made me survive in the toughest of the times and without their support I couldn’t have done this.
ACKNOWLEDGEMENTS

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td></td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td></td>
<td>xxi</td>
</tr>
<tr>
<td>CHAPTER ONE: INTRODUCTION</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>Fundamental Features</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>LCs in nature</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>History of LCs</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Types and Phases of LCs</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Lyotropic LCs</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Thermotropic LCs</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>Metallotropic</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>Theoretical treatment of LCs</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>Molecular arrangements and ordered parameters</td>
<td>10</td>
</tr>
<tr>
<td>13</td>
<td>Theoretical Models for LCs</td>
<td>13</td>
</tr>
<tr>
<td>15</td>
<td>LCs in external field</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>LCs under electrical field</td>
<td>15</td>
</tr>
<tr>
<td>16</td>
<td>LCs under magnetic field</td>
<td>16</td>
</tr>
<tr>
<td>17</td>
<td>LCs under surface tension and at interface</td>
<td>17</td>
</tr>
<tr>
<td>18</td>
<td>Freedrcksz Transition</td>
<td>18</td>
</tr>
<tr>
<td>19</td>
<td>Optical properties of LCs under polarized light</td>
<td>19</td>
</tr>
<tr>
<td>19</td>
<td>Polarization of light waves</td>
<td>19</td>
</tr>
<tr>
<td>21</td>
<td>Birefringences in LC</td>
<td>21</td>
</tr>
<tr>
<td>23</td>
<td>LCs under polarized light</td>
<td>23</td>
</tr>
<tr>
<td>25</td>
<td>Texture of LCs</td>
<td>25</td>
</tr>
<tr>
<td>26</td>
<td>Defects in LCs</td>
<td>26</td>
</tr>
</tbody>
</table>
Time line of LC applications .................................................................................................................. 26
Primary application of LCs ......................................................................................................................... 29
Liquid crystal and self-assembly .................................................................................................................. 30
LC interface as sensing platforms ................................................................................................................ 32
LC droplets as sensors ................................................................................................................................... 35
Thesis objectives and outline ....................................................................................................................... 36
CHAPTER TWO: GENERALIZED EXPERIMENTAL APPROACH ................................................................. 39
Materials Used ............................................................................................................................................... 39
Experimental .................................................................................................................................................. 40
  Synthesis of the Droplets .............................................................................................................................. 40
  Analyte Incubation ........................................................................................................................................ 41
Characterization .............................................................................................................................................. 42
  Optical Microscopy ....................................................................................................................................... 42
  Optical Microscopy Data Analysis .................................................................................................................. 43
  Fluorescence and Confocal microscope ........................................................................................................ 44
  Photoluminescent spectroscopy ................................................................................................................... 45
  UV-Vis spectroscopy ...................................................................................................................................... 46
  Infra Red (FT-IR) Spectroscopy .................................................................................................................... 46
  Dynamic Light Scattering .............................................................................................................................. 47
  Zeta Potential ............................................................................................................................................... 48
  Transmission Electron Microscope .............................................................................................................. 49
Data representation ......................................................................................................................................... 50
CHAPTER THREE: STABILIZATION AND SENSITIVITY OF POLYELECTROLYTE COATED LC DROPLETS ................................................................................................................... 51
Introduction ..................................................................................................................................................... 51
Experimental .................................................................................................................................................... 53
Results and discussion.............................................................................................................................................. 99

SDS and CTAB stabilized 5CB droplets.................................................................................................................. 101
LCA sensing using SDS or CTAB coated droplets.............................................................................................. 103
Conclusion............................................................................................................................................................... 114

CONCLUSION...................................................................................................................................................... 116

REFERENCES: ...................................................................................................................................................... 119
LIST OF FIGURES

Figure 1: Molecular arrangement in (a) solid, (b) LC and (c) liquid (d) optical appearance under polarized light. .................................................................................................................. 1

Figure 2: Nature’s colorful displays (a) sunset moth, (b) a panther chameleon, (c) morpho butterfly and (d) DNA single crystal under polarized light. Photo credit a to c: earths featured creatures blogspot, d: M Davidson, FSU (micro.magnet.fsu.edu)...................................................... 2

Figure 3: Hierarchy of morphologies and different phases formed during the self-assembly of surfactants to lyotropic LC; (a) surfactant molecule, (b) micelles, (c) cubic, (d) hexagonal, (e) columnar and (f) bi-layer. .......................................................................................................................... 5

Figure 4: Different phases of LCs: (a) nematic, (b) smectic A, (c) Smectic C, (d) Chiral nematic and (e) Smectic C*. Image Credit: Barreett Research Group, McGill Uni. ............................................. 7

Figure 5: The chiral phases, (a): half pitch and (b): pitch of chiral LC. Image credit: Liquid crystal group U. Colorado at Boulders. ........................................................................................................ 8

Figure 6: (a) lattice structure, (b) typical platelet pattern of blue phase LC under polarized light. Photocredit a: condensed matter theory group, Oxford Uni, b:LC group Kent State Uni............. 9

Figure 7: Discotic LC of (a) nematic and (b) columnar phases. Image Credit: Barreett Research Group, McGill Uni................................................................. 9

Figure 8: Molecular shapes of LCs (a) rod, (b) disc, (c) banana and (d) DNA .................. 11

Figure 9: The concept of Order Parameter (S); (a) Local ordering of the mesogens, (b) the temperature dependence of S. ......................................................................................... 12

Figure 10: Effect of electric field on the alignment of LC (a) along the applied field and (b) perpendicular to it, for Δε >0 and Δε < 0 respectively. ............................................................... 16
**Figure 11:** Alignment of LC molecules under magnetic field (a) perpendicular with more distortion, (b) parallel with less distortion................................................................. 17

**Figure 12:** Alignment of LC molecules on planar surfaces (a) parallel, (b) perpendicular orientation due the functional groups. ................................................................. 18

**Figure 13:** Freedricksz Transition phenomenon in LCs when the configuration (n) gradually rotates from substrates to the middle of the cell in (a) OFF and (b) On condition. ............... 19

**Figure 14:** polarization of light waves (a) linear propagation of light waves, (b) linearly polarized light at 45° and (c) circularly polarized light. Image credit: Polymers and Liquid Crystals Case Western Reserve Uni. .................................................................................. 20

**Figure 15:** Light waves between two polarizers (a) parallel polarized or bright field condition, (b) cross-polarized condition. Image credit: Polymers and Liquid Crystals Case Western Reserve Uni.............................................................................................................. 21

**Figure 16:** Basis of birefringence in optically anisotropic LCs .............................................. 22

**Figure 17:** A typical ‘schlieren’ texture in a nematic LC showing the dark brush regions, which meet at different points of disclination. Photo credit: LC group, kent state uni ............... 24

**Figure 18:** Nematic LC textures showing (a) planar (b) homeotropic anchoring. Photo credit: Prof Lavrentovich, Kent State University................................................................. 26

**Figure 19:** The disclination line and the change of director around it a: schematic b: electron micrograph. Photo credit: Polymer and LC lab, CWRU .................................................. 26

**Figure 20:** The time line showing the most significant discoveries in the field of LC.............. 28

**Figure 21:** (a) A schematic, (b) An image of LC based digital display. Photo credit: Dr J. Fernsler, Cal Poly........................................................................................................... 30
**Figure 22:** Actual and schematic image of a privacy window. Image credit: Inhabitat.com/super smart privacy glass. 

**Figure 23:** Timeline for the development of soft matter in general and LC based sensor in particular.

**Figure 24:** LC thin film based sensor. schematics showing the (a) perpendicular, (b) planer anchoring before and after the exposure to the enzyme and (c) optical image of the sensor panel showing the gradual change in the intensity of transmitted light with time, as the enzymatic reaction progresses. Photo credit a and b Woltman et al. Nature Materials 2007, 6, 929 and c Prof Abbott and co-workers in U. Wisconsin.

**Figure 25:** The polarizing light microscope (a) Olympus BX51 and (b) the schematic of the image formation.

**Figure 26:** (a) Acu-scope 3031 inverted microscope and (b) Zeiss Axioscope-2 motplus confocal microscope.

**Figure 27:** (a) Jasco spectrofluorometer and (b) Thermo UV-Vis spectrometer used in our measurements.

**Figure 28:** (a) Nicolet IR100 to obtain IR-spectra and (b) Melvern Zeta Sizer Nano for particle size and zeta-potential.

**Figure 29:** (a) TEM Jeol 1011 (b) Origin and (c) Image J used for processing microphotographs.

**Figure 30:** Molecular structure of the polyelectrolytes used as stabilizing agents.

**Figure 31:** Layer-by-layer assembly of polyelectrolytes triggered alternate bipolar/radial transition in PSS/PDADMAC coated LC droplets.
Figure 32: The effect of PSS concentration on the LC droplet formation. (a) The transition of the LC droplets from unstable Radial to stable Bipolar as a function of PSS concentration. (b) The effect of additional PSS on the number of droplets formed. ................................................. 55

Figure 33: A schematic showing (a) the molecular arrangement of PSS and (b) π-π type intermolecular interactions between the 5CB and PSS molecules at the 5CB-water interface. ........... 57

Figure 34: Polarizing optical microscope images of (a) PSS-coated 5CB droplets, (b) PDADMAC/PSS-coated 5CB droplets, (c) PSS/PDADMAC/PSS-coated 5CB droplets and (d) (PDADMAC/PSS)2-coated 5CB droplets. ................................................................. 58

Figure 35: (a) The ζ-potential of polyelectrolyte-coated 5CB droplets as a function of layer numbers, (b) TEM image of (PDADMAC/PSS)8 capsule shells dried on a carbon-coated copper grid. .......................................................................................................................... 59

Figure 36: (a) The director of configuration transition of PDADMAC/PSS-coated 5CB droplets after being exposed to PSS solution with different concentrations. (b) The director of configuration of 5CB droplets as a function of number of PDADMAC/PSS bilayer coatings after being exposed to a PSS solution with a 0.25 mg/mL. ................................................................. 61

Figure 37: Polarizing optical microscope images of (PDADMAC/PSS)6-coated 5CB droplets at (a) pH 10, (b) pH 4.0, (c) pH 3.0 and (d) The direction of the polarizer and analyzer is indicated by white arrows. The director of configuration of (PDADMAC/PSS)6-coated 5CB droplets as a function of pH values. A schematic representation of the possible internal structure of a PDADMAC/PSS-coated 5CB droplet at low pH values (inset in Figure 37d)......................... 63
Figure 38: (a) The director of configuration of PDADMAC/PSS-coated 5CB droplets as a function of NaCl concentration. (b) collapsing of the PDADMAC/PSS-coated 5CB droplets with the excess of NaCl. .......................................................... 64

Figure 39: The molecular structure of (a) PAMAM, (b) 5CB and (c) PSS. ......................... 67

Figure 40: The structure and morphology of (a) BSA, (b) CPMV and (c) TMV. ............... 70

Figure 41: (a) A schematic of interactions between the PSS droplets and G5 dendrimer. The polarized light microscope image of the droplets (b) before and (c) after the exposure to G5. ... 71

Figure 42: (a) The change in surface charge due the increasing concentration of dendrimers, (b) the interaction of amine and sulfonate groups. ................................................................. 72

Figure 43: The concentration dependent transition of the PSS coated droplets for all four generations of PAMAM dendrimers for four different set of droplet with average sizes (a) 0.5, (b) 0.75, (c) 1.0 and (d) 1.4 μm. ................................................................. 74

Figure 44: The size distributions for four different sets of droplets with varying sizes. ........ 75

Figure 45: The effect of (a) droplet size and (b) dendrimer generation on the complete or 100% transition concentration ................................................................. 76

Figure 46: The variation in zeta-potential with pH for droplets with average sizes (a) 0.7 μm and (b) 1.0 μm. ................................................................. 78

Figure 47: The effect of multilayering (a) G5 induced concentration dependent transition of the PSS coated LC droplets with various numbers of surface layers. (b) The corresponding 50% transition concentration as function of the number of layers........................................ 79
Figure 48: BSA detection using LC droplets (a) the concentration dependent transition of the PEI and PDADMAC coated droplets in presence of BSA, (b) A schematic showing the binding of BSA onto the droplet surface leading to the transition ................................................................. 80

Figure 49: Detection of viruses using LC droplets (a) spherical shaped CPMV and (b) longitudinal shaped TMV ............................................................................................................. 81

Figure 50: (a) The amino acid sequence in MP and MP-X polypeptides, (b) the coil-like substructure that MP-X forms in solution and its amphoteric nature of MP-X polypeptides .......... 84

Figure 51: Different conformations of MP-X; (a) normal polypeptide structure, (b) backbone structure of membrane-bound MP-X, (c) tightly membrane-bound structure, (d) weakly membrane-bound structure and (e) bound to protein G, a family of immunoglobulin binding protein. Image credit: a: Biochem, 1992, 31, 5654; b to e: Biophys J, 2006, 91, 1368 ............. 85

Figure 52: A schematic of the cell wall structure of (a) Gram positive and (b) Gram negative. Image credit: Dept. Microbiology. Cornell University ................................................................. 86

Figure 53: MP-X stabilized the 5CB droplets (a) immediately after synthesis (b) after incubation ................................................................................................................................. 91

Figure 54: The stabilization of LC droplets using MP-X: (a) the effect of the MP-X concentrations on the number of stable droplets obtained and (b) polarizing microscope image of stable droplets stabilized with 50 nM MP-X ........................................................................................................ 92

Figure 55: The effect of surfactant exposure to the MP-X coated droplets (a) the polarizing light microscope image showing the transition to bipolar and (b) the concentration dependence of the transition. .......................................................................................................................... 93
Figure 56: Interaction of *E. coli* with LC droplets, (a & b) MP-X- coated droplets, (c & d) SDS coated droplets; and (e & f) CTAB coated droplets; (a, c, e) polarizing light and (b, d, f) bright field images................................................................................................................. 94

Figure 57: (a) A schematic showing the detachment of MP-X polypeptides from the droplet surface and binding to cell wall of *E. coli*; therefore triggering the radial to bipolar transition and (b) the percentage transition as function of the number of *E. coli* in the solution......................... 95

Figure 58: The stabilization effect of LCA (a) polarizing micrograph of LCA coated 5CB droplets; molecular structures and schematic of (b) 5CB and (c) LCA, (d) a schematic showing molecular arrangement of LCA at 5CB droplet interface resulting a bipolar structure, (e) effect of LCA stabilizing concentration on the number of stable droplets, (f) the size distribution of LCA stabilized droplets using 1 mM of LCA......................................................................................................................... 100

Figure 59: The surfactant stabilized LC droplets: molecular structure of (a) SDS and (b) CTAB, (c) polarizing micrograph of SDS stabilized 5CB droplets, (d) effect of SDS and CTAB concentration on the number of stable droplets formed; the size distribution of (f) SDS and (g) CTAB stabilized droplets both using 1 mM of stabilizing concentrations......................... 102

Figure 60: A schematic illustration of the replacement of SDS or CTAB molecules from the 5CB-water interface by LCA molecules, triggering the radial to bipolar transition. The inset shows molecular arrangement at the interface................................................................. 104

Figure 61: The concentration dependent transition of (a) SDS and similar molecules and (b) CTAB and similar molecules coated droplets in presence of LCA. The LCA concentrations resulting in 50% transitions for different chain lengths of (c) SDS and (d) CTAB type surfactants. .............................. 106
**Figure 62:** (a) The molecular structure of 18:1 PE, (b) the concentration dependent transition of PE coated droplets and (c) comparison of the 50 % transition concentrations between CTAB and PE coated droplets.

**Figure 63:** (a) Molecular structure of NBD-PE; (b) fluorescent microscope image of PE-NBD stabilized droplets as made, (c) after exposure to LCA, the brightness and contrast of the image is enhanced for clarity and (d) the corresponding bright field image.

**Figure 64:** Chitosan stabilized droplets and their surfactant triggered transition; (a) molecular structure of chitosan, (b) polarizing micrograph of SDS stabilized 5CB droplets; (c) the size distribution of chitosan coated droplets; (d) effect of SDS and CTAB concentration on the transition of the chitosan coated droplets; (e) 50 % transition concentrations for SDS and CTAB.

**Figure 65:** (a) LCA triggered transition of both types of chitosan stabilized droplets modified with SDS, (b) LCA concentrations required for 50 % transition for both medium and low molecular weight chitosan and (c) a schematic showing the sequence of transitions.
LIST OF TABLES

Table 1: List of different types and phases of LC................................................................. 4

Table 2: The polyelectrolytes used to stabilize the LC droplets and their configurations......... 56

Table 3: Showing the molecular weight, size and surface charges of different generations of PAMAM Dendrimers........................................................................................................... 68
CHAPTER ONE: INTRODUCTION

Fundamental Features
Liquid crystals (LC) are a special state of matter that can flow like a liquid and yet can maintain an ordered molecular arrangement like crystalline solids (Figure 1). Therefore they are aptly named as LCs. The ordered molecular structure allows it to interact with light (especially with polarized light) in a very unique way. The optical properties of LCs make them an interesting system for scientific studies and industrial applications. The field of LC is more than hundred years old. Extensive studies have been carried out to understand different aspects of the field. The molecular mechanisms responsible for the optical property of LCs were studied with even greater emphasis and have been well documented\(^1\). All of these molecular mechanisms and complex properties of LCs can be summarized by its ability to maintain periodic molecular arrangement, and its interaction with light.

Figure 1: Molecular arrangement in (a) solid, (b) LC and (c) liquid (d) optical appearance under polarized light.
**LCs in nature**
The term LC is generally associated with the electronic liquid crystal displays (LCD) that surround us. But, LC is one of the most abundant states of material in nature. It is now known that most of the nature’s colors are from LC like molecular arrangements. The wings of many species of insects, butterflies and chameleons have naturally occurring LCs. All of these splendid colors arise from precise periodical arrangements that interact with light to produce such a wide range of colors (Figure 2). Thus, the LCs are quite ubiquitous in nature.

![Figure 2: Nature’s colorful displays (a) sunset moth, (b) a panther chameleon, (c) morpho butterfly and (d) DNA single crystal under polarized light. Photo credit a to c: earths featured creatures blogspot, d: M Davidson, FSU (micro.magnet.fsu.edu)](image)

**History of LCs**
Yet it was not until late nineteenth century that LCs were first discovered. It was in 1888, when Austrian physiologist Friedrich Reinitzer first came across a colorful biological substance, a cholesterol derivative and became curious about it. Later in collaboration with Otto Lehman, a physicist studied the material and observed that it has crystalline features, yet it flows and is able to rotate circularly polarized light. This was reported as the first study on such a material and named as LC. After Lehman, scientists like Georges Friedel, Carl Oscen and F.C. Frank carried forward the investigation on the physical behavior of LCs. Daniel Vorlander, a German chemist on the other hand, synthesized several artificial LCs. But mostly for about next half a century or
more the field of LC research remained quite dormant. The research in the field was pursued purely for scientific curiosity (if not for their fascinating colors under polarized light). This is primarily because all the LCs known then were active (in LC state) only at high temperatures (>150˚C) and thus found no use in real life. All of this changed in 1969, when Hans Kelker synthesized a room temperature LC (in nematic phase), MBBA (N-(4-Methoxybenzylidene)-4-butylaniline). Around this time, physicists became interested in ‘soft matters’ and Nobel laureates like Pierre-Gilles de Gennes came up with theory for ‘soft matters’ and extended the approach to understand the physics of LCs. This was the point the field of LCs got completely changed. Shortly after this, George Grey, a well-known British organic chemist, reported the synthesis of more room temperature LCs, especially the cyanobiphenyl derivatives. Together with Ken Harrison, he developed the first LC displays in 1973. In the following decade the field of LCs received great scientific and industrial research attention mostly due to electronics display based applications. In modern days, most of LCs are man-made and are extensively used in displays. Even after years of research, the field of LCs still remains popular in pure and applied sciences.

Types and Phases of LCs
The field of LC research is now more than a century old. During this time, effects were made not just to improve the display units but the fundamentals of the field has been studied well too\textsuperscript{3,4}. Significant discoveries have been made in unraveling the molecular mechanisms that govern the optical and related phenomena in LCs\textsuperscript{5-7}. It is now understood that the skin of a chameleon and an electronic display unit essentially operates on the same molecular mechanism. In both cases
the colors appear due to carefully controlled periodic arrangement of molecules and their interaction with light waves. However, they remain distantly different in structure and arrangement. This gives rise to differences in LCs. Hence, LCs have been categorized in different types depending on the parameters that govern the molecular arrangement. Each type of LC may have different molecular patterning based on which they are sub-divided in different Phases. The following Table 1 describes different Types and Phases of LCs.

**Table 1: List of different types and phases of LC**

<table>
<thead>
<tr>
<th>Liquid Crystals</th>
<th>Lyotropic (concentration drive)</th>
<th>Thermotropic (Temperature drive)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cubic</td>
<td>(Different type of each phase)</td>
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<td></td>
<td>Columnar</td>
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<td></td>
<td>Bi-layer</td>
<td>• Semiatic C/C*</td>
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<td>(Based on the nature of the molecule)</td>
<td>1. Rod like</td>
<td>• Blue phase</td>
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<td>2. Disc like</td>
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<td>3. Chiral</td>
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**Lyotropic LCs**

Most of natural LCs are lyotropic in nature and nature has devised ways to control their optical properties by simply manipulating the concentration (and also by temperature)\(^8,9\). Lyotropic LCs usually have two components viz. a solute and a solvent. The solute molecule is amphiphilic in nature and when dissolved in the solvent at a concentration more than the critical micelle concentration (CMC) the molecules self-organize, to form a sub-structure called micelle. The solvent on the other hand simply acts as matrix and therefore provides the fluidity to the system.
Under suitable conditions and concentration, the micelles further self-assemble themselves into a lattice like periodic structure. This concentration driven periodic arrangement of micelles is the central basis of lyotropic LCs. Depending upon the concentration and temperature, the arrangement of micelles can either be cubic, hexagonal, lamellar or bi-continuous. Each such arrangement is known as a ‘phase’ of lyotropic LCs. There can be slight variations in the arrangement within one phase leading to sub-phases and therefore more diversities in the arrangement. The lyotropic LC does undergo a phase transition from one phase to another due to the change of concentration. The phase and their concentration dependence are schematically shown in Figure 3. This essentially means that all systems with amphiphilic solute dissolved in a solvent can form LC state. This is probably why lyotropic LCs are so abundant in nature. However, they are yet to find industrial applications but remain an active field of research for biologists and soft matter physicists for their relevance in biological systems like cell membranes and fat trafficking in our blood.

**Figure 3:** Hierarchy of morphologies and different phases formed during the self-assembly of surfactants to lyotropic LC; (a) surfactant molecule, (b) micelles, (c) cubic, (d) hexagonal, (e) columnar and (f) bi-layer.
**Thermotropic LCs**

Most man-made LCs of industrial relevance are thermotropic in nature where the molecular ordering is temperature driven\(^1\). For thermotropic LCs, the material is in a state between that of a crystalline solid and liquid. At a particular temperature, a crystalline solid melts and starts flowing. However, for thermotropic LCs it will still preserve most of its ordered molecular structure. If the temperature is raised higher, all the ordering gets destroyed and the material transforms into a liquid. The intermediate temperature range is where the thermotropic LC is stable. However, the pattern in which the molecules are arranged may change. Based on this the thermotropic LC are divided into several phases and each phase can transform into another when the temperature is changed suitably. Different phases of thermotropic LCs and their molecular arrangement are shown in Figure 4.

Nematic Phases: LCs in nematic phases have no positional ordering but all the molecules are arranged along one particular direction i.e. along the direction vector \(n\). This gives them a ‘thread like’ arrangement, and hence the name ‘Nematic’.

Sematic Phases: The word ‘sematic’ means ‘soap-like’, which refers to the layering of the molecules. In each layer all the molecules are arranged along one direction and they maintain positional ordering. Depending on the tilt direction of the molecules with respect to the layer normal the ‘Sematic’ phase is divided in two sub-divisions

Sematic A: where all the molecules in each layer are aligned along the direction of the plane normal.
Sematic C: where all the molecules are tilted at an angle with respect to the plane normal. The schematic molecular arrangement and optical appearances for each such phase is shown in Figure 5.

![Figure 4: Different phases of LCs: (a) nematic, (b) sematic A, (c) Sematic C, (d) Chiral nematic and (e) Sematic C*. Image Credit: Barreett Research Group, McGill Uni.](image)

**Chiral Phases:**

Chirality means handedness. When the LC molecules or mesogens have handedness or lack of inversion symmetry, they result in formation of such phases. This is frequently observed in cholesterol and its derivatives hence this phase is also known as cholesteric LC. Due to the chiral nature of the molecules, they get arranged in an asymmetric manner. For chiral nematic phase each mesogen progressively twists more than its predecessor around the director, (in a manner to a winding stairs, with the director being along the vertical direction). This twisting pattern remains same for sematic C* (or chiral sematic), but in this case it is the layer of mesogens that progressively twist as shown schematically in Figure 4e. In this case molecular ordering is long-range and the direction of molecules or the layer of molecules gradually rotate around the director and complete several turns. The linear distance between each turn where two
molecules or layers complete turn of $360^0$ is known as the Pitch length (p) (Figure 5). So for every half-pitch (p/2) length, the molecules complete a 180 tilt and appear in equivalent positions (due to their 2 fold rotational symmetry). The pitch (or half pitch) is an important parameter in chiral LCs as it has important implications in the optical properties of the LCs. The pitch can be altered by adding dopants, which imparts great optical tunability to the chiral phases.

![Figure 5: The chiral phases, (a): half pitch and (b): pitch of chiral LC. Image credit: Liquid crystal group U. Colorado at Boulders.](image)

**Blue Phases:**

This is another phase of LC that appear within a brief temperature range between chiral and nematic phases. The uniqueness of this phase is its special molecular arrangement. In this case the mesogens are arranged periodically in a cubic lattice with a lattice constant (spacing between two nearest neighbor) of several hundred nanometers. Such wider spacing of the mesogens allows a periodic arrangement of defect points between them making two inter-penetrating cubic lattices (much like B1 or NaCl type crystal structure) (Figure 6a). This periodic arrangement of both defects and mesogens in one lattice helps them exhibit selective Brag’s reflection. There applications were quite limited until recently when their stability range as increased to a wider
temperature range to fall in room temperature. So, now they find applications in optical switches and even LCDs due to their tunable photonic crystal like behavior.

![Figure 6: (a) lattice structure, (b) typical platelet pattern of blue phase LC under polarized light. Photocredit a: condensed matter theory group, Oxford Uni, b:LC group Kent State Uni.](image)

**Discotic:** Most of the LCs in use have molecules that are longitudinal or rod shaped. But LCs with disc shaped molecules could are also possible. In this case too, the mesogens can arrange in nematic or in smectic architectures. But interestingly, the disc shaped mesogens can also self-assemble to form pillars like structures, which in turn can organize to form a columnar structure (Figure 7) and impart a new phase.

![Figure 7: Discotic LC of (a) nematic and (b) columnar phases. Image Credit: Barreett Research Group, McGill Uni.](image)
Metallotropic

Another new type of LC is metallotropic in which LC phase can be achieved in low-melting inorganic phases like ZnCl$_2$ or other organo-metallic structures. This area is quite new and has not progressed enough to find suitable applications.

Theoretical treatment of LCs

Molecular arrangements and ordered parameters

For materials scientists and crystallographers, crystallinity means repetition of a particular pattern in all three directions. In other words, crystal structure refers to periodic arrangement of atoms that is found in simple crystalline materials like metals, minerals or ceramics. But for LCs it is not the atoms but the molecules that assume an organized arrangement similar to the crystalline solids. The molecules of LCs, known as mesogens, usually are of simple shapes like rod or disc$^{1,11}$. But they can also have more complex shapes like banana to extremely complex shapes (Figure 8). Such complex shapes like in twisted polypeptides, proteins and coiled DNA fragments are usually found in natural LCs. But regardless of their shapes and sizes they are able to interact with the neighboring molecules and the surrounding environment through intermolecular forces like dipole-dipole interaction, ion-dipole interactions, van der walls force etc. It is this inter- (and intra-) molecular interaction that imparts them with the ability to order themselves in a periodic manner (unlike the atoms of liquid, which are almost randomly arranged)$^{11}$. These weak forces therefore allow the LC to flow like a liquid without completely loosing the molecular arrangements.
However, the lack of stronger bonding (like a covalent or ionic bond) makes the arrangement rather ‘local’ and more than one type of arrangement may prevail over smaller length scales compared to some crystalline solids. However there are regions in LCs where most the mesogens are arranged along a particular direction are known as domains (analogous to grains for crystalline solids). The vector representing the cumulative direction of the mesogens (similar to the grain orientation for the crystalline solids), is known as the director of configuration (or simply configuration or director) and is represented as \( n \). It is also worth noting that mostly the mesogens align in a direction close to the director vector \( n \) but not exactly along it. In order to define how closely the molecules follow the director of configuration, an Order Parameter (\( S \)) is used which defines the goodness of the arrangement (Figure 9). The order parameter is defined and explained schematically as shown below

\[
S = \langle P_2(\cos \theta) \rangle = \left\langle \frac{3 \cos^2 \theta - 1}{2} \right\rangle
\]

\text{eq. 1}
Figure 9: The concept of Order Parameter (S); (a) Local ordering of the mesogens, (b) the temperature dependence of S.

For a liquid the value of S is 0 and for solid it is 1 representing random and perfect arrangements respectively. For LCs the value of S lies between 0.3 and 0.9 and is a complex function of temperature and molecular movement. The value of S goes to a minimum at the boundaries between two domains, where the molecules arrange themselves from one direction to another one. These regions are known as defect regions (analogous to grain boundaries). Other than that the molecular arrangement within a domain can get influenced or disturbed by the packing disorders, substrate or interface or even some inclusions, which are aptly known as defect points.

Since the molecular arrangements in LCs are not rigid like solid crystalline materials three different types of ordering is needed to completely describe the arrangement, namely Positional Order, Orientational Order and Bond Orientational Order. The Position order, like in solid crystalline states, describes the cumulative translational symmetry in the arrangement. The Orientational Order on the other hand is the measure of cumulative alignment of the molecules
along the director of configuration in long range. Bond Orientational Order on the contrary describes the short-range alignment of each molecule with respect to its nearest neighbors.

**Theoretical Models for LCs**

Once the arrangement of the molecules could be precisely represented using the order parameters, further theories have been developed to predict the molecular arrangements under different conditions. Some of such theoretical approaches are Onsager hard rod model, Maier-Saupe mean field theory and McMillian’s model. Though each of these models has been developed to study different types of transitions or elastic changes in LCs, the basic approach essentially remains the same in all of them. In general, the parameters or factors are first identified which are the primary driving force for the phase transition. Then, a relation between the parameters and the change in order parameters due to the transition is derived. For example, Osager’s hard rod model uses the minimization of positional entropy by assuming the LC molecules as hard cylindrical objects. This approach is often used to understand phase transition in lyotropic LC. This approach has been the pioneering approach to lead other entropy derived self-assembly theoretical models to study the isotropic to lyotropic LC transition behavior. McMillan’s model, which basically extends the Maier-Saupe model a step further, considers short-range inter-molecular forces like dipole-dipole interactions and calculates the mean-field average of such interactions using statistical mechanics approach. This is a very successful approach and is very successfully used to predict the nematic to smectic transition with good agreement to experimental results.
On the other hand, Elastic continuum theory is used to predict the orientation of the LC within a single phase (often nematic and chiral) to predict their response to external forces\textsuperscript{12}. In this particular case, the molecular details are ignored for simplicity and the LC is treated as a solid elastic body that may undergo a distortion under the influence of externally applied forces. The total distortion is dissolved in three different forms viz. twist, splay and bend and the total distortion energy (which is a measure of Helmholtz Free Energy) due to all three component is summed up. This yields the Frank free energy density ($F_d$), which is given as following

$$
F_d = \frac{1}{2}K_1(\nabla \cdot \hat{n})^2 + \frac{1}{2}K_2(\hat{n} \cdot \nabla \times \hat{n})^2 + \frac{1}{2}K_3(\hat{n} \times \nabla \times \hat{n})^2 \tag{2}
$$

where, $K_i$ are three components (twist, splay and bend) of the elastic constant and are also known as Frank’s constant. If, $K_1 = K_2 = K_3 = K$, then equation 1.2 can be given as

$$
F_d = \frac{1}{2}K((\nabla \cdot \hat{n})^2 + (\nabla \times \hat{n})^2) = \frac{1}{2}K\partial_\alpha n_\beta \partial_\alpha n_\beta \tag{3}
$$

A fourth term may also be included in the equation which is known as the Saddle-splay component ($F_{sd}$) of $F_d$ that describes the surface energy of the droplets. It is given below, but is often ignored as it most cases it is considerably smaller than other elastic components.

$$
F_{sd} = \frac{1}{2}K_{24}\nabla \cdot ((\hat{n} \cdot \nabla)\hat{n} - \hat{n}(\nabla \cdot \hat{n})) \tag{4}
$$
This approach is an extremely successful model for understanding and predicting the general behavior of LC under external fields like electrical and magnetic fields, which are forces the LCs are most often subjected to during their applications as display units.

**LCs in external field**

As discussed above for most of the applications LCs are subjected under several forces, which cause them to undergo some physical change. This essentially result the tunable optical properties of LCs for which are so sort after. At the molecular level, the ordered molecular organizations in LCs are due to weak forces like van der walls, dipole-dipole interactions. So upon application the external field may interact with the intermolecular forces and may alter the molecular ordering of the LCs and therefore their physical and optical properties. The following describes the cases for different fields.

**LCs under electrical field**

LC molecules can be either polar or non-polar molecules. For polar molecules, there are functional groups like -C≡N that impart a permanent dipole moment to the molecule due to the slight shift of shared electron cloud. For non-polar LC molecules, dipole moments are created when electric field is applied. These induced dipoles are much weaker than permanent dipoles but they behave in a similar manner (same type of force but with different amplitude) under the applied electric field. Usually, the LC molecules are longitudinal in shape. So, when the direction of the dipole (permanent or induced) lies along (or parallel) to the long molecular axis, then the molar circular dichroism becomes positive i.e. $\Delta \varepsilon > 0$. In this case, the molecules orient
themselves along the direction of applied electrical field. But when, the dipole is perpendicular to the long molecular axis, thus $\Delta \varepsilon < 0$ and they orient normal to the applied field direction as shown schematically in Figure 10.

**Figure 10:** Effect of electric field on the alignment of LC (a) along the applied field and (b) perpendicular to it, for $\Delta \varepsilon > 0$ and $\Delta \varepsilon < 0$ respectively.

It is important to note that since the applied electrical field exerts the same force (in direction and amplitude) to all the free LC molecules so, all the molecules collectively reorient and the relative ordering between the molecules remain the same. The director of configuration ‘n’ shifts according to the direction of applied electrical potential, causing distortions in director vector ‘n’.

**LCs under magnetic field**

Magnetic field is basically another form of electrical field and thus produces a similar effect on the LC orientations. However, most LC molecules are diamagnetic, they orient themselves
perpendicular to the magnetic field (applied parallel) to minimize distortion of the field and vice-versa as shown schematically in Figure 11.

**Figure 11**: Alignment of LC molecules under magnetic field (a) perpendicular with more distortion, (b) parallel with less distortion.

**LCs under surface tension and at interface**

In absence of an external field, the director $n$ is not forced to align along a particular direction. However, when LC molecules are spread on substrate or at an interface, the surface tension of the substrate (or of the interface) plays an important role in deciding the director of configuration $n$. The type of surface, its surface charge, presence of any defects on the surface and other factors that contribute to the surface tension eventually decides the surface free energy and thus the way LC molecules orient themselves at the surface. When the surface has any epitaxy (preferred orientation or directionality) the LC molecules follows it and align along the same direction. In general the LC molecules can either lie parallel to the surface or stand perpendicular to it, which one called planar or homeotropic anchoring, as shown in Figure 12.
**Figure 12:** Alignment of LC molecules on planar surfaces (a) parallel, (b) perpendicular orientation due the functional groups.

**Freedricksz Transition**

Freedricksz transition is a phenomenon observed in LC molecules placed between two flat substrates with a potential difference applied between them. It signifies the competitive behavior between the surface tension and the electrical forces when applied perpendicular to each other. In absence of any external electrical field the LC molecules align themselves purely under the influence of surface tension. But when electrical field is applied perpendicular to the direction of the surface tension, it begins to influence orientation of LC molecules. At a certain voltage, known as threshold voltage, the electrical force become strong enough to cause deformation in the orientation of the LC molecules. On or above this voltage, the LC molecules lying away from the substrate gets orientated along the direction of the electrical field. However, the LC molecules lying next to the surface tension force (as they experience more force) and each subsequent layer of LC molecules gradually rotate to accommodate this difference of orientation i.e. they gradually tilt 0°-90°, much like a chiral LC as shown in Figure 13. This gradual change of orientation under an electrical field is called Freedricksz transition. The same phenomenon can occur under magnetic or similar forces. This is one of the basic mechanisms used in LC
displays. However, it is not something that will be encountered in our experiments thus will not be discussed in more details.

**Figure 13:** Freedicksz Transition phenomenon in LCs when the configuration (n) gradually rotates from substrates to the middle of the cell in (a) OFF and (b) On condition.

**Optical properties of LCs under polarized light**

Light is an electro-magnetic wave. Thus, its electrical and magnetic components influence LCs orientations in a way similar to what was discussed before. Moreover, visible light has wavelengths between 0.3-0.7 µm, which is comparable to molecular spacing of the LCs. So when light propagates through LC, it gets diffracted from the periodic arrangement of LC molecules. It is mutual interaction between light waves & LCs and thus primarily been utilized both in nature and in man-made display industries\(^{10}\). But for most display units, the optical properties observed uses polarized light. So, for the same of clarify the light waves and the polarization will be explained in brief.

**Polarization of light waves**
Light waves are essentially transverse electro-magnetic wave due to mutually perpendicular, fluctuating electric and magnetic fields, as shown below in figure. A white light comprises of waves that vibrate in all possible angles and directions. But when the waves fluctuate only in one specific plane it is called linearly polarized light, which can be obtained by passing the white light through a polarizer. A polarizer is a material that allows light to pass through only with a specific angle of vibration. The direction of this specific angle of vibration is called the “easy” axis of the polarizer and often lies parallel to its optical axis.

![Figure 14: polarization of light waves (a) linear propagation of light waves, (b) linearly polarized light at 45° and (c) circularly polarized light. Image credit: Polymers and Liquid Crystals Case Western Reserve Uni.](image)

When light waves pass through 2 polarizers, its final intensity depends on the angle between the two polarizers. When the optical axes of the 2 polarizers lie parallel to each other the light pass through each of them and the intensity is maximum. But when they are at 90° to each other, known as cross polarized condition, the polarized light from the 1st gets extinguished by the 2nd and the intensity is minimum, as shown in Figure 15.
Birefringences in LC

Due to the patterned arrangement of (usually) rod shaped LC molecules, they are anisotropic in nature. This anisotropic structure also makes them birefringent, as they demonstrate double refraction i.e. have two indices of refraction. It is easier for polarized light to pass through the LC when the director (n) is parallel to the direction of the polarized light compared to when it is perpendicular. Hence, depending on the relative orientation of the director to the direction of polarization the speed of light gets changed. So, when polarized light travels through a randomly oriented LC (or any optically anisotropic material) it gets split into two components, namely, the fast or the ordinary ray and the slower or the extra-ordinary components. This produces a phase difference since they travel at different speeds. So when they finally recombine and emerge out of LC, the state of polarization has changed due to the difference in phase, as shown schematically in Figure 16.
Birefringence of a material is therefore measured by the difference ($D_n$) of indices of refraction of ordinary & extra-ordinary rays. i.e.

$$D_n = n_e - n_o$$ \hspace{1cm} eq. 4

For a nematic LC, which is a positive uni-axial material $n_o \approx 1.5$ & $D_n$ lies usually between 0.05 and 0.5. For birefringent materials, the optical path in the media (i.e. the optical thickness of the Birefringent material that the light has to cross) is also important as this will decide how much change in polarization state would eventually take place, as the light wave progress through the media. So, the optical path for a wave traveling a distance $L$ is given by $n.L$ and the optical path difference for the two wave components

$$p = L.D_n.$$ \hspace{1cm} eq 5

and the resultant phase difference between the ordinary and extra-ordinary components

$$\Delta \lambda = \frac{2p.L}{\lambda_v} D_n$$ \hspace{1cm} eq 6

where $\lambda_v = \text{wavelength in vacuum}$. 

So, when linearly polarized light enters LC, the following can be controlled:

Extra-ordinary index of refraction
The optical path by changing the thickness

The outgoing polarization state by controlling the optical length

This provide means to control the interaction of polarized light with LCs by controlling these parameters thus, obtaining interesting optical properties. This allows the use of LCs using polarized light for both theoretical studies and practical applications.

**LCs under polarized light**

Let’s imagine that a LCs sample is placed between crossed polarizers (i.e. between two polarizers with their axes of polarization perpendicular to each other). The transmission of the polarizer is aligned at an angle that falls between the fast and slow direction of the LC. Due to the birefringent nature of LC, the incoming linearly polarized light becomes elliptically polarized (as the fast component moves more than the slower therefore changing the axes). So, when this wave reaches the second polarizer, there is now a component (due to elliptical asymmetry & phase difference) that can pass through and therefore the region appears bright, as shown schematically in figure. Now, for monochromatic light, the phase difference world depends on the sample thickness. If the sample is very thin (i.e. $L \to 0$), the phase difference is negligible (i.e. $p \to 0$), according to equation 1.6. But if the sample is thick the phase difference is large. If the samples have the thicknesses which cause the phase difference to become $360^\circ$, then the wave overlaps to its original polarization state and gets blocked by the second polarizer. Therefore the sample thickness and hence the phase shift determines the intensity of transmitted light.
However, if the transmission of 1st polarizer lie along the ordinary or extraordinary component direction, light does not break into components (perpendicular component is zero), thus there is no phase difference. Hence no change in polarization, leading to compute blockage of light by the end polarizer and the region appears dark. So, the intensity of the transmission light depends on the angle ($\phi$) between the LC and polarizer ($I = k\sin^2\phi$) for a typical LC sample the alignment or the director (n) changes from one domain to another (analogous to grain orientations as described previously) changing the difference. The sample thickness is also not constant. Thus, when a typical nematic LC sample is looked under cross-polarized light regions of light & dark shades appear as shown in figure. This change of shade corresponds to difference in director orientation due to difference in domains, birefringence or length. Such a contrasting texture is a characteristic of nematic LC and is known as Selieren texture. The dark regions correspond to extinction orientation where the director (n) lays either parallel or perpendicular the polarizer or analyzer axes there removing any phase difference. These dark regions are called “brushes”, as per their appearance.

![Diagram](image.png)

**Figure 17:** A typical ‘schlieren’ texture in a nematic LC showing the dark brush regions, which meet at different points of disclination. Photo credit: LC group, Kent State Uni
The points where 2 or 4 such brushes (or dark regions) meet correspond to a unique director vector and are known as disclination in the structure. Each such disclination is characterized by its strength (S) which basically denotes how many brushes meet at the point of disclination. The point where four of them meet correspond to $S=\pm 1$ (and is a high strength) and that where they are two, $S=\pm \frac{1}{2}$, which are the two typical values of $S$ for nematic phase. The sign of strength denotes how the brushes rotate with respect to the sample. The negative sign corresponds to the same & the positive denotes to the opposite direction. Usually, two neighboring singularities have opposite signs.

**Texture of LCs**

Texture in crystallography refers to alignment along a particular direction. For LCs, an alignment of its molecules along one direction is known textures. Each mesophase of LCs when all the molecules and orientated (under the influence of any governing forces) parallel to the surface they are known as planar texture. And they are all perpendicular it is called the homeotropic texture, as shown below. But texturing is often not stretched along the entire length of the sample (of mesoscopic ~ cm length scale), as the director vector change from domain to domain. So, one can observe both textures and an alignment in between them. This gradual change of intensity is very typical to nematic phase.
**Figure 18:** Nematic LC textures showing (a) planar (b) homeotropic anchoring. Photo credit: Prof Lavrentovich, Kent State University.

**Defects in LCs**
When the change of brightness is abrupt, it signifies the presence of a defect in the LC. This basically signifies to a sudden change in the director of orientation \( n \). Such a drastic change in \( n \) along a line or at a point (singularity point) is known as a disclination and is regarded as defects (line or point defects). At disclination the director of orientation is undefined.

**Figure 19:** The disclination line and the change of director around it a: schematic b: electron micrograph. Photo credit: Polymer and LC lab, CWRU

**Time line of LC applications**
As discussed before, that even though the LC state was discovered in late nineteenth century, the applications of LCs came only in 1970s and later. Figure 20 gives a time line of the significant discoveries in the field of LCs. The discovery of the LCs with cholesteryl benzoate in 1888 did not ignite much research attention. In the next fifty years, more materials were isolated or synthesized that showed LC behavior. But there was no molecular understanding of the phenomenon, due to the lack of advanced characterization tool available at that time. However some rudimentary trends like elongated structure, multiple melting points, were beginning to
emerge. Finally in 1930s the molecular structure of cholesteryl benzoate and similar molecules were discovered. This promoted organic chemists to synthesis molecules that gave LC behavior. Thus in the next couple of decades a better understanding of the molecular structure and the phase transition behavior was obtained. By 1960, chemists could synthesize synthetic molecules or its concoctions that showed remained stable in a wider range of temperatures and the physicists could come to some molecular theory to understand and predict the phenomenon. This predicted the opto-electrical properties of LCs. The big breakthrough came in 1969; Kelker and co-workers synthesized MBBA whose solid to LC transition temperature was at room temperature. At the same time Grey and co-works developed cyanobiphenyl derivatives that too showed room temperature transitions. Subsequently, the tunable optical properties as predicted by theory were also studied for the cyanobiphenyl derivatives. All these eventually lead to development of the first digital numerical display in 1973, which marked the first technological application of LCs.

These digital numerical displays become increasingly popular especially in digital watches calculator screens and marked the beginning of digital age. The low power consumption, attractive features along coincidence with the rise of electronic chip industries combined all the elements to make the LC based displays a technologically success story. In 1975 Mayer and co-workers reported the perroelectric behavior of LCs in p-decyloxybenzylidine-p’ amino-2 methylbutyl cinnamate (DOBAMBC). Further, research with the opto-electrical properties of ferroelectric LCs helped researchers tune the optical behavior of LC with the applied electrical field. These LCs were better than the previously used twisted nematic type due to their ultra-fast
response time and better energy-efficiency. In a device thousands of tiny cells containing the LCs called ‘pixels’ were assembled and each of which was connected to thin-film transistors. This allowed the color and intensity of each pixel to be controlled individually and digitally using electrical signal and the flat panel liquid displays (LCD) were born. This is lead to the revolution in the digital displays. They were thus been used in televisions; computer screen or any such display devices and soon made the bulkier cathode ray based displays obsolete. Further research discovered better LC materials like supertwisted nematic, chiral sematic and even blue phases that enabled bigger and better displays. The displays kept getting better and within thirty years of the fabrication of first device the LC based displays have spread to all possible direction and are rather ubiquitous.

Figure 20: The time line showing the most significant discoveries in the field of LC
**Primary application of LCs**

Since their discovery, electronic displays or LCDs have remained the primary application of LCs. Over the last couple of decades it has undergone various design upgrades mainly to improve the resolution, response time and decrease the width. But in principle the basic working model remains the same. Figure 21 describes the basic working principle remains the same. Each display is composed of several thousand or even few million pixels connected and controlled together digitally. Each pixel is a working unit cell and works similar to the first display. In a typical LCD, light waves are sent across the cell from its rear side. The light waves then encounter two polarizers, set at cross-polarizing conditions and in normal conditions cannot immerse from the other side. Between them is a patterned layer of LC, which is connected to two electrodes. The potential applied between the electrodes induces LC to change its anchoring form planar to perpendicular (homeotropic) and thus ‘bend’s (introduce phase difference due the optical anisotropy) light that is passing through it. This helps light waves to overcome the cross-polarization condition and illuminates the cell when looked from the other side. Other than electronic displays, LCs find applications in privacy windows (Figure 22), tunable filters and spatial light modulators, all of them for their opto-electrical properties.
Figure 21: (a) A schematic, (b) An image of LC based digital display. Photo credit: Dr J. Fernsler, Cal Poly

Figure 22: Actual and schematic image of a privacy window. Image credit: Inhabitat.com/super smart privacy glass.

**Liquid crystal and self-assembly**

With the advent in the LC technology in early 1970s, physicists got more interested in the condensed matter theories for interfaces, polymeric and similar non-crystalline materials. The properties and thermodynamic behavior of such materials especially LCs and polymers seemed different than so far encountered in metallic or crystalline materials. Around the same time, chemists were working together with biologists put more emphasis on understanding the chemical aspects of the biological processes. A decade or so later, a new phenomenon had began to emerge which gradually was known as self-assembly. It produced mesoscopic materials,
which behaved much different than either macro or microscopic materials. The physics of this was understood a decade later. From there, the theory of soft material began to take shape after Pierre-Gilles de Gennes was awarded the Nobel Prize in pioneering the field. As the field got more research attention, researchers found remarkable similarity between self-assembled soft structures with biological components like cell membranes and even optical properties of LCs and a common theory was developed to predict the behavior of such soft matters in general. For the first time physical properties of biological interfaces and sub-structures like cell walls, proteins, surfactants, colloidal particles, LCs and polymers came under one scientific umbrella. This probably prompted researchers to use synthetic soft matters to understand the more complex biological structures that lay higher in the morphological hierarchy.

**Figure 23:** Timeline for the development of soft matter in general and LC based sensor in particular.
**LC interface as sensing platforms**

LCs were beginning to find more use than just opto-electrical displays or similar devices. The fact that LCs had extremely sensitive interface made them extremely amiable to be used in study of interfaces\(^{14}\). By 1990s, nematic LCs emerged as a simple yet very effective tool in understanding the study of monolayers, their interactions and similar soft matter studies. Following the scientific progress of the past (Figure 23), LC was waiting to be re-discovered for a completely new type of application. In 1998, Abbott and co-workers reported the first LC based sensing tool to study the binding of ligands to the receptors in cell wall using an optical microscope\(^{15}\). The tool was a small two-dimensional cell similar to a small display cell, which gradually changed become bright when exposed to the ligands. In 2003, Fang and co-workers reported the use of LCs for imaging cells. These two discoveries marked the beginning of LC based sensing units that could be used to measure biological phenomena. Subsequently, numerous studies been reported mostly by Abbott and team in the field to detect several biologically important species like surfactants, proteins, enzymes and to monitor interfacial phenomenon like binding of ligands, coupling of two proteins\(^{16-27}\).

Figure 24 schematically describes the way these biosensors work. Typically a 200 mesh copper grid is used as a substrate, on to which a thin film of 5CB, a room temperature nematic LC, is coated. The 5CB LC is oil like organic molecule; hydrophobic in nature. This is then dipped in aqueous environment within the cell, leading to a homeotropic or perpendicular anchoring of the 5CB molecule at the aqueous interface. The perpendicular anchoring in planer surface cannot
bend the polarized light. The cell appears dark under cross-polarized conditions in this case. This marks as the ‘set’ state of the device, which is ready for the detection process. When the cell environment is replaced with a lipid-rich aqueous solution, the lipids bridge the 5CB-water interface. In this case, the long chain amphiphilic lipid molecules arrange themselves in way that the long hydrophobic chains remain buried inside the hydrophobic 5CB layer and the polar head groups face the water, making the lipids ‘stand straight’ at the 5CB-water interface. This induces the 5CB molecules to along align perpendicular to the interface in homeotropic manner, as shown schematically in Figure 24a. This marks the ‘reference state’ before the exposure of the analyte, an enzyme in this case. Since the molecular ordering remains homeotropic, i.e. same as the ‘set’ condition, the cell continues to remain dark. But when the lipid interacting enzyme solution is exposed to the cell, the enzyme molecules begin to interact with the lipids at the 5CB-water interface. This results in the engulfment of the hydrophobic tails of the lipids. This locally destroys the standing arrangement of the lipid molecules at the interface. This induces the 5CB molecules undergo transition of alignment from homeotropic to planar, as shown in the Figure 24b. The regions where such transitions occur can now bend the polarized light and thus they glow up under polarized light. The process is slow as the diffusion takes time in confined cellular environment. A transition of configuration could be observed in a time dependent manner as the dark cell gradually changed to bright patterns. Depending on the analyte, the reference point can be made bright (due to planer anchoring) in the beginning and it gradually change to dark (due to planer to homeotropic transition) when a species of interest is exposed to it. Any either of the way, the basic detection mechanism remains the same. The upon exposure to the analyte of
interest, the configuration of the LC molecules undergo a transition which changed the intensity of the light passing through the cell in a concentration dependent manner.

**Figure 24:** LC thin film based sensor. schematics showing the (a) perpendicular, (b) planer anchoring before and after the exposure to the enzyme and (c) optical image of the sensor panel showing the gradual change in the intensity of transmitted light with time, as the enzymatic reaction progresses. Photo credit a and b Woltman et al. Nature Materials 2007, 6, 929 and c Prof Abbott and co-workers in U. Wisconsin.

This indeed was great leap in the use of LCs; Abott and his co-workers remain pioneers in using this particular technique. The best merit of this technique is its ability to observe and detect cellular mechanisms and biologically important phenomena that are sub-microscopic or even nanoscopic, simply using polarizing light microscopy with reasonable accuracy and sensitivity. For the next almost a decade more improvement in the design took place and numerous species and phenomena could be observed using this novel LC based detection tool. This technique had several other advantages too like it was simple and easy to use, portable and even could be made specific. But it has certain disadvantages too like
it is extremely delicate architecture, lack of robustness in design and less surface area due the two-dimensional planer geometry.

**LC droplets as sensors**

LC droplets on the other hand proved to be a better sensing platform than their 2-D counterpart. By this simple improvement in dimension and design most of the disadvantages of the LC thin film based sensing unit could be addressed. Hence, LC droplets have very recently become attractive due to their large surface areas and tunable director of configurations, which are critical for developing their applications in biosensors. It has been shown that the liquid-crystal droplets dispersed in aqueous solution can be stabilized by the adsorption of surfactants and polymers at the LC-aqueous interface. The director of configuration of liquid-crystal droplets reflects the balance between the elasticity and the surface anchoring of the LC inside the droplets. The adsorption of chemical and biological species at the surface of the stabilized LC droplets may change the surface anchoring and disturb the balance, triggering the *director of configuration* transition of the LC inside the droplets. Therefore, the liquid-crystal droplets with stimuli-responsive *director of configurations* provide a unique platform to detect the chemical and biological events occurring at the surface of liquid-crystal droplets\(^{45-48}\).

Recently, Abbott and coworkers have shown that poly(allylamine hydrochlorid) (PAH)/poly(styrenesulfonate sodium) (PSS)\(^{49,50}\) multilayer coated-LC droplets can be used in detecting lipid-enveloped viruses by simply observing their *director of configuration* transitions with a polarizing optical microscope\(^{51-54}\). They find that the bipolar-to-radial configuration
transition in detecting lipid-enveloped viruses is a result of the transfer of lipids from the viruses to the PAH/PSS multilayer coated-LC droplets. Yang and coworkers have reported an immunoassay in which immunoglobulin G (IgG) are immobilized on the polyethylene imine-coated LC droplets. The formation of anti-IgG/IgG immunocomplex at the surface of the LC droplets is found to induce the configuration transition of the LC inside the droplets\textsuperscript{54}.

**Thesis objectives and outline**

This thesis focuses on designing novel biosensors using surface functionalized the LC droplets. Stable LC droplets have defined director of configuration and a particular optical pattern observed through polarizing light microscope. The director of configuration of LC droplets is found to be extremely sensitive to any changes in their solution environment. A change in pH and the presence of salt can also trigger the change in a concentration dependent manner. This motivates the designing sensing applications based on these LC droplets. The use of light microscope for observation highlights the simplicity and cost effectiveness of this system. Another main objective of this thesis is to mature this area of sensing with sensitivity and specificity and make it a robust and stable system for clinical and technological sensing.

Chapter 3 focuses on synthesis and stabilization of LC droplets. Effect of different types of polyelectrolytes with various functional groups, used to synthesize stable LC droplets on director of configuration is discussed in this chapter. The sensitivity of these droplets towards any kind of surface modification and environmental change was also investigated.
The polyelectrolyte stabilization not just provided a charge onto the LC droplets but also imparted them with a well-defined director of configuration, which served as the reference for the detection studies. Chapter 4 discusses the use PSS coated droplets to sense charged molecules. Poly-amido amine (PAMAM) Dendrimers was used a proof of principle. Following this, polyelectrolyte coated droplets were used to detect biologically relevant species like proteins and viruses.

Detection of pathogenic bacteria is of great clinical and technological importance. Certain strains of *E. coli* are virulent and are commonly associated with food poisoning. The use of LC droplets to detect Escherichia coli (*E. coli*) is elaborated in Chapter 5. The presence of *E. coli* in solution was detected using a synthetic polypeptide decorated droplets.

Lithochloric acid (LCA) is a bile acid and is involved in lipid and cholesterol transport in our body. However, when the bile acid clearance from the body is affected, due to any physiological conditions, its concentration in blood increases, making it genotoxic and mutagenic enhancing. Thus, higher concentration level of LCA is used as a specific biomarker for the diagnosis of colon cancers. Chapter 6 explores the use of surfactant stabilized LC droplets detect LCA concentration.

In this work, the primary focus was to observe whether the surface engineered LC droplets can be used to as a detection platform for different analytes and also to achieve high sensitivity.
Here, by sensitivity or detection limit we mean the lowest possible concentration of the analyte that can be detected.

Finally, the conclusion and future research directions are discussed in Chapter 7 of this thesis.
CHAPTER TWO: GENERALIZED EXPERIMENTAL APPROACH

Materials Used

In all of the experiments, n-pentyl-cyanobiphenyl (5CB) was used as the model liquid crystal. It melts form solid state to liquid crystal state at 18°C and remains so until 34.2°C, at which its transition to liquid takes place. Hence, it remains in nematic phase at room temperature, which suits experimental conditions. The liquid crystal molecules of 5CB were purchased from EMD Licrystal and were used as received.

The analytes for detection ranged from simple polyelectrolytes to pathogens like bacteria in some cases. All the polyelectrolytes were purchased from Sigma-Aldrich and were used without any further purification. The same is true for all the surfactants and bile acids. The details of stabilizing concentrations and the procedures are given in individual chapters. All the lipid molecules were obtained from Avanti Polar Lipids and the biological species like bacteria and viruses were given by our collaborators. Further details of all the analytes are provided in individual sections, which highlight their detection.

All the experiments were conducted in aqueous media like saline or buffer solutions or just in de-ionized (DI) depending on the analyte. The water used in our experiment was DI water from Easy-Pure and had a resistivity of ~18.2 MΩcm-1.

Most of the experiments involved optical microscopy, since whole of the work is on light microscopy based detection. Transparent glass slides and cover slips used were from Fisher
Scientific made from Swiss glass. The glass slides were cleaned in two steps. In the first step, they were immersed in ethanol and cleaned using bath sonicator for about 5 minutes. They were then transferred into DI water and sonicated for about 5 minutes and then transferred into a dry glass slide holder and air-dried. Subsequently, they were preserved in sealed containers to avoid the exposure of dust particles.

**Experimental**

*Synthesis of the Droplets*

Independent of stabilizing agents (polyelectrolytes or surfactants) the basic procedure for liquid crystal droplets remained the same. The following describes the general procedure of synthesis and the minor details are discussed in the respective chapters. In a typical synthesis 10 mL of stabilizer solution of adequate concentration (Usually ~ 10 mg/mL for polyelectrolytes or 1 mM for surfactants) is taken in a 15 mL plastic tube. To this 10 µL (i.e. 1 µL/mL) of 5CB is added carefully and mixed well by vortexing the tube for about 10-15 seconds. The vortexing helps the viscous oil-like 5CB to break into smaller droplets, which can be seen suspended in the solution. The tube is then placed into the bath sonicator (Branson 2510) without any delay. The water bath of the sonicator is kept at around 23 °C. The sample is sonicated for a total of 15 minute, with vortexing it after every 5 minutes for about 10 seconds. The vortexing helps a homogeneous distribution of the droplets and keeps the droplet size distribution narrow. After sonication the transparent solution turns milky white indicating the formation of tiny droplets as they scatter light. The droplet solution thus formed contained free stabilizers in the solution. Thus, the droplets were washed using DI water twice by centrifugation. The speed and time of the
centrifugation depends on the stabilizer used. The “clean” droplets were then preserved carefully for further experimental studies. Droplets more than 2 days old were not used for experiments as they could have became less sensitive with time, even though some of droplets were stable for months.

For certain studies, droplet samples had more than one layer of coating on their surface. For the subsequent layers of coating, the droplets coated with first layer of stabilizer were incubated with adequate concentrations of surfactant or stabilizer in buffer or DI water. After about 30 minutes, they were removed and washed by centrifuging. The details of concentrations, incubation media and washing steps are elaborated in each experimental section. This cycle of incubation and washing was repeated till the desired number of layers of coating was obtained.

**Analyte Incubation**

Once the droplets with adequate surface functionalization are obtained, they were incubated with analyte or the species of detection. In a typical incubation, about 50 µL of surface functionalized droplet solution was taken in a 500 µL microcentrifuge tube. To this a calculated volume of analyte solution of desired concentration was added carefully. Finally, the volumes of all the samples were raised to same level (usually 100 µL) with DI water or buffer saline solution, as required. The tubes were then slightly vortexed to ensure thorough mixing of the droplets with the analyte. The tubes were allowed to stand for 30 minutes (or more if needed) at room temperature. After the incubation time, the samples were vortexed lightly again to mix up the contents, before observing them under light microscope in polarizing and bright field mode.
Characterization

Optical Microscopy

The entire detection system proposed here is based on basic light or optical microscopy, which is the primary basis of the simplicity in this approach. For our experiment, we used Olympus BX51 as our optical microscope with polarization features. Figure 25 explains the operations of the microscope. The light source is heated at the bottom and luminosity can be controlled. The light passes through a polarizing lens (42 mm Prinz polarizer Japan), which polarizes the light along X-Y planes. The polarized light passes through the sample, containing liquid crystal droplets. Once transmitted through the sample, the light has to pass through another polarizer, whose plane of polarization lies perpendicular to the first polarizing lenses. Most of the light gets blocked by this cross-polarizing condition (as explained in Chapter 1). The presence of liquid crystals within the droplets bends the light when the later pass through the droplets. The ability of liquid crystal to bend the polarized light (due to its optical anisotropy) allows the droplets to be viewed under cross polarized condition. Depending on the arrangement of molecules within the droplets i.e. planar or homeotropic anchoring, each droplet seems to bear their optical signature for either radial or bipolar under cross-polarized light. This essentially serves as the optical signal for our sensing mechanism. After passing through the sample, the light comes to the eye pieces for visual observation and could also be documented by photography using, Steindorf camera. When, the polarized images are not required, simple bright field images are captured. In this case the second polarizer is removed from the light path to avoid the cross-polarized condition. For bright field, the intensity of light is decreased to suitable level since no
light is lost through the absorption into polarizing lenses, as in case of crossed-polarized image. Bright field images are usually taken for general observation of the samples; it is the cross-polarized images that reveal the sensing phenomenon.

**Figure 25:** The polarizing light microscope (a) Olympus BX51 and (b) the schematic of the image formation.

*Optical Microscopy Data Analysis*

For every data point, at least 20-25 captured images were manually analyzed to have a total droplet count of about 100-200. For transient condition (radial to bipolar or bipolar to radial transition), more droplets were analyzed to minimize the errors. During the analysis, number of bipolar or radial droplets are counted from each optical photograph and tabulated to maintain the statistics. Droplets that did not appear to be distinctly radial or bipolar are tabulated as errors. Once the tabulation is computed, the numbers of bipolar and radial droplets were converted to percentage values, which give the percentage transition for each condition along with an error-bar.
Fluorescence and Confocal microscope

Fluorescent microscope is a modified version of light microscope except that it uses UV or visible light waves and filtering mechanism to observe fluorescent dyes or fluorophores. It is a tool used extensively in chemistry and biology when specific details within samples are needed to be viewed. In a rudimentary way, a light wave of particular frequency called absorption frequency (compared to white light in light microscopy) is shone on the sample containing some fluorophores. The fluorophores absorb this frequency, gets excited and subsequently release emission, in order to come back to ground state. This emission of wavelength makes the fluorophores glow, making them visible specific to a particular frequency. So, when any particular feature from the entire sample features needs to be observed, that particular feature is conjugated with a fluorophores. This enables only the region of interest to glow and rest remains dark, when viewed through fluorescent microscopy. Since, all the sensing in this work, is based on the attachment (or detachment) of the analyte /species of interest onto the droplet surface therefore triggering the change of orientation of the liquid crystal molecules inside the droplets (which is thus observed in the cross-polarized microscopy). Fluorescent-labeled analytes were used to observe the attachment/detachment mechanism and a fluorescent microscope was used to reinforce the observations (or hypothesis) from the polarized light microscope, and to characterize the binding behavior. For simple fluorescent studies, Acu-scope 3031 inverted microscope with mercury lamp was used and for confocal imaging, Zeiss Axioscope-2 mot plus with Argon- Krypton laser (Melles Griot) was used.
Photoluminescent spectroscopy

Photoluminescent spectroscopy operates on the same mechanism of exciting a fluorophore and recording its emission. However, it records the intensity of emission rather than observing its image. Photoluminescent spectroscopy is widely used as sensing units and the intensity of emission is often made to relate to the concentration of the analyte. Comparing the data from liquid crystal based sensing and fluorescent spectroscopy based sensing, provided good accountability to this novel sensing technique. Jasco FP-6500 spectrofluorometer was used for all the measurements.
**UV-Vis spectroscopy**

UV-Vis spectroscopy was used to observe the optical absorption or transmittance in UV-Vis light. Thermo electron corporation UV1 UV-Vis spectrometer was used for characterization. The amount of light scattered by the liquid crystal droplets were measured using the instrument. They were then normalized with respect to the maximum absorbance and converted to transmittance values by subtracting them from 1 (as 100% absorbance= 0% Transmittance). The transmittance intensity was related to the concentration of droplets in a complex fashion, as more droplets scatter more light and hence, have less transmittance. UV-Vis spectroscopy provided an approximate idea about the droplet concentration. The actual droplet counts were done using optical microscopy.

**Infra Red (FT-IR) Spectroscopy**

Similar to any other spectroscopy technique IR spectroscopy measures the absorbance in the Infra-Red wavelength and Fourier Transformation (FT-) gives the entire spectral feature. Since most chemical bonds have energies correspond to IR wavelength, so it can be used to analyze the change in the chemical bonds. Since, IR spectroscopy is used to characterize molecular interaction and changes in functional groups; Nicolet IR100 was used to understand the mechanism interaction between the analyte of interest binds and the surface functionalized liquid crystal droplet.
Figure 28: (a) Nicolet IR100 to obtain IR-spectra and (b) Melvern Zeta Sizer Nano for particle size and zeta-potential.

Dynamic Light Scattering

Particles suspended in a fluid media when hit by a beam of light, scatter light in all directions, known as Rayleigh scattering. The particles in the solution move randomly (Brownian motion) and it influences the scattering of light. This eventually produces fluctuations in the intensities of the scattered light. The rate of fluctuation basically depends on the motion of the particles, which in a turn depends on the size of the particles. Monitoring the fluctuation of the intensities of scattered light can give an estimate of the size of the particles. This essentially is the basis of dynamic light scattering. Usually, a monochromatic and coherent laser source is used and a time-dependent fluctuation in scattering-intensity is recorded which is then analyzed through a review of co-relation functions to eventually obtain the size of the particles. DLS was used to measure the size of droplets using Melvern Zeta Sizer Nano. The size or the diameter of the droplet is quadratically related to the surface area. Since, detection mechanism is basically a surface phenomenon it is important to known to have a good estimate of the surface area therefore the size of the droplets.
Zeta Potential

Zeta potential is a measure of surface charge and therefore the stability of colloidal systems. The surface charge of each colloidal particle makes them repel (electrostatic repulsion) each other stabilizing the colloidal system. This surface charge attracts counter-ions that form the electrical double layer. The zeta potential corresponds to the surface charge of the second layer, known as the slipping plane. The zeta potential measurements were carried out in Melivern Zeta Sizer Nano. The basic principle of the measure is similar to the measurement of DLS (which is why they often used the same machine just with different operating conditions). The sample is loaded in a polycarbonate cuvette fitted with two electrodes on each side. Through these electrodes a known electric potential is applied which includes the charged colloidal particles to move towards oppositely charged electrodes. This induced motion is proportional to the surface charge and effect the scattering of light and thus the fluctuation if the intensity of scattered light. Therefore, by monitoring the applied potential and light scattering, the surface charge of the colloidal particles can be measured.

Zeta potential measurements were done to characterize the surface charge and stability of the surface engineered liquid crystal droplets. These measurements were conducted before and after the exposure to the analyte to observe the electrostatic interactions between the droplets and the analyte. This helped to understand the mechanism that triggered the transition of configuration. Thus, zeta-potential measurements were a very important tool to understand and hypothesize the underlying mechanism responsible for the detection.
Transmission Electron Microscope

Transmission Electron Microscope (TEM) is like light microscope but it uses accelerated electron beams instead of light. Electron microscopes have a better resolution due to smaller wavelength of the electron beam and thus used for finer details in a sample. The resolution can be tuned by changing the acceleration of the electrons (thus their wavelength) to observe images in different resolutions. TEM is extensively used to explore sub-micron size features of a sample in materials science and related fields. However, TEM characterization of soft materials has limitations and is challenging, as the electron beam sometimes can be powerful enough to destroy samples. However, it was used to study the binding of biological species like virus onto the droplet surface and to characterize the multi-layered droplets. For TEM characterization, samples analyte treated droplets were placed on holy carbon coated copper grids (Electron microscopy sciences) of 200 mesh size, by placing a drop of the suspension onto the carbon coated side of the grid for about 15-20 minutes. The excess liquid was then removed and the grids with the droplet sample on them were allowed to dry in air at room temperature. The TEM used was JEOL 1011, a low resolution TEM with 100 kV of acceleration voltage, sufficient enough for our resolution and not strong enough to destroy the samples.
Figure 29: (a) TEM Jeol 1011 (b) Origin and (c) Image J used for processing microphotographs.

Data representation
The data was represented using Origin 8.0 as the plotting platform. Most of the data analyzed was from optical microscope and care was taken to represent overall sample volume. More droplets were analyzed to minimize the errors. For transition curves, the data-points were joined using B-splines, which resulted in S-curves. For the size distribution of droplets or similar analysis histograms were used and fitting functions were chosen to obtain the Gaussian curves. For bright field images, the fluorescent microscopy images and other analytical calculation like size, number of droplets ImageJ was used. ImageJ is image analysis software been developed and recommended by the National Institute of Health.
CHAPTER THREE: STABILIZATION AND SENSITIVITY OF POLYELECTROLYTE COATED LC DROPLETS

Introduction

Polyelectrolytes are polymers with repeating units bearing an electrolyte group. The electrolyte group dissociates in aqueous solution, making the polymers charged. Polyelectrolytes can be good stabilizing agents due to both stearic and electrostatic stabilization. It has been previously reported that negatively charged poly(styrenesulfonate sodium) (PSS) can stabilize LC droplets in aqueous solution. The PSS stabilized liquid-crystal droplets can be further modified. Interestingly, such modifications can help bind different functionalities to the LC droplets, which may have an effect on the optical properties of the LC inside the droplets.

To further develop applications of polyelectrolyte functionalized LC droplets, it is important to understand how the director of configuration of the LC within the droplets is effected by the nature of polyelectrolytes. In this chapter, the stabilization of LC droplets using polyelectrolytes as stabilizing agents is demonstrated. Several polyelectrolytes, both positively and negatively charged, were used to demonstrate this. Figure 30 illustrates the molecular structures of all the polyelectrolytes that we used to stabilize LC-water interface. It was observed that all the polyelectrolytes (except those with amine groups at higher pH) produced bipolar configurations. The polyelectrolyte coating onto the LC droplet surface not just provided stability but also imparted them with a well-defined director of configuration.
Figure 30: Molecular structure of the polyelectrolytes used as stabilizing agents.

The LC droplets coated with single layer of polyelectrolytes could further be surface modified using the layer-by-layer assembly of polyelectrolytes. Here, PSS stabilized droplets were coated with PAH and PDADMAC to obtain two different sets of polyelectrolyte multilayer coated LC droplets. For the PSS-PAH pair, where both polyelectrolytes bind tightly to each other, did not show any change of configuration due to the change in their solution environment. But due to the higher coupling distance between PDADMAC and PSS, the PSS/PDADMAC multilayer-coated LC droplets were found to be sensitive to their environments. Herein, we observed that the layer-by-layer adsorption of positively charged PDADMAC and negatively charged PSS on to the LC droplets triggered alternate bipolar/radial transition (Figure 31). The configuration encapsulated 5CB droplets were found to be sensitive to the concentration of polyelectrolytes added into the solution. Furthermore, it was found that the director of configuration of the 5CB droplets coated by PDADMAC/PSS multilayers was also sensitive to the pH values and salt concentrations of the droplet solution.
**Figure 31:** Layer-by-layer assembly of polyelectrolytes triggered alternate bipolar/radial transition in PSS/PDADMAC coated LC droplets

**Experimental**

**Experimental Methods**

The basic synthesis protocol remained the same as is described in chapter 2. The multilayer coating of the PSS-stabilized 5CB droplets was done by the layer-by-layer adsorption of positively charged PDADMAC and negatively charged PSS. During the layer-by-layer adsorption, 1 mL PDADMAC solution with a concentration of 1 mg/mL and 0.1 M NaCl was added into 1 mL of the PSS-stabilized 5CB droplet solution. The mixture was incubated for 20 mins to ensure the adsorption of a PDADMAC layer. The PDADMAC/PSS-coated 5CB droplets were purified by centrifuging the solution for 15 mins, then washing them with water for 2 times, followed by the adsorption of a PSS layer. The PSS solution with a concentration of 1 mg/mL and 0.1 M NaCl was used in the PSS layer coating. This process was repeated until the desired number of PDADMAC and PSS layers was achieved on the 5CB droplets.
**Characterization**

The director of configuration of the 5CB inside the polyelectrolyte coated droplets was characterized by a polarizing optical microscope (Olympus BX40). TEM measurements were carried out for PDADMAC/PSS multilayer capsules dried on carbon-coated grids. The $\zeta$-potential measurements were also carried to observe the surface charge.

**Results and Discussion**

The adsorption of the polyelectrolytes was suitable to stabilize the LC droplets. However, the stability of the droplet was dependent on the concentration of the polyelectrolyte added. This suggests that a critical thickness of polyelectrolyte layer is necessary to stabilize the droplets. Otherwise the LC droplets do not have enough polyelectrolyte at the interface to provide the necessary stability and also a stable director of conformation. Figure 32a shows the concentration dependent stabilization of 5CB droplet with PSS. Further addition of PSS resulted in increased number of 5CB droplets in the solution. This is because more polyelectrolyte is available to stabilize more surface area. Thus, all the 5CB droplets formed due to the sonication, gets stabilized due to the high concentration of the PSS in the solution (Figure 32b). However, addition of excess PSS results only in the increase of free polyelectrolytes in the solution, which only makes the purification process more cumbersome.
Figure 32: The effect of PSS concentration on the LC droplet formation. (a) The transition of the LC droplets from unstable Radial to stable Bipolar as a function of PSS concentration. (b) The effect of additional PSS on the number of droplets formed.

Similarly, the use of PAA, PAH, chitosan, PEI, PDADMAC also provided good stability to the 5CB droplets. They all produced a very well defined director of configuration. Table 2 provides the list of polyelectrolytes, their surface charge and the director of configuration that it imparted to the droplets. It can be observed from the table that most polyelectrolytes produced bipolar droplets regardless of their surface charge, except for PEI and PDDAC. For PEI and PDDAC, both with quaternary amine groups, imparted a radial conformation to the LC droplets at basic pH.
Table 2: The polyelectrolytes used to stabilize the LC droplets and their configurations

<table>
<thead>
<tr>
<th>Polyelectrolyte</th>
<th>Functional group</th>
<th>Configuration</th>
<th>$\zeta$-potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSS</td>
<td>SO$_3^-$</td>
<td>Bipolar</td>
<td>- 43 mV</td>
</tr>
<tr>
<td>PAA</td>
<td>COO-</td>
<td>Bipolar</td>
<td>- 42 mV</td>
</tr>
<tr>
<td>Chitosan</td>
<td>NH$_2^+$</td>
<td>Bipolar</td>
<td>+ 47 mV</td>
</tr>
<tr>
<td>PAH</td>
<td>NH$_2^+$</td>
<td>Bipolar</td>
<td>+ 41 mV</td>
</tr>
<tr>
<td>PDADMAC</td>
<td>N+(C)$_4$</td>
<td>Radial</td>
<td>+ 47 mV</td>
</tr>
<tr>
<td>PEI</td>
<td>N+(C-NH$_2$)</td>
<td>Radial</td>
<td>+ 45 mV</td>
</tr>
</tbody>
</table>

It is known that the bipolar configuration represents the planer anchoring of the LC molecules at the LC interface and often is a result of $\pi$-$\pi$ type interaction or a lack of dipole-dipole interaction. Since most of the polyelectrolyte imparted a bipolar structure so, it is rational to assume that the polyelectrolytes coating of the LC droplets favor a $\pi$-$\pi$ type interaction between the hydrophobic alkyl tail and bi-phenyl core of the 5CB molecule with the hydrophobic polymer backbone of the polyelectrolyte. Thus, at molecular level, the polyelectrolyte chains can be assumed to warp the LC droplets like threads with the polymer backbone lying along the interface (Figure 33).
Figure 33: A schematic showing (a) the molecular arrangement of PSS and (b) \(\pi-\pi\) type inter-molecular interactions between the 5CB and PSS molecules at the 5CB-water interface.

PSS-stabilized 5CB droplets with the bipolar configuration were further coated by the layer-by-layer adsorption of positively charged PDADMAC and negatively charged PSS in 0.1 M NaCl solution. This procedure was repeated until 16 layers (8 PDADMAC layers and 8 PSS layers) were achieved on the 5CB droplets.

After the adsorption of each layer, the director of configuration of the 5CB inside the droplets was observed with a polarizing optical microscope in aqueous solution at room temperature. As can be seen in Figure 34a, all the PSS-coated 5CB droplets show a bipolar configuration, which is independent on their diameters. The bipolar configuration of the PSS-coated 5CB droplets suggests a parallel anchoring at the interface. However, the bipolar configuration of the PSS-coated 5CB droplets transits the radial configuration after the adsorption of a PDADMAC layer (Figure 34b), corresponding to a homeotropic anchoring at the interface. Subsequent deposition of a PSS layer on the PDADMAC/PSS-coated droplets switches the director of configuration of
the 5CB back to the bipolar configuration (Figure 34c). The bipolar configuration of the 5CB in the PSS/PDADMAC/PSS-coated droplets turns into the radial configuration after the deposition of the second PDADMAC layer (Figure 34d). The alternate bipolar/radial configuration transition of the 5CB in the droplets is observed for the 16 deposition cycles we studied here.

![Figure 34: Polarizing optical microscope images of (a) PSS-coated 5CB droplets, (b) PDADMAC/PSS-coated 5CB droplets, (c) PSS/PDADMAC/PSS-coated 5CB droplets and (d) (PDADMAC/PSS)2-coated 5CB droplets.](image)

This is surprising as it was expected that, the first PSS layer would only control surface anchoring of the 5CB in the droplets. The \( \zeta \)-potential measurements during the layer-by-layer coating on the 5CB droplets show alternating changes (Figure 35a). The potential is negative when PSS is the outer layer and positive when PDADMAC is the outer layer, respectively. The alternating changes in the \( \zeta \)-potential suggest the surface charge reversal during the layer-by-layer coating on 5CB droplets. In order to further confirm that the 16 polyelectrolyte layers are indeed coated on the 5CB droplets, \((\text{PDADMAC}/\text{PSS})_8\) capsules were observed with a transmission electron microscope (TEM) after removing the 5CB core, according to the method reported in the literature. Briefly, approximately 1 mL of ethanol was added to ~ 1 mL of the \((\text{PDADMAC}/\text{PSS})_8\)-coated 5CB droplet solution. The mixture was agitated with a vortex mixer.
for 15 min and then centrifuged. This process was repeated three times. Figure 35b shows a TEM image of the resultant hollow (PDADMAC/PSS)₈ capsules dried on a carbon-coated copper grid. The wall thickness of the capsules is ~ 72 nm, which is fairly consistent with the thickness of (PSS/PDADMAC)₈ layers reported in the literature.

Figure 35: (a) The ζ-potential of polyelectrolyte-coated 5CB droplets as a function of layer numbers, (b) TEM image of (PDADMAC/PSS)₈ capsule shells dried on a carbon-coated copper grid.

The layer-by-layer adsorption of negatively charged PSS and positively charged PDADMAC represents charge arrangements on the 5CB droplets. The high polarity of (PDADMAC/PSS)₄ multilayers was confirmed. Therefore, it was inferred that the entire capsule shell made by an odd number of layers is polarized. The interaction of the polar 5CB and the polar shell is expected to induce a homeotropic surface anchoring, which agrees with the radial configuration observed in our experiments. The entire capsule shell made by an even number of layers becomes nonpolar. The director of configuration of the encapsulated 5CB droplets switches back to the bipolar configuration, which is fixed by the first, PSS layer.
It is worth pointing out that the adsorption of PAH on the PSS-coated 5CB droplets for 24 hours does not trigger the bipolar-to-radial transition. This may reflect the difference in the layer architecture between the PDADMAC/PSS pair and the PAH/PSS pair. It has been shown that the coupling of PAH and PSS is much stronger than that of PDADMAC and PSS\textsuperscript{59}. The closest distance between the cationic and anionic charge in the PSS/PAH pair is estimated to be \(\sim 0.25\) nm. While the closest distance between the cationic and anionic charge in the PSS/PDADMAC pair is \(\sim 0.5\) nm due to the presence of the methyl groups of the ammonium cations. It has been shown that the polarity of \((\text{PAH}/\text{PSS})_4\) multilayers is much smaller than that of the \((\text{PDADMAC}/\text{PSS})_4\) multilayers. It is likely that the interaction between the polar 5CB and the polar PAH/PSS shell is not sufficient to switch the director of the 5CB droplets which is fixed by the first PSS layer\textsuperscript{60-64}.

The sensitivity of the director of configuration of the \((\text{PDADMAC}/\text{PSS})_n\)-coated 5CB droplets was further tested by studying the concentration of PSS in solution, which is necessary to trigger the radial-to-bipolar configuration transition of the 5CB in the droplets, where \(n\) represents the number of PDADMAC/PSS bilayers. The \((\text{PDADMAC}/\text{PSS})_n\)-coated 5CB droplets with the PDADMAC outer layer were exposed to PSS solutions with different concentrations for a constant time (20 mins). Figure 36a shows the experimental results of the \((\text{PDADMA}/\text{PSS})_1\)-coated 5CB droplets exposed to PSS solutions with different concentrations. By the statistical analysis of \((\text{PDADMA}/\text{PSS})_1\)-coated 5CB droplets, it was found that all the 5CB droplets switch to the bipolar configuration when the concentration of PSS is larger than 0.75 mg/mL and maintain the radial configuration when the concentration is lower than 0.25 mg/mL. While, in
the range of 0.25 - 0.75 mg/mL, both the bipolar and radial configurations are observed. In addition, the examination of individual (PDADMA/PSS)$_1$-coated 5CB droplets with different sizes shows, the triggered radial-to-bipolar transition of small droplets is faster than that of large droplets in this concentration range, indicating that the sensitivity of the (PDADMA/PSS)$_1$-coated 5CB droplets increases with the decrease of droplet sizes. Furthermore, the concentration dependent-director of configuration is found to depend on n (Figure 36b). For the (PDADMA/PSS)$_2$-coated 5CB droplets exposed to PSS solution with a 0.25 mg/mL for 20 mins, approximately 30% of them transit into the bipolar configuration. In the case of (PDADMA/PSS)$_3$-coated 5CB droplets, approximately 50% of them become bipolar. All the (PDADMA/PSS)$_4$- and (PDADMA/PSS)$_5$-coated 5CB droplets become the bipolar configuration after being exposed in PSS solution with a 0.25 mg/mL for 20 mins. The sensitivity dependence of (PDADMA/PSS)$_n$-coated 5CB droplets on n may be a result of the layer ordering of the (PDADMA/PSS)$_n$ shells, which is known to increase with n.

Figure 36: (a) The director of configuration transition of PDADMAC/PSS-coated 5CB droplets after being exposed to PSS solution with different concentrations. (b) The director of configuration of 5CB droplets as a function of number of PDADMAC/PSS bilayer coatings after being exposed to a PSS solution with a 0.25 mg/mL.
PDADMAC/PSS multilayer coating makes the director of configuration of the 5CB droplets sensitive to pH changes. The results for the (PDADMA/PSS)₆-coated 5CB droplets are shown in Figure 37. At pH values above 5.4, ~ 100% of the (PDADMAC/PSS)₆-coated 5CB droplets show the radial configuration (Figure 37a). At pH values blow 3.5, ~ 100% of the (PDADMAC/PSS)₆-coated 5CB droplets became bipolar (Figure 37b). While in the narrow pH range from 3.5 to 5.4 both radial and bipolar droplets are observed (Figure 37c). The percentage of the bipolar droplets increases rapidly with the decrease of pH in this narrow range (Figure 37d). The pH-dependent configuration of the 5CB droplets is found to be independent on the number of PDADMA/PSS bilayers. All the (PDADMA/PSS)₂- and (PDADMA/PSS)₄-coated 5CB droplets switch to the bipolar configuration in acidic solution (pH ~ 3.0). The radial-to-bipolar transition can also be explained by the polarity change of the entire PDADMA/PSS shells. In acidic solution, the majority of ammonium groups of PDADMAC is protonated, which makes the entire PDADMAC/PSS capsule shells less polar because there is only a limited number of ionic coupling sites between PDADMAC and PSS (see the inset in Figure 37d). In this case, the interaction of the 5CB with the first PSS layer is dominant and the director of configuration of the 5CB droplets switches back to bipolar in acidic environment. The pH-induced configuration transitions of the PDADMAC/PSS-coated 5CB droplets are fully reversible.
Figure 37: Polarizing optical microscope images of (PDADMAC/PSS)$_6$-coated 5CB droplets at (a) pH 10, (b) pH 4.0, (c) pH 3.0 and (d) The direction of the polarizer and analyzer is indicated by white arrows. The director of configuration of (PDADMAC/PSS)$_6$-coated 5CB droplets as a function of pH values. A schematic representation of the possible internal structure of a PDADMAC/PSS-coated 5CB droplet at low pH values (inset in Figure 37d)

Salt is another important stimulus to tune the structure of polyelectrolyte multilayers by attenuating the intermolecular interactions in polyelectrolyte multilayers. Finally, the effect of salt concentrations on the director of configuration of the PDADMA/PSS-coated 5CB droplets was studied. It has been shown that the thickness of PDADMAC/PSS multilayers increases after being exposed to NaCl solution. The PDADMA/PSS-coated 5CB droplets with the radial configurations were incubated with NaCl solution with varied concentrations from 0.01 to 2.5 M for 2 hours. The salt-induced swelling is expected to take place on the PDADMAC/PSS coated the 5CB droplets. The director of configuration of the coated 5CB droplets was found to be sensitive to the presence of NaCl. The radial-to-bipolar transition of PDADMA/PSS-coated 5CB
droplets starts when the concentration of NaCl in solution increases to ~ 0.5 M. As can be seen in Figure 38a, 60~80% of the PDADMA/PSS-coated 5CB droplets transit into the bipolar configuration for concentrations higher than 1.0 M. The salt-triggered configuration transition of the 5CB droplets was found to be independent on the number of PDADMA/PSS bilayers as well as the size of the droplets.

**Figure 38:** (a) The director of configuration of PDADMAC/PSS-coated 5CB droplets as a function of NaCl concentration. (b) collapsing of the PDADMAC/PSS-coated 5CB droplets with the excess of NaCl.

The influence of salt concentrations on the director of configuration of the 5CB droplets also support the hypothesis that the polarity changes of the entire PDADMAC/PSS capsule shells may be a main factor that triggers the configuration transition. It has been shown that the incorporation of the salt ions within polyelectrolyte multilayers can be expressed by the equilibrium between the intrinsically charge compensated polyelectrolyte complex, Pol−Pol+, where internal charge is balanced by polymer segments and the extrinsically compensated form, Pol+ Cl− and Pol− Na+, where salt counterions participate in charge neutralization61,62,66,67.
Therefore, the increase in salt concentrations will compensate polyelectrolyte charge within the multilayer to transform from the intrinsic to the extrinsic form. The transformation leads to the decrease in the intrinsic compensation coupling sites between PDADMAC and PSS and reduces the polarity of the entire PDADMA/PSS shells (Figure 38b), triggering the observed radial-to-bipolar transition of PDADMA/PSS-coated 5CB droplets. The remaining intrinsic compensation keeps the cohesion of the PDADMA/PSS system. However, if the concentration exceeds 2.5 M, the PDADMA/PSS-coated 5CB droplets collapses since the few remaining intrinsic compensations are no longer sufficient to keep the integrity of PDADMA/PSS shells.

Conclusions
In conclusion, the layer-by-layer adsorption of PSS and PDADMAC is able to trigger the alternate bipolar/radial configuration transition of the encapsulated 5CB droplets. The sensitivity of the director of configuration of the PDADMAC/PSS multilayer-coated 5CB droplets increases with the increase in number of PDADMAC/PSS bilayers. The effect of pH values and salt concentrations on the director of configuration of the polyelectrolyte multilayer-coated 5CB droplets has been investigated by incubating the droplets in solution with varied pH values and salt concentrations. The results show that the PDADMAC/PSS multilayer-coatings make the director of configuration of the encapsulated 5CB droplets sensitive to pH values and salt concentrations. The possibility of using the layer-by-layer adsorption of PDADMAC and PSS to design functional liquid-crystal droplets and make their director of configuration sensitive to chemical environments is highlighted in all the results.
CHAPTER FOUR: SENSING BIOLOGICALLY RELEVANT SPECIES THROUGH ELECTROSTATIC INTERACTION USING POLYELECTROLYTE COATED LC DROPLETS

Introduction
It has been previously discussed that it is also possible to alter the director of configuration by changing the surface potential of the LC droplets. This was achieved by layer-by-layer assembly of negatively charged PSS and positively charged PDADMAC, which resulted alternate bipolar to radial configuration. Such a transition was also observed with the change in pH and salt concentration of the solution (Chapter 3). These results led to the hypothesis whether detection of an analyte is possible based on the electrostatic interaction between the droplet and a species of interest. It seemed possible that the analyte and the LC droplets may interact due to electrostatic interaction. If such interactions happen, then it may alter the interfacial energy of the LC inside the droplets in a way similar to the layer-by-layer assembly of polyelectrolytes. Therefore, like polyelectrolyte layer deposition, the interaction of oppositely charged analyte and LC droplet may trigger a change in the director of configuration of the LC droplets. Thus, it may be possible to detect the analyte simply by monitoring the change in the optical pattern using polarizing light microscope.

In order to validate such a possibility, a positively charged dendrimer molecule was used as a model charged species and was interacted with the negatively poly-styrene sulfonate (PSS) coated 5CB droplets. The choice of poly-amido amine (PAMAM) dendrimer molecule, as the charged species, provides the ability to alter the surface charge and size of the molecule by
changing the dendrimer generation. Figure 39a shows the molecular structure of PAMAM dendrimer of generation 2. It has 16 amine groups at its terminals. Table 3, lists the molecular weight, size and the number of surface groups for all the 4 generations of PAMAM dendrimer molecules we have used in our experiments. PSS was used as the polyelectrolyte stabilizer. The droplets coated with multiple layers, had alternate layers of PDADMAC/PSS, with PSS as the terminal layer. The terminal PSS layer provides a negative surface charge to the 5CB droplets suitable for the interaction with the positively charged dendrimer molecule through electrostatic interaction. It was observed that the PSS coated bipolar droplets changed to radial configurations upon exposure to the dendrimer solution. The transition occurred in a dose dependent manner and seemed favorable by higher dendrimer generation and smaller droplet size.

The transition was much rapid for droplets with multiple layers compared to a single PSS layer. Since the transition essentially resulted from the electrostatic interaction between the droplets and the dendrimer and most of the phenomenon occurring in nature also relies on similar principle (along with a specific binding of course).

Figure 39: The molecular structure of (a) PAMAM, (b) 5CB and (c) PSS.
Table 3: Showing the molecular weight, size and surface charges of different generations of PAMAM Dendrimers

<table>
<thead>
<tr>
<th>Dendrimer Generation</th>
<th>Molecular weight</th>
<th>Diameter (Å)</th>
<th>Number of surface amine groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3,256</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>14,215</td>
<td>45</td>
<td>64</td>
</tr>
<tr>
<td>4.5</td>
<td>21,520</td>
<td>56</td>
<td>96</td>
</tr>
<tr>
<td>5</td>
<td>28,826</td>
<td>67</td>
<td>128</td>
</tr>
</tbody>
</table>

The detection of dendrimers using the LC droplets based on the electro-static interaction was quite motivating. The next set of experiments was designed to observe if electro-static interaction based detection mechanism can be extended to other charged macro-molecules. Proteins and viruses are also charged macro-molecules of great biological significance. Since proteins are one of the fundamental species to any biological activity so their concentration reveals the a great deal about biological processes in general. Thus the significance of protein detection is magnanimous in the context of health care and medicine. Viruses on the other hand are one of the primary causes of several infectious diseases and importance of their detection is needless to explain. Viruses have an external shell known as capsid, which is essentially made of proteins; hence, viruses can be structurally regarded as self-assembled protein structures. Interestingly, both proteins and viruses have surface charges and their often use electro-static interactions to initiate their activity. They make ideal charged macro-molecules for electro-static interaction based detections.
In order to demonstrate the working of the detection mechanism, bovine serum albumin (BSA) was used as a model protein. BSA is a globular protein with negative surface charge and is a significant plasma protein. The detection of BSA is significant as it is used in most of the bio-analytical tools. It is also regarded as the first step towards the detection of proteins which is often done using more complex techniques. Further, BSA is a much robust protein and can be handled easily at a wide pH and temperature ranges. Similar to proteins viruses are an important part in health care and their detection too needs highly specialized detection tools. Plant viruses are non-pathogenic and are easy and save to handle in laboratory environments. So as a model virus, we choose two plants viruses namely, cowpea mosaic virus (CPMV) and tobacco mosaic virus (TMV). Both the viruses are negatively charged but have slightly different morphology. The TMV is elongated in shape and CPMV is spherical as shown in figure 40.

Since the species of interest in this case were negatively charged so positively charged polyelectrolyte coated droplets were used for the detection. Both, poly-ethylene-imine (PEI and poly-diallyl-dimethyl-ammonium chloride (PDADMAC) provided stable positively charged droplets. It was observed that the exposure to BSA, CPMV and TMV to the PEI and PDADMAC coated droplets indeed transform them from radial to bipolar in a concentration dependent manner. We found that the sensitivity of polyelectrolyte-coated LC droplets in detecting these charged biological molecules is associated with the size of LC droplets, and also on the surface charge and the morphology of the species. It was observed that only 200 µg/mL of BSA, 100 µg/mL of CPMV, and 400 µg/mL of TMV could completely transform the droplets.
Experimental

The PSS stabilized 5CB droplets were synthesized following the same protocol as mentioned before. The PAMAM dendrimers of generation 2, 4, 4.5 and 5 used in the experiments were purchased from Dentritech. The dendrimers were dissolved in water and incubated overnight at 60 °C to remove methanol and transfer dendrimer into water. Adequate amount of water was then added to them to prepare 10 mg mL⁻¹ of dendrimer solution in water. For the incubation with the droplets, calculated amount of dendrimer stock solution was added to polyelectrolyte coated 5CB droplet solution and mixed well. The mixture was allowed to incubate at room temperature for an hour to ensure complete adsorption of dendrimer molecules onto the droplet surface.

Results and Discussion

Detection of dendrimer

The PSS layer adsorbed at the 5CB-water interface allows the droplets to be stable in aqueous media. Figure 41b shows the polarized light optical image of PSS coated 5CB droplets being
bipolar. However, upon exposure to 2 mg/mL of G5 solution all of them turned radial as shown in figure 41c. This shows presence of positively charged PAMAM dendrimer of G5 can trigger the transition of the director of configuration of LC in the droplet from bipolar to radial due to the electrostatic interactions as shown schematically in figure 41a.

![Figure 41: (a) A schematic of interactions between the PSS droplets and G5 dendrimer. The polarized light microscope image of the droplets (b) before and (c) after the exposure to G5.](image)

It is known that the director of configuration depends on the balance between the surface anchoring energy and the bulk elastic energy of the liquid crystal molecules within the droplets\textsuperscript{76,77}. Incubation of positively charged dendrimer molecules with negatively charged droplets allows the dendrimer molecules to bind to the surface of the droplets due to the electrostatic interactions. The dipole moment generated due to the binding interacts with the cyano group of the 5CB molecule triggering favoring a perpendicular anchoring and hence leading to the transition of configuration.
The native PSS coated droplets are negatively charged but the surface charge of the droplets gradually increase to a less negative value when they are exposed to an increasing concentration of G5 solution (figure 43). This increase in the surface charge of the droplets continued until it reached to a positive value (~15 mV) and all droplets get coated with the positively charged dendrimer molecules. This dendrimer coated PSS shell now becomes polar which favors perpendicular anchoring thus eventually resulting all the droplets to become radial in configurations. Therefore, bipolar to radial transition of the droplets is due to the electrostatic interaction between the dendrimer and the PSS coated droplets and it needs a certain concentration of dendrimer to trigger this change making the transition a concentration dependent phenomenon.

**Figure 42:** (a) The change in surface charge due the increasing concentration of dendrimers, (b) the interaction of amine and sulfonate groups.
Since bipolar to radial transition occurs in a concentration dependent manner, the droplets were incubated with all four generations of PAMAM dendrimer solutions whose concentrations ranged from 0 to 2 mg/mL. Figure 43a, shows the effect of concentration on the extent of transition for all the four generations of the dendrimers for droplets with an average size of about 0.5 μm. The transition did occur for all four generations of dendrimers in a concentration dependent manner. No or very little transition occurred at concentrations below 0.5 mg/mL and almost all of them transformed to radial configurations beyond 1.2 mg/mL. Between these two concentrations both the configurations coexisted together and the percentage of transition increased quite rapidly for a relatively smaller increase in the dendrimer concentrations eventually resulting in S-shaped transition curves\textsuperscript{34,45,49}. 
Figure 43: The concentration dependent transition of the PSS coated droplets for all four generations of PAMAM dendrimers for four different set of droplet with average sizes (a) 0.5, (b) 0.75, (c) 1.0 and (d) 1.4 μm.

As discussed previously, configuration transition of the droplet depends on the balance between the surface and the bulk forces. A change in the droplet size leads to change in the bulk elastic energies of the 5CB molecules and also a change in their surface area. Therefore the size of the droplet seems an important parameter in understanding this phenomenon of radial to bipolar transition. In order to understand the effect of size on the transition behavior, droplets with four average sizes of 0.5, 0.7, 1.0 and 1.4 μm were synthesized (size distribution shown in figure 44).
Figure 43a shows the concentration dependent transition of the droplets having a size of about 0.5 μm. A similar trend of concentration dependent S-type transition curves was observed for the other three droplet sizes as well (Figure 43b, 43c and 43d). However, upon a careful observation it reveals that the S curves gradually shift to the right with the increasing droplet size.

Figure 44: The size distributions for four different sets of droplets with varying sizes.

More careful analysis of figure 45 makes it possible to know the concentration of dendrimers required to induce close to 100% transition for all the four different droplet sizes. Figure 45a shows the effect of average droplet size on the 100% transition concentration. It can be seen from the figure that the 100% transition concentration gradually increases with the droplet size. The trend remains same for all the four generations of dendrimer. Thus it can be said, that one of the ways to achieve higher sensitivity is to use smaller droplets when the number of droplets in the suspension remains almost same. However, use of very small droplets (< 0.3 μm) makes
them difficult to visualize in the microscope and therefore distinctly identify the configuration. The smaller size of the droplets also increases the bulk elastic energy of the 5CB molecules within the droplet and droplets too small in size may make the 5CB rigid enough not to undergo the transition at all.

Figure 45: The effect of (a) droplet size and (b) dendrimer generation on the complete or 100% transition concentration.

The size and surface charge of a dendrimer depends on its generation. As shown in the table 3, the number of surface amine groups increases with the increase of the dendrimer generations. Thus, larger the generation of the dendrimer the more surface charge it has. Since, the transition of the droplets from bipolar to radial conformation is essentially a result of the electrostatic interaction between the positively charged dendrimer molecule and negative charged droplet surface. Next question is how the generation of the dendrimer molecule or its surface charge effects the transition. As it can be seen in Figure 45b, that as the dendrimer generation increases,
the transition occurs at a much lesser dendrimer concentration. The results also suggest that analytes with higher surface charge are more easily detectable and have better sensitivity.

The surface charge of a species often varies with the pH of the solution. Since, the entire phenomenon is based on the surface charge of two species and their electrostatic interaction, it is important to understand the effect of pH on the interaction. The PSS coated 5CB droplets were exposed to the dendrimer solution whose pH varied from 2 to 12. PSS coated 5CB droplets were exposed to the same pH range to compare the binding effects as function of pH. No significant effect of the pH onto the dendrimer-triggered transition of the droplets was observed. The PSS coated bipolar droplets did transform completely into radial ones when exposed to dendrimer solution just as they did at neutral pH. However, at very extreme pH conditions (<2 and >11) stability of the droplets were found be slightly compromised. The zeta potential measurements of the samples in this pH range were carried out for the droplets both with and without the dendrimer exposure. Figure 46 shows the variation in the zeta potentials of the droplets with and without the dendrimer exposure for pH ranging from 2 to 12. It can be seen from figure 46a that the zeta potential values of the PSS coated droplets of size 0.7 µm exposed to the droplets remained almost constant within the pH range of 4-10. Beyond this range, they change only slightly but not enough to show any effect on the transition of the droplets. The same was found for the droplets of size 1.0 µm (figure 46b). Therefore, it can be stated that the pH did not play any major role in the optical transition of the droplets when exposed to the dendrimer solutions. This also signifies that the binding of the dendrimer or a similar molecule to the PSS coated droplets can be carried out at a wide range of pH which only makes the system more robust.
Figure 46: The variation in zeta-potential with pH for droplets with average sizes (a) 0.7 µm and (b) 1.0 µm.

Droplets coated with polyelectrolytes multilayer were used to observe how extended surface modification of droplets with multiple number of polyelectrolyte layers would affect the dendrimer-triggered transition. Droplets with varying number (from 1 to 9) of PDADMAC-PSS layers were synthesized. The terminal layer was of PSS layer to have bipolar configuration and negative surface charge. Figure 47a shows bipolar to radial transition of the 5CB droplets of size 0.7 um with different number of surface layers when exposed to an increasing concentration of G5 solution. It can be seen from the figure that the initial increase in the number of layers results an increase in the rate of transition resulting in stiffer S curves. It however, fell to saturation after first few layers only. This can be seen well in figure 47b, in which the 50% transition concentration gradually reaches to a saturation value when the number of layers increases beyond 5. It is known from the studies conducted on polyelectrolyte multi-layers that after the initial few layers polyelectrolyte layers get more regular and uniformly arranged. No significant change in the molecular arrangement takes place after that. The results are consistent with this as
the sensitivity does not increase much after the first 5 layers. Moreover, the process of multilayering is time consuming process and suffers from loss of droplets due to the washing steps. Therefore single or few layering of the droplets makes them ideal detection units, making this technique simple and easy to be put into use.

Figure 47: The effect of multilayering (a) G5 induced concentration dependent transition of the PSS coated LC droplets with various numbers of surface layers. (b) The corresponding 50% transition concentration as function of the number of layers.

Detection of Proteins and Viruses

The dendrimer study signifies the use polyelectrolyte coated LC droplets for detecting charged species based on their electrostatic interaction. To validate the hypothesis, positively charged PEI and PDADMAC coated droplets were used to stabilize the LC droplets. The PEI and PDADMAC coated droplets showed radial director of configuration. But when they are incubated separately in BSA solution the direction of the configuration is found to have changed to radial. Figure 48a shows, concentration dependent transition the director of configuration in
presence of BSA. The transition is due to the binding of the BSA onto the surface of positively charged polyelectrolyte coated droplets due to simple electro-static interaction as shown schematically in figure 4.10b. This simple and non-specific detection technique can detect BSA as low as 150 µM, using a simple polarizing microscope. Bovine serum albumin (BSA) was used as a model for proteins and concentrations as low as 200 mg/mL could be sensed.

Two different plant viruses CPMV and TMV were used as model viruses using the same principle. They both showed concentration dependent transition (figure 49). However, the CPMV was more sensitive than TMV. It may be due to the fact that the two viruses have different morphologies. The CPMV is spherical in shape and the TMV is longitudinal (figure 4.2). It may be possible that CPMV being spherical in shape can pack easily and interact better with the polyelectrolyte layers to bring about the change. On the contrary TMV may face both stearic and orientational constrains to interact with the polyelectrolytes. Moreover, the ζ-potential of CPMV (\(-\sim 38\) mV) was slightly more than TMV (\(-\sim 30\) mV), which may also add to it.

**Figure 48:** BSA detection using LC droplets (a) the concentration dependent transition of the PEI and PDADMAC coated droplets in presence of BSA, (b) A schematic showing the binding of BSA onto the droplet surface leading to the transition.
Conclusion

The presence of dendrimer, a charged macro-molecule, triggers the polyelectrolyte coated 5CB droplets to transform from bipolar to radial configuration in a concentration dependent manner. The size and surface charge of the dendrimer molecule could be easily manipulated providing an opportunity to understand the underlying mechanism responsible for the bipolar to radial transition. The electrostatic interaction between the positively charged dendrimer molecule and the negatively charged PSS coated droplets leads to the optical transition. The sensitivity was better for droplets with smaller surface area and for dendrimers with higher surface charge. This could happen in a wide range of pH as well. The polyelectrolyte coated LC droplets were then used to detect biologically important species. Proteins like BSA and viruses like CPMV and TMV were put to the test. The detecting limit was found to be ~150 µM for BSA, ~30 µg/mL for CPMV, and ~200 µg/mL for TMV. It is evident from these results that polyelectrolyte coated LC

Figure 49: Detection of viruses using LC droplets (a) spherical shaped CPMV and (b) longitudinal shaped TMV.
droplets can indeed be used as platforms to detect biologically relevant species, since most of the natural phenomena are partially based on electrostatic interactions.
CHAPTER FIVE: POLYPEPTIDE DECORATED LC DROPLETS FOR ULTRA-LOW DETECTION OF BACTERIA

Introduction
Pathogens are microscopic organisms that are known to cause diseases and health conditions. One of the most common forms of pathogens is bacteria which cause many common health disorders and diseases to humans and other animals which is why their detection is so important in health care industry. Bacteria are present in air and water and surround us in all possible ways. But fortunately, not all of them are pathogenic or toxic. It is only a few handful of them are pathogenic and can cause serious health concerns in unhygienic conditions where they flourish. But when they do flourish they may bring epidemic and the effects can be really devastating. Often, the pathogenic bacteria are water borne and they propagate through aqueous media. Thus, it is very important to device a simple and easy way to detect bacteria especially in water.

In this work, the use of LC crystal droplets for non-specific detection of bacteria is demonstrated. In order to achieve the bacterial detection the surface of the LC droplets were modified with a specific polypeptide known as Mastoporan X (MP-X), which is known for its lipid binding abilities.

Mastoporan (MP) is a polypeptide derived from wasp venom. In 1983, Mizayawa and co-workers first reported the isolated and purified MP from the venom of Japanese yellow hornet (Vespa Xanthoptera). It is a 14 amino acid long poly-peptide with the sequence Ile-Asn-Leu-
Lys-Ala-Leu-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH2 (Figure 50a). Later researchers have found that MP does act as biochemical activator in biological conditions.\textsuperscript{101} The mechanism and process of activation and the resultant effects depends on the type of cell and their environment\textsuperscript{102}. It was also observed that this activation behavior is related to the conformations of MP. MP being a long macromolecule can undergo random coil to helix conformation change depending on its environment\textsuperscript{103,104}.

\[(a)\]
\[
\text{MP: Ile – Asn – Leu – Lys – Ala – Leu – Ala – Ala – Leu – Ala – Lys – Lys – Ile – Leu - NH}_2
\]
\[
\]

**Figure 50:** (a) The amino acid sequence in MP and MP-X polypeptides, (b) the coil-like substructure that MP-X forms in solution and its amphoteric nature of MP-X polypeptides.

The X in MP-X is designated for the wild type. This is a special type of MP polypeptide where the amino acid sequence is slightly different (Ile-Asn-Trp-Lys-Gly-Ile-Ala-Ala-Met-Ala-Lys-Lys-Leu-Leu-NH2). This slight change in the sequence makes this polypeptide unique in nature, especially in the change of conformation and its biochemical activation. It is known to form coil like \(\alpha\)-helix structure in aqueous solution\textsuperscript{101,105-107}. One side of the \(\alpha\)-helix is hydrophilic in nature and the other one is hydrophobic, making it amphoteric in nature (Figure 50b). One of the special features of MP-X is its affinity to lipid membranes\textsuperscript{103}. It is now believe that the bio-
activation of certain cells through MP-X is related to the way the later interacts with the lipid bi-layer of the cell membrane in one way of the other. Though the exact mechanism and orientation is subjected to debate, it unanimously believed that the MP-X molecules undergoes to a alpha-helix conformation in presence of lipid bi-layer and accommodates itself within the bi-layer by replacing some of the lipid molecules (Figure 51). This unique phenomenon has made MP-X and its interaction with cell-membranes a very interesting and well studied system.

**Figure 51:** Different conformations of MP-X; (a) normal polypeptide structure, (b) backbone structure of membrane-bound MP-X, (c) tightly membrane-bound structure, (d) weakly membrane-bound structure and (e) bound to protein G, a family of immunoglobulin binding protein. Image credit: a: Biochem, 1992, 31, 5654; b to e: Biophys J, 2006, 91, 1368.

Bacterial membrane structure contains layer of sugar (peptidoglycan or lipopolysaccaride) and one or two lipid bi-layers. The structure may vary depending on the type and species of bacteria. Usually, bacteria are divided in two classes based on their membrane structure viz. Gram Positive (G +ve) or Gram Negative (G –ve). This division is based on the Grams’s staining
technique that manages to stain some of the bacteria the (G+ve) and others which do not get stained are called (G-ve). This is primarily due to the difference in the cell membrane structures between the two. The G-ve bacteria usually have an outermost shell of thick layer of lipopolysaccharide (a long macro-molecule that is consists of lipids and polysaccharides). Underneath this there is a lipid-bilayer that is known as the outer membrane. This like the other cell membranes hosts the trans-membrane proteins and valve systems known as Porins. There is a thinner layer (compared to G-ve) of peptidoglycan, which acts like an accommodating layer for lipo-proteins and Omp A (outer membrane protein A). There is another layer of lipid-bilayer underneath this OmpA, which is known as the inner membrane as it surrounds the cytosol. The cell wall structure of gram –ve bacteria is schematically shown in Figure 52.

Figure 52: A schematic of the cell wall structure of (a) Gram positive and (b) Gram negative. Image credit: Dept. Microbiology, Cornell University.

The cell wall structure of G +ve bacteria, on the contrary, is simpler but very rigid (figure 52b). The outer part if their cell wall is made of a very thick layer of peptidoglycan. Peptidoglycan is a
polymer consisting sugar and amino acid molecules that are heavily cross-linked to each other to form a rigid 3D mesh like structure. The cross-linking imparts high rigidity to the G+ve cell wall making it more resistant to the antibiotics. Underneath the peptidoglycan layer, there is a lipid bilayer containing the lipo-proteins and surrounds the cytosol of the G+ve bacteria. Thus, the two basic features that makes significant difference between the G-ve and the G+ve bacterial cell wall are the presence of thick layer of peptidoglycan (the rigid well cross-linked biopolymer) on G+ve and the presence of less rigid lipopolysaccharide layer on the G-ve surface and the two layers of lipid bi-layers within the cell wall of the G-ve bacteria.

As explained above, the bacterial cell walls are structurally much thicker than animal cell membranes (which are usually much thinner and consist of a lipid bi-layer) even though both of them consist of at least one set of lipid bilayer. Scientists have explored the interaction between MP (and its forms) with bacterial cell wall probably to understand whether the presence of thick coatings of structural biopolymers (like lipopolysaccharides and peptidoglycan) inhibits the MP from interacting with the bacterial cell membranes. Recent reports highlight that different forms of MP not just interact strongly with the bacterial cell wall but also brings in anti-bacterial effect\textsuperscript{108,109}. However, the exact mechanism of interaction is debatable but the presence of MP (or its modified forms) produces blast-like extrusions on bacterial surface and therefore killing them through disrupting the cell walls. This was observed for both G+ve and G-ve strains. Researchers have observed that modified forms of MP can even selectively interact with bacterial cell walls. All this makes the interaction of MP (or MP-X) with bacteria worth exploring forward.
In this work, MP-X has been used as the means to surface engineer LC droplets. The droplets of 5CB were dispersed in water by stabilizing them with MP-X. The MP-X coated LC droplets were then exposed to bacterial cultures in aqueous media to study whether the MP-X coated droplets interacted with the bacteria and if that will induce any change in the optical properties of the droplets. The presence of *E.coli* triggered the MP-X coated LC droplets to change their director of conformation from radial to bipolar in a dose dependent manner. In fact, the entire system was found to be quite sensitive and very low levels of bacterial contamination could be sensed using these surface engineered droplets. The present work therefore highlights the surface modified LC droplets as a good sensing platform for simple yet efficient ways to detect bacteria or such pathogens in aqueous media.

**Experimental**

*Synthesis of MP-X coated LC droplets*

The MP-X coated droplets were synthesized much the same way as other stable LC droplets. In a micro-centrifuge tube containing, 2 mL of 1 μM MP-X solution, 2 μL of 5CB was added. The mixture was then sonicated for 15 minutes in a bath sonicator. This results the solution to turn into a whitish suspension. The suspension was washed through centrifugation, just once to minimize the loss of droplets. This yields MP-X coated 5CB droplets, which was carefully stored no more than 24 hours in room temperature to carry out further experimentations and characterizations.
**Bacterial culture**

Escherichia coli was used as the model G-ve bacteria. The bacterial culture was carried out using the standard protocols. In short, for every 100 mL Luria Bertani (LB Broth) culture media, 1 g Bacto-tryptone, 0.5 g Bacto-yeast extract, 1 g NaCl was dissolved in about 90 mL DI water, with constant stirring. The solution pH was then adjusted to pH 7.4 using dilute NaOH solutions and the solution volume was made up to 100 mL. The solution was autoclaved to avoid any contamination. Once the solution was cooled to room temperature, one colony of e-coli was picked from the culture disc using a sterilized loop and added to the LB culture media and sealed with a cotton cap. This was then incubated at 37°C in a standard bacterial incubator (proper humidity, light and temperature control) with shaking (250 rpm). An overnight incubation (about 8 hours) turns the transparent media into a translucent one. The concentration of e-coli (which is counted in terms of Colony Forming Units (CFU)) in CFU was obtained by measuring the Optical Density (OD) of the media, using a UV-Visible spectroscopy set a 600 nm wavelength. The media containing the e-coli was diluted 10 times with DI water to avoid measurement errors. The bacterial suspension was then diluted adequately to have OD of 1 (i.e. the absorbance value in the UV-Vis at 600 nm is 1.0), which corresponds to about 109 CFUs of e-coli per mL of the media. The suspension was then centrifuged at 3000 rpm for 15 minutes to precipitate out the e-coli cells. The precipitate was then suspended in saline solution (0.19 M NaCl solution). This step was repeated once more to get rid of culture media that may act as a contaminant to further experiments. Finally, the OD was adjusted to 1 and bacterial suspension was serially diluted to
have different concentrations (10^3, 10^5, 10^7 CFU/mL) of e-coli in saline and preserved for incubation and detection experiments.

**Bacterial Detection**

For the bacterial detection experiments, known volumes of droplet suspension were mixed well and then incubated with calculated concentrations of e-coli suspension in sealed micro-centrifuge tubes at 4°C for an hour. Care was taken to have small number of samples to avoid high analysis time as that may induce great errors in the measurement. The bacteria were alive (and even happily swimming), the samples were incubated and observed carefully with minimum possible time to minimize the growing of the e-coli (since it doubles itself in every 20 minutes under optimum conditions).

**Optical characterization**

The optical characterizations were similar to the previously described methods. The samples were analyzed under microscope in cross-polarized light. Several images of the sample were taken and then each droplet image was manually examined for their director of configurations. Minimum of 200 droplets were analyzed for each data points and the percentages of bipolar or radial droplets were plotted with the experimental conditions like e-coli concentration to obtain the concentration dependent S curves.
Results and Discussions
The adsorption of MP-X at the 5CB-water interface initially imparted the droplets with a bipolar conformation (Figure 53a), but with time configuration changed to radial (Figure 53b). This is perhaps due the change of conformation of MP-X at the 5CB-water interface from random coil to aggregate.

![Figure 53: MP-X stabilized the 5CB droplets (a) immediately after synthesis (b) after incubation.](image)

To determine the critical stabilized concentration of MP-X the droplets were synthesized with different concentrations of MP-X. A concentration of 100 μM of MP-X produced extremely high numbers of LC droplets (∼10^{10}) and the number of droplets dropped as the concentration of MP-X decreased (Figure 54). Interestingly, only 5 nM MP-X could impart a good stability to the droplets, even though the number of droplets yielded was quite less (∼10^5). Below 5 nM, there was not enough MP-X to stabilize the droplets completely. Such low stabilizing concentration of MP-X also demonstrates the affinity of MP-X towards lipid or similar hydrophobic surfaces such as 5CB.
Figure 54: The stabilization of LC droplets using MP-X: (a) the effect of the MP-X concentrations on the number of stable droplets obtained and (b) polarizing microscope image of stable droplets stabilized with 50 nM MP-X.

In order to observe the lipid-loving nature of MP-X, LC droplets stabilized with MP-X were exposed to different concentrations of CTAB and SDS solutions. As shown in the Figure 55, the exposure of MP-X coated droplets to surfactants resulted in change of configuration from radial to bipolar. The transition took place in a concentration dependent manner and extremely low concentrations of surfactants could be sensed using these droplets. The surface charge of surfactants did not show much effect on the transition curves, which is consistent to the lipid binding behavior and not electrostatic interactions. However, it was interesting to observe that the droplets tend to aggregate once exposed to the surfactant. The zeta potential suggested a better conformation; however, the stability of droplets was compromised when exposed to the surfactant solution. This is perhaps due to the detachment of MP-X from the droplet surface to bind the surfactant. The detachment probably imparted the change of conformation to the droplets and also made them unstable.
This motivated the use MP-X coated droplets to sense the presence of *E. coli* in aqueous media. The radial MP-X droplets changed to bipolar when exposed to *E. coli* solution (Figure 56a). The bright field image shows the presence of *E. coli* and LC droplets together in the solution, corresponding to the change of conformation (Figure 56a). In a control experiment, SDS and CTAB stabilized droplets were exposed to *E. coli* and the interaction was observed (Figure 56c to f). The negatively charged *E. coli* cells did not respond to the negatively charged SDS but got attracted to positively charged CTAB coated droplets. But even such an attachment did not result in any transition of the configuration. This proved that the transition is not due to the electrostatic interaction but is specific to MP-X and probably due to the detachment mechanism (Figure 57a).

In order to observe if the transition of conformation for the MP-X coated droplets in presence of *E. coli* was concentration dependent, the droplets were incubated with increasingly low concentrations of *E. coli*. It was observed that as low as 10 bacteria per mL could be detected.
using these droplets (Figure 57b). This is an extremely low concentration and very few detection methods can achieve such high sensitivity and none of them can do so with a simple tool like optical microscope. Such a low concentration of bacteria is not visible in bright field making this detection technique unique and interesting. Presently, the experiments are being performed for other type of bacteria to have a generalized mechanism for bacterial detection.

**Figure 56:** Interaction of *E. coli* with LC droplets, (a & b) MP-X- coated droplets, (c & d) SDS coated droplets; and (e & f) CTAB coated droplets; (a, c, e) polarizing light and (b, d, f) bright field images.
Figure 57: (a) A schematic showing the detachment of MP-X polypeptides from the droplet surface and binding to cell wall of *E. coli*; therefore triggering the radial to bipolar transition and (b) the percentage transition as function of the number of *E. coli* in the solution.

Conclusions
A synthetic polypeptide, MP-X was used as the stabilizing agent in this particular work. MP-X is a 14 amino acid long polypeptide that is known to form helical structure and is amphoteric in nature. The polypeptide provided excellent stability to the LC droplets in water and produced radial configurations when reached equilibrium. It was found that the MP-X stabilized droplets changed to bipolar configuration when exposed to surfaces due to the high affinity of MP-X towards lipid. It was also found that the incubation of MP-X stabilized droplets with *E. coli* also results in a change of configuration. This is perhaps due to the detachment of the polypeptide form the droplet surface to bind to the lipid bilayer present in the bacterial cell wall. This
detachment-based mechanism of sensing could detect an extremely low level (~ 10-100 CFU/mL) of e-coli. Achieving such a high sensitivity using a simple light microscope is technologically significant and makes this polypeptide stabilized LC droplet based sensing technique simple yet effective sensing platform.
CHAPTER SIX: DETECTING LCA, A COLON CARCINOGEN, WITH SURFACE ENGINEERED LIQUID CRYSTAL DROPLETS

Introduction
Bile acids are physiologically important metabolites in biological fluids, which are produced by the degradation of cholesterol in the liver and then stored in the gall bladder for the digestion of fatty foods. However, they can also be toxic to the gastrointestinal tract\textsuperscript{110,111}. It has been found that individual suffering from intestinal diseases often has a sharp increase in the concentration of bile acids because the hepatic synthesis and clearance of bile acids are disturbed\textsuperscript{112-114}. The concentration of bile acids found in a healthy individual is lower than 7 \(\mu\text{M}\), while those suffering from intestinal diseases are found to have a concentration level in the range of 10-100 \(\mu\text{M}\)\textsuperscript{115}. Therefore, the concentration level of bile acids provides a specific biomarker for the diagnosis of intestinal diseases. However, the simple, sensitive, and specific detection of bile acids still remains to be a difficult task because they show very low ultraviolet/visible absorbance. Currently, chromatography techniques are a common method for the detection and quantification of bile acids in biological fluids. Although the precision and selectivity of chromatography techniques in the detection of bile acids are high, the downside of chromatography measurements is time consuming as well as the need for expensive instruments that is not widely available. Chemical and optical sensors are more attractive in detecting bile acids\textsuperscript{115-118}.

Lithocholic acid (LCA) is known to be the most toxic bile acid with genotoxic and mutagenesis-enhancing properties. Unlike primary bile acids, LCA in the small intestine is poorly reabsorbed
into enterohepatic circulation and moves into the colon where it accumulates. Individual who develops colon cancer often has high levels of LCA. The application of surfactant-stabilized liquid crystal droplets in detecting LCA in aqueous solution is exploited here. The detection is based on the replacement of the surfactants from the liquid crystal/water interface by LCA, which triggers the radial-to-bipolar configuration transition of the liquid crystal in the droplets. The concentration level of LCA, which are found from individual suffering from intestinal diseases, can be detected by surfactant-stabilized liquid crystal droplets.

**Experimental**

*Synthesis of stabilized droplets*

For chitosan and LCA the solution was made in dilute acetic acid (Fluka) of pH 3-4 and in 10 mM sodium hydroxide (Sigma-Aldrich) of pH 12 respectively. Ten microliters of 5CB was then added to 10 mL of the stabilizer solution and vortexed vigorously to break 5CB into smaller droplets. Next, this suspension was sonicated (Bradson 2510) for 15 minutes at room temperature (~23-25 °C) with vortexing after every 5 minutes. The sonication breaks the coarse droplets of 5CB into tiny droplets, which are then coated by the stabilizer. This results in suspension turning milky white. To remove the excess stabilizers the droplets were washed twice through centrifugation by replacing the clear supernatants with DI water. Finally, the cleaned stabilized droplets suspended in water are carefully stored. The chitosan-coated droplets were preserved at pH 4-4.5 and the LCA ones at pH 11. For further characterizations and
experimentations droplets no older than two days were used. All the incubation studies were carried out at room temperature in DI water for 30 minutes unless otherwise mentioned.

**Characterization**

A polarizing optical microscope (Olympus BX40) in transmission mode was used to observe the director of configuration of the 5CB droplets. The ζ-potential was measured using Zetasizer Nano ZS90 (Malvern Instruments Inc.) at room temperature. Dynamic light scattering (Precision Detector PD 2000DLS) was used to measure the size of droplets. To obtain the absorption from the droplet suspension The UV-Visible spectrometer (Cary Win UV) was used. An average intensity in the range 490-510 nm was selected as the absorption value, which was the normalized to obtain the intensities of transmitted light from a given droplet suspension. For the transition curves, minimum 200 droplets were manually analyzed to check for their optical patterns (from the photographic images) and calculated for percentage transition for individual data-points. The data-points were connected through B-SP lines to obtain smooth transition curves. The 50% and 100% transition concentrations were obtained from these transitions curves for each condition.

**Results and discussion**

LCA stabilized 5CB droplets: The nature of LC-water interface in presence of LCA molecules was analyzed by stabilizing LC droplets with LCA molecules. As shown in Figure 58a, the LCA stabilized 5CB droplets resulted in a bipolar configuration. The bipolar configuration of the droplets implies that the LC molecules are anchored parallel to the LCA-water interface. It is
know that the LCA molecule has two sides viz. the convex hydrophobic and the concave hydrophilic (Figure 58c). It is reasonable to assume that hydrophilic side of the LCA molecule will be facing water molecules at the interface and the hydrophobic side towards the 5CB molecules. Such an arrangement would therefore induce the 5CB molecules to lie down at the water-5CB interface resulting in a bipolar configuration as shown schematically in Figure 58d.

![Figure 58](image)

**Figure 58**: The stabilization effect of LCA (a) polarizing micrograph of LCA coated 5CB droplets; molecular structures and schematic of (b) 5CB and (c) LCA, (d) a schematic showing molecular arrangement of LCA at 5CB droplet interface resulting a bipolar structure, (e) effect of LCA stabilizing concentration on the number of stable droplets, (f) the size distribution of LCA stabilized droplets using 1 mM of LCA.
The effect of LCA concentration on the droplet stabilization was assessed and it was observed that the concentration of LCA did not have any effect on the nature of the interface. LCA self-assembles into white tubular structures at concentrations more than 1 mM and concentrations of less than 0.1 μM of LCA fail to stabilize 5CB droplets (hardly any stable droplets could be observed under the microscope), hence, the stabilization studies were carried out at in the range of 1 mM to 0.1 μM concentrations. All the droplets obtained through different stabilizing concentrations of LCA had bipolar configuration. The only thing that changed was the number of stable droplets formed in the suspension. The number of droplets (and their total surface area which is the total area of the 5CB-LCA-water interface) kept decreasing with the decreasing concentrations of LCA. The intensity of the transmitted light passing through the droplet suspension depends on the number of the droplets present in it. More the number of droplets, more light is scattered and therefore, the transmitted light intensity is less. Figure 58 e shows increase in the transmitted light intensity with decrease in the concentration of LCA. The size of the droplets stabilized by different concentrations of LCA was found to remain almost constant. The average diameter of the droplet was measured to be about 0.72 μm and did not show a very wide distribution of size (Figure 58f).

**SDS and CTAB stabilized 5CB droplets**

Contrary to the LCA molecule, which is laterally divided into hydrophobic and hydrophilic parts, SDS and CTAB are molecules that have a long hydrophobic chain and a small hydrophilic terminal group (Figure 59a & b). The terminal groups of SDS and CTAB are respectively negatively and positively charged and they represent the most common type of surfactants that
are used to stabilize oil-water interfaces. They were chosen as representative molecules to observe the nature of 5CB-water interface stabilized by long chain polar molecules.

**Figure 59:** The surfactant stabilized LC droplets: molecular structure of (a) SDS and (b) CTAB, (c) polarizing micrograph of SDS stabilized 5CB droplets, (d) effect of SDS and CTAB concentration on the number of stable droplets formed; the size distribution of (f) SDS and (g) CTAB stabilized droplets both using 1 mM of stabilizing concentrations.

SDS and CTAB both were observed to be excellent stabilizers of the 5CB droplets in water. They formed stable droplets of 5CB with zeta potentials of –45 mV and +42 mV for CTAB and SDS respectively. The droplets had radial configuration compared to the bipolar for LCA stabilized droplets (Figure 59c). The effect of stabilizer concentration onto the number of
droplets remained similar to that of LCA. The transmitted light intensity decreased drastically with decrease in the concentration used for the stabilization (Figure 59d). Once again the concentration of stabilizer used had a direct effect on the total area of the 5CB-water interface. The size of the droplet also was found not to be a function of the stabilizing concentration. The average size was measured to be about 0.56 and 0.54 μm for SDS and CTAB stabilized 5CB droplets respectively (Figure 59e and f).

**LCA sensing using SDS or CTAB coated droplets**

Incubation of SDS and CTAB coated droplets with adequate amount of LCA resulted in the change of conformation from radial to bipolar. For SDS and CTAB coated droplets, it is likely that the long hydrophobic part of these surfactants spread inside the 5CB droplets and the tiny hydrophilic head groups face the water. Such an arrangement allows the 5CB molecules to align themselves perpendicular to the 5CB-water interface (and parallel to the hydrophobic chains) therefore imparting a radial configuration. It is also notable that the director of configuration remained the same (i.e. radial) for both SDS and CTAB stabilized droplets, indicating that the nature of charge (either negative for SDS or positive for CTAB) of the terminal head group does not seem to have effect on the director of configuration. It can be concluded that the way in which the hydrophobic and hydrophilic parts are arranged within the stabilizer molecule and their orientation at the interface that dictates the director of configuration. Upon incubation with LCA, the LCA molecules are perhaps replacing the linear surfactants from the 5CB-water interface, due to a better stabilizing ability. This replacement of surfactant by LCA removes the long chain hydrophobic tails from the core of the 5CB droplets, which may induce the 5CB
molecules to lie down at the interface than standing perpendicular. Thus, the competitive behavior of two types of surfactants at the interface brings about the transition, as illustrates schematically in Figure 60.

![Figure 60: A schematic illustration of the replacement of SDS or CTAB molecules from the 5CB-water interface by LCA molecules, triggering the radial to bipolar transition. The inset shows molecular arrangement at the interface.](image)

The transition was found to be concentration dependent. Figure 61a shows the percentage change in the conformation of the SDS coated 5CB droplets as a function of LCA concentration. The transition curve showed S-shape trend, reaching to saturation at ~120 μM. For the CTAB coated droplets, the trend of the transition curve remained the same but it shifted more towards right (Figure 61b).

Further analysis of the transition curve reveals that for SDS stabilized droplets it took about 40 μM of LCA to induce 50% transition where as the value was found to be 95 μM for the CTAB
coated droplets (Figure 61c). To completely change droplets to bipolar (100% transition), the values were found to be ~117 μM and ~ 160 μM of LCA for SDS and CTAB coated droplets, respectively (Figure 61d). It also noteworthy that the type of charges, the terminal polar groups of the SDS and CTAB molecules have, also play an important role in such replacement reaction. LCA is negatively charged in basic pH due to the deprotonated carboxyl (COO⁻) terminal. This may induce a repulsive force to the negatively charged SDS molecules (due to the SO₃⁻ terminal) easing the process of replacement. However, for CTAB such repulsion is not possible, owing to its positively charged amine terminal (NH³⁺). Moreover, some LCA molecules may even bind to the CTAB molecules and therefore do not get to reach the 5CB-water interface. Furthermore, the CTAB molecule has a longer (C₁₆) hydrophobic chain compared to SDS (C₁₂), which may help the former to anchor more strongly at the interface. Possibly, it is a combined effect of the nature of charge and the chain length that result the difference in the transition curves for the SDS and CTAB coated droplets.
Figure 61: The concentration dependent transition of (a) SDS and similar molecules and (b) CTAB and similar molecules coated droplets in presence of LCA. The LCA concentrations resulting in 50% transitions for different chain lengths of (c) SDS and (d) CTAB type surfactants.

Phospholipids are natural surfactants that bridge the hydrophobic-hydrophilic interface. But, 18:1 PE is a synthetic lipid and has two long hydrophobic tails compared to one in SDS or CTAB (Figure 62a). Adsorption of PE lipid at the 5CB-water interface also resulted stable lipid-coated droplets, which were radial in conformation. This is consistent with the assumption that the long
hydrophobic tails are anchored inside the 5CB core with their polar amine terminals facing the water. This makes the 5CB molecules align perpendicular to the interface, resulting in a radial conformation. The PE lipid coated 5CB droplets when incubated with LCA solutions also triggered a change in conformation like its single tailed counterparts. Therefore, the PE lipid molecules get replaced with LCA molecules to lower the 5CB-water interfacial energy. In this case also, the radial to bipolar transition occurred in a concentration dependent manner resulting in an S curve (Figure 62b). But in this case the transition occurred at slightly higher concentration at about 87 μM LCA (for 50% transition) compared to 85 μM LCA for CTAB (Figure 62c), though they both have amine terminals. This may be due the difference in the molecular structures of the two surfactants and therefore their arrangement at the 5CB-water interface. It is reasonable to think that each molecule of PE lipid molecule with its two tails would need more space at the 5CB-water interface, compared to a single tailed CTAB molecule. Therefore the molecular density (number of molecules per unit interfacial area) of the 2-tail PE lipid at the 5CB-water interface is much less compared to the 1-tail CTAB. This means that number of molecules of PE lipid that needs to be replaced from the interface by LCA molecules also goes down significantly. Moreover, its primary amine terminal is feebly less positive in charge than the tertiary amine terminal of CTAB making the attraction less intense. So, the number of LCA molecules lost, if any, for binding to the oppositely charged lipid molecule would also be less. However, having two tails may also lead to a better anchoring ability of the PE, which may counter some part of the above factors. Interplay of all such factors result the transitions to occur at slightly lesser concentrations of LCA in case of the 2 tailed PE lipids.
In order to better understand the replacement of surfactants at the 5CB-water interface, the change of conformation was simultaneously compared with fluorescent microscopy. For this fluorescent dye (NBD) conjugated PE lipid or PE-NBD lipid, was used as the stabilizing agent whose molecular structure is shown in Figure 63a. The PE-NBD lipid coated 5CB droplets were stable in water and had radial conformation. Under bright field the 5CB droplets could be seen floating in the aqueous media (Figure 63b). Since all the droplets were not in one focal-plane and their positions varied along the Z-axis, they appear to have a large difference in their diameters. When the PE-NBD lipid coated droplets were viewed under fluorescent microscope they appeared brightly glowing balls in a dark background (Figure 63c). The glow showing the droplet out line is due to the fluorescent PE-NBD lipid that coats the droplet surface. During the imaging, multiple frames along Z-axis were captured individually and then compiled together into one image automatically using the imaging software. Each frame, capturing one cross-
section of the sphere, appeared as a ring and when they all are compiled together; the image reveals the droplet surface as bright circles. When LCA solution is introduced to PE-NBD coated droplets the glowing outline from the droplet surface starts to fade off. Thus the droplets are hardly visible in under fluorescent microscopy. When identical droplets are viewed under polarized light they had undergone a change of conformation form radial to bipolar as well. If the fluorescent microscopy image is compared with the polarized light microscopy image then the fading of the fluorescent glow can linked directly to the change of conformation of the droplets. The decrease of fluorescence from the droplet surface would occur only when it is deprived of the fluorescent PE-NBD molecules and gets replaced by non-fluorescent LCA molecules. Hence it can be said that the replacement of the surfactants at the 5CB-water interface, as observed through fluorescent microscopy, is the reason for the change in director of conformation of the 5CB molecules within the droplets as seen in polarized light microscopy.
**Figure 63:** (a) Molecular structure of NBD-PE; (b) fluorescent microscope image of PE-NBD stabilized droplets as made, (c) after exposure to LCA, the brightness and contrast of the image is enhanced for clarity and (d) the corresponding bright field image.

Chitosan is a natural polymer that is known for binding with LCA or such similar molecules. Chemically it is an amino-polysaccaride with connected pentose sugar rings as the backbone with hydroxyl and amine peripheral functional groups (Figure 64a). Chitosan is also often used a functional polymer, so we used medium molecular weight chitosan (mid-chitosan) to stabilize the 5CB droplets in water. The mid-chitosan coated droplets were mono dispersed with average diameter of about 0.71 μm (Figure 64b). They were found to very stable at a pH 5 or less and had a zeta potential of about +45 mV. The conformation for the chitosan coated 5CB droplets was found to be bipolar, implying that the 5CB molecules are lying parallel to the interface. Thus, the chitosan backbone is probably oriented parallel to the interface facing the 5CB core and the peripheral amine terminals facing the water molecules. This also explains the positive surface charge of the droplets and their instability at a basic pH as the amine groups looses the H⁺ ions.
**Figure 64:** Chitosan stabilized droplets and their surfactant triggered transition; (a) molecular structure of chitosan, (b) polarizing micrograph of SDS stabilized 5CB droplets; (c) the size distribution of chitosan coated droplets; (d) effect of SDS and CTAB concentration on the transition of the chitosan coated droplets; (e) 50 % transition concentrations for SDS and CTAB.

When mid-chitosan coated droplets were exposed to solutions of SDS or CTAB, the director of conformation was found to change from bipolar to radial. Once again the transition was concentration dependent for both SDS and CTAB. But the transition curve was much steeper for SDS compared to CTAB (Figure 64c). Thus, less amount of SDS was necessary for the same percentage of transition. For example, it took only about 12 μM of SDS to induce 50% transition compared to about 22 μM for CTAB (Figure 64d). It is the repulsive force between the
positively charged CTAB and chitosan that can be attributed for such difference much the same way as for the replacement with LCA.

In this case SDS or CTAB does not completely remove or replace chitosan from the surface of the droplets, as the SDS treated chitosan droplets starts to aggregate at higher pH values. This implies that the chitosan still remains on the surface of the droplets and the surfactant molecules possibly penetrate through the mess like polymer layer. Therefore chitosan acts as an anchoring layer for the surfactant molecules to bind to and then interact with the 5CB-water interface. This seems consistent with the fact that it needs more CTAB molecules to overcome the electrostatic repulsion to gain access to the interface and therefore induce a transition.

Similar trend was observed with droplets that were coated with low molecular weight chitosan (Low-Chitosan). They too turned to radial when exposed to SDS or CTAB solutions in a concentration dependent manner. Further, the two sets of droplets coated with Mid- and Low-chitosan were exposed to SDS solutions (SDS is chosen over CTAB as the transitions needs less amount of SDS) containing just enough SDS (~65 μM) to induce a complete transition. This resulted in mid- and low- chitosan stabilized 5CB droplets that are radial in orientation due to the SDS exposure. To this LCA solution was introduced. Interestingly, a repetitive change of orientation from bipolar to radial then back to bipolar was observed for sequential exposure of surfactants as shown schematically in Figure 65a. This time also, the transition occurred in a concentration dependent manner for both mid- and low- chitosan coated droplets (Figure 65b). But the transitions in this case occurred at a much lower LCA concentration compared to only
SDS coated droplets. For 50% of the droplets to convert to bipolar from radial to bipolar only took about 18 μM LCA when chitosan coating was present compared to 40 μM for droplets coated only with SDS. The molecular weight of chitosan also plays a role as one can observe a steeper transition curve for the mid-chitosan. The 50% transition for the low-chitosan required slightly more (~23 μM) of LCA. The low-chitosan owing to the lower molecular weight has less dense network. This makes the polymer layer on the 5CB droplet more pervious to the surfactant molecule. But this also reduces the interaction sites where the LCA molecules can anchor themselves. So, the higher molecular weight of mid-chitosan molecule would provide a platform with more polymer chains as anchor for the LCA molecules. This would therefore increase the local concentration of LCA molecules at the droplet surface, as if LCA molecules would get tangled into the polymer network. This local increase in LCA concentration coupled with a higher binding constant facilitates LCA molecules to replace SDS molecules at the 5CB-water interface, which eventually brings about the transition. Thus the affinity of chitosan towards LCA allowed us to detect much lower levels of LCA by a simple alteration of droplet surface.
**Figure 65:** (a) LCA triggered transition of both types of chitosan stabilized droplets modified with SDS, (b) LCA concentrations required for 50 % transition for both medium and low molecular weight chitosan and (c) a schematic showing the sequence of transitions.

**Conclusion**

In this work, LCA was successfully detected to a clinically significant concentration. It was observed that LCA being a natural surfactant can also stabilize the LC droplets and imparts them with a bipolar conformation. It was observed that the linear surfactants like SDS and CTAB coated droplets could also stabilize the LC droplets but in this case there were radial. The incubation of SDS and CTAB coated droplets with LCA triggered a radial to bipolar conformation to the LC within the droplets in a concentration dependent manner. The LC droplets stabilized with two tail lipids also resulted in radial conformation, which changed to bipolar when incubated with LCA. The transition was dependent on the length, surface charge and molecular architecture of the surfactants. The shorter chain length and similar surface charge yields better sensitivity. Stabilization of the droplets with chitosan provided good stability to the droplets and subsequent treatment with surfactant yielded radial conformation which changed to bipolar in the presence of LCA. The sensitivity was better possibly due the presence to chitosan...
platform that improves the local concentration of LCA at the water-5CB interface. The chitosan modification provided a better sensitivity and with this LCA concentrations as low as 25 M could be sensed using the LC droplets. Such sensitivity is clinically significant making LC droplets as a good candidate for future sensing applications.
CONCLUSION

Liquid Crystal (LC) droplets are new class of functional materials with sensitive optical properties, high surface area and great portability. PSS, a surfactant, was used to obtain stable 5CB droplets in water. Interestingly, deposition of alternate layers of positively charged PDADMAC, and negatively charged PSS through the layer-by-layer assembly process induced the director of configuration to alter between radial (for PDADMAC terminal layer) to bipolar (for PSS terminal layer). Change of configurations due to the change in pH and salt content in the droplet solution was also observed. Thus, LC droplets optically responded to any changes at their interface or solution environment. This motivated to investigate the use of LC droplets as potential sensing units.

Two distinct mechanisms were exploited to detect different biologically relevant species. In the first study, PSS coated LC droplets were used to sense dendrimer molecules, the dummy charged species. Four different generations of PAMAM Dendrimers, with different sizes and surface charges were studied and it was observed that higher surface charge have better sensitivity. The study demonstrated the use of LC droplets to detect charged species based on their electrostatic interactions. Since most of the natural phenomena are partially based on electrostatic interactions, this experiment provided a critical insight about how such an interaction would trigger the transition in LC droplets and laid the platform to detect biologically more relevant species like proteins and virus using LC droplets. BSA was used as a model for non-specific detection of proteins and concentrations as low as 200 mg/mL could be sensed. Two different plant viruses viz. cow pea mosaic virus (CPMV) and tobacco mosaic virus (TMV) were studied
as model viruses. The sensitivity in this case depends both on the morphology and surface charge of the virus. The droplets were more sensitive to spherical virus (CPMV) compared to the tubular one (TMV) and the detectable concentrations were 100 μg/mL and 400 μg/mL respectively. Thus, low concentrations of proteins and viruses using surface modified LC droplets could be detected, based on their electrostatic interaction. Studies are currently under way to achieve better sensitivity and specificity.

In the second study, both surfactants and lipids were used to stabilize the LC droplets. The species to detect was chosen to be lithochloric acid (LCA), a bile acid. LCA did induce a change in the director of configuration, thus making its detection possible using LC droplets. Fluorescent-labeled lipids were used to understand the transition. It was observed that LCA partially replaced the lipids at LC-water interface thereby inducing the transition. This replacement-induced transition was also observed for linear surfactants and the replacement was found to be more favorable for surfactants with smaller chain length and same (negative) surface charge. Thus, by choosing the proper surfactant molecules as the stabilizing agent, extremely low levels (~35 μM) of LCA could be detected using the LC droplets. This is very close to the LCA concentration (>20 μM) observed in colon cancer patients.

The basic objective of this work is to establish the fact that LC droplets can indeed be used to detect technologically relevant molecules. Herein we demonstrate that surface modified LC droplets can indeed be used to sense biologically relevant species. Moreover, they can be used to sense low concentrations of important molecules in a qualitative manner in solution phase with
relative ease and simplicity, as needed for most biosensing applications. The study also addresses the fundamental principles and molecular phenomenon that cause the detection possible, which will help improve this sensing technology in future. In conclusion, with their tunable optical properties, coupled with high surface area and portability, LC droplets make good potential candidate for next generation sensing applications.

Further studies are underway to address the selectivity aspect of the detection. Presently, more specific protein detection has been planned. Some experiments are being carried out to selectively determine each bile acid and cholesterol. Studies are also underway to detect more species of bacteria. Improvements in the stabilization and the sensor design is also been carried forward to improve the overall performance of the sensing unit.

This broadens the future scope of this sensing platform and further studies on different types of analytes in the field of health care and environmental management will strengthen it and may establish surface engineered LC droplets as a real-time detection platform.
REFERENCES:


