INVESTIGATION OF THE PHYSICAL PROPERTIES OF CHOLESTERYL ESTER LIQUID CRYSTAL AND THE INTERACTION WITH CELLS

WAN IBTISAM BINTI WAN OMAR

A thesis submitted in
fulfilment of the requirement for the award of the
Degree of Master of Electrical Engineering

Faculty of Electrical and Electronic Engineering
Universiti Tun Hussein Onn Malaysia

JANUARY 2016
For my dearest mother, father, husband and family for their encouragement and blessing...

To my beloved friend and their support and caring......

-Never tired and give up to gain knowledge and life is a journey of learning-
ACKNOWLEDGEMENT

Alhamdulillah, I am grateful to ALLAH S.W.T the most merciful and the most compassionate for the guidance and knowledge bestowed upon me, for without it I would not been able to come this far. Peace be upon him, Muhammad the messenger of god.

I would like to express my gratitude to honourable Assoc. Prof. Dr Soon Chin Fhong, my supervisor of Master’s project. Under her supervision, many aspects regarding this project has been explored and with the knowledge, idea and support received from her. I also would like to thank my co-supervisor Dr Hatijah Basri for sharing her knowledge to me.

Many thanks and grateful to the Malaysia Ministry of Higher Education for research funding support (FRGS Phase 1 Vot No. 1050) and also Universiti Tun Hussein Onn Malaysia for providing post graduate incentive research grant (GIPS Vot 1111).

Finally, I would like to dedicate my gratitude to my parents, my family, my husband and friends who helped me directly and indirectly in the completion of this project. This encouragement and guidance mean a lot to me. Their sharing and experience foster my belief in overcoming every obstacle encountered in this project.

Guidance, co-operation and encouragement from all people above are appreciated by me in sincerely. Although I cannot repay the kindness from them, I would like to wish them to be well and happy always.
LIST OF ASSOCIATED PUBLICATIONS

Journals


Conference Proceeding:


LIST OF AWARDS

1. Silver Medal in Research & Innovation Festival 2012 (R & I Fest UTHM)

2. Gold Medal in Persidangan dan EXPO Ciptaan Institusi Pengajian Tinggi Antarabangsa 2013 (PECIPTA)

3. Silver Medal in Research & Innovation Festival 2013 (R & I Fest UTHM)
   Thong Kok Tung, Wan Ibtisam Wan Omar, Rosliza Mohamad Zin, Yap Huing Yin. “Scaffoldless Technique to Culture 3D Keratinospheroids.”
ABSTRACT

Cholesteryl ester liquid crystals (CELC) were demonstrated with application in biosensing and microtissue regeneration. The affinity of the cells to this liquid crystal is unclear and required further investigation. This study focused on characterising the physical properties of CELC and interaction of human keratinocytes with CELC. The physical properties of CELC were characterised by a custom built contact angle measurement system and bubble pressure measurement apparatus. Other methods such as pendant drop were applied to determine the critical surface tension of the CELC. Then, the characterization of the CELC was continued by using Differential Scanning Calorimeter (DSC), X-ray Diffraction (XRD), Polarising Microscopy (POM) and Fourier Transform Infrared Spectroscopy (FTIR). Nonetheless, the morphology of cells interaction with CELC after it reached confluency was studied using Field Emission Scanning Electron Microscopy (FESEM) and non-contact mode of Atomic Force Microscopy (AFM). The results showed that the critical surface tension of the liquid crystal using contact angle was 37.5 mN/m and the surface tension measured using pendant drop method was found to be 23.6 mN/m. Both results indicate that the surface of the liquid crystal was moderately hydrophobic. From the DSC, CELC was found stable at room and incubator temperature. From XRD results, the compound of CELC interacts in cell culture media self-assembles into lyotropic layer. POM and FTIR analysis showed CELC after immersion in media displayed lyotropic smectic phases. The AFM and FESEM images indicated good adhesion of cells on the CELC. This research thus showed that the hydrophilic layers of lyotropic phase of cholesteryl ester liquid crystal were demonstrated with biophysical properties that support the adhesion of cells.
ABSTRAK

CONTENTS

TITLE i
DECLARATION ii
DEDICATION iii
ACKNOWLEDGEMENT iv
LIST OF ASSOCIATED PUBLICATION v
LIST OF AWARD vi
ABSTRACT vii
ABSTRAK viii
TABLE OF CONTENTS ix
LIST OF TABLES xiv
LIST OF FIGURES xv
LIST OF SYMBOLS xx
LIST OF ABBREVIATIONS xxii

CHAPTER 1 PROJECT OVERVIEW
1.1 Project background 1
1.2 Problem statement 2
1.3 Objective 3
1.4 Scope of project 3
1.5 Thesis organisation 4
1.6 Thesis contribution 4
CHAPTER 2  LITERATURE REVIEW

2.1  Introduction to liquid crystal 6
2.2  Types of liquid crystal 7
  2.2.1  Thermotropic liquid crystal 7
    2.2.1.1  Nematic phases 8
    2.2.1.2  Cholesteric phases 8
    2.2.1.3  Smectic phases 10
  2.2.2  Lyotropic liquid crystal 10
2.3  Application of liquid crystal in biosensing 12
2.4  Human keratinocytes (HaCaTs) 14
2.5  Cellular adhesion 15
2.6  Surface tension measuring technique 16
  2.6.1  Contact angle measurement 17
  2.6.2  Bubble pressure 19
  2.6.3  Pendant drop 20
  2.6.4  Surface tension of liquid crystal 22
2.7  Spectrophotometer 23
2.8  Differential scanning calorimeter (DSC) 23
2.9  X-ray diffractometer (XRD) 24
2.10  Fourier transform infrared spectroscopy (FTIR) 26
2.11  Microscopy 27
  2.11.1  Phase contrast microscope 28
  2.11.2  Atomic force microscopy (AFM) 29
  2.11.3  Field emission scanning electron microscope 31

CHAPTER 3  METHODOLOGY

3.1  Introduction 34
3.2  Development of a contact angle measurement system 36
  3.2.1  Experiment for validating contact angle measurement system 44
3.3 Characterising the physical properties of cholesteryl ester liquid crystal (CELC)
   3.3.1 Preparation of cholesteryl ester liquid crystal (CELC) 45
   3.3.2 Measuring surface tension of CELC 46
      3.3.2.1 Contact angle method 46
      3.3.2.2 Bubble pressure method 47
      3.3.2.3 Pendant drop method 49
   3.3.3 Thermal stability analysis 50
      3.3.3.1 Preparation of CELC for differential scanning calorimeter (DSC) 50
      3.3.3.2 Preparation of CELC for optical emission spectroscopy (OES) 51
   3.3.4 Preparation of CELC for polarising microscopy 52
   3.3.5 Liquid crystal sample preparation for x-ray diffraction (XRD) 53
   3.3.6 Liquid crystal sample preparation and fourier transform infrared analysis 54
3.4 Studying the cells interaction with CELC 55
   3.4.1 Cell culture 55
   3.4.2 Cell culture on the liquid crystal and glass substrate 56
   3.4.3 AFM of cells on the liquid crystal and glass substrate 57
   3.4.4 FESEM of cells on the liquid crystal and glass substrate 58

CHAPTER 4  RESULT AND DISCUSSION
4.1 Introduction 60
4.2 The contact angle measurement system 60
4.2.1 Calibration results of contact angle measurement system 63

4.3 Physical properties of cholesteryl ester liquid crystal (CELC)

4.3.1 Cholesteryl ester liquid crystal (CELC) 66

4.3.2 Surface of cholesteryl ester liquid crystal

4.3.2.1 Contact angle 66

4.3.2.2 Bubble pressure 68

4.3.2.3 Pendant drop 70

4.3.3 Thermal stability of cholesteryl ester liquid crystal 72

4.3.4 Polarising optical microscopy (POM) 74

4.3.5 Crystallinity study of cholesteryl ester liquid crystal 76

4.3.6 Chemistry elements in cholesteryl ester liquid crystal 77

4.4 Cells interaction with cholesteryl ester liquid crystal

4.4.1 Morphological effect and adhesion characteristic of cells to CELC 79

5.1 Conclusion 89

5.2 Future works 90

REFERENCES 92

APPENDIX A Architecture of the Arduino UNO microcontroller board

APPENDIX B Specification of L293D motor driver
APPENDIX C  Arduino coding
APPENDIX D  Matlab coding
APPENDIX E  The data of contact angle method
APPENDIX F  The data of bubble pressure method
<table>
<thead>
<tr>
<th>TABLE NO</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Application of the liquid crystal as biosensor</td>
<td>13</td>
</tr>
<tr>
<td>2.2</td>
<td>Probe liquids and their interfacial tension</td>
<td>18</td>
</tr>
<tr>
<td>2.3</td>
<td>Surface tension values for liquid crystal</td>
<td>22</td>
</tr>
<tr>
<td>4.1</td>
<td>Interfacial tension value for CELC</td>
<td>71</td>
</tr>
<tr>
<td>4.2</td>
<td>Percentage of transmission calculated from Beer’s law</td>
<td>73</td>
</tr>
<tr>
<td>4.3</td>
<td>Differences of AFM and FE-SEM for the application in cells imaging</td>
<td>88</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE NO</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Basic structure of a liquid crystal molecule</td>
<td>7</td>
</tr>
<tr>
<td>2.2</td>
<td>A graphical illustration of cholesteric phase</td>
<td>8</td>
</tr>
<tr>
<td>2.3</td>
<td>Types of cholesteric derivatives</td>
<td>9</td>
</tr>
<tr>
<td>2.4</td>
<td>Standard thermotropic liquid crystal phases</td>
<td>10</td>
</tr>
<tr>
<td>2.5</td>
<td>Typical phases of lyotropic liquid crystal</td>
<td>11</td>
</tr>
<tr>
<td>2.6</td>
<td>Layer of skin at epidermis consists of keratinocytes</td>
<td>14</td>
</tr>
<tr>
<td>2.7</td>
<td>A schematic diagram of cellular adhesion</td>
<td>15</td>
</tr>
<tr>
<td>2.8</td>
<td>Contact angle measurement of a droplet of liquid</td>
<td>17</td>
</tr>
<tr>
<td>2.9</td>
<td>Apparatus for surface tension measurement using bubble pressure</td>
<td>19</td>
</tr>
<tr>
<td>2.10</td>
<td>A pendant drop system from AST Products, INC</td>
<td>20</td>
</tr>
<tr>
<td>2.11</td>
<td>The pendant drop geometry</td>
<td>22</td>
</tr>
<tr>
<td>2.12</td>
<td>Light transmitted or reflected through a sample</td>
<td>23</td>
</tr>
<tr>
<td>2.13</td>
<td>Schematic DSC heating curve of a semi-crystalline polymer</td>
<td>24</td>
</tr>
<tr>
<td>2.14</td>
<td>PANalytical X’Pert Powder X-Ray Diffraction</td>
<td>25</td>
</tr>
<tr>
<td>2.15</td>
<td>Setup for a scattering experiment utilizing X-ray diffraction</td>
<td>25</td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>2.16</td>
<td>Fourier transform infrared spectroscopy (Perkin Elmer Spectrum 100)</td>
<td>26</td>
</tr>
<tr>
<td>2.17</td>
<td>Sample in contact with evanescent wave</td>
<td>27</td>
</tr>
<tr>
<td>2.18</td>
<td>Light path of a phase contrast microscope</td>
<td>28</td>
</tr>
<tr>
<td>2.19</td>
<td>A Nikon Eclipse TS100 phase contrast microscope</td>
<td>29</td>
</tr>
<tr>
<td>2.20</td>
<td>Schematic diagram of an AFM</td>
<td>30</td>
</tr>
<tr>
<td>2.21</td>
<td>Atomic force microscopy XE-100 Park System</td>
<td>30</td>
</tr>
<tr>
<td>2.22</td>
<td>FE-SEM schematic diagram</td>
<td>32</td>
</tr>
<tr>
<td>2.23</td>
<td>Field Emission Scanning Electron Microscopy (JEOL JSM-7600F series)</td>
<td>32</td>
</tr>
<tr>
<td>3.1</td>
<td>The project flow chart</td>
<td>35</td>
</tr>
<tr>
<td>3.2</td>
<td>Flow chart of surface tension methods</td>
<td>36</td>
</tr>
<tr>
<td>3.3</td>
<td>Setup for contact angle measurement technique</td>
<td>37</td>
</tr>
<tr>
<td>3.4</td>
<td>Circuit diagram of contact angle motor controller</td>
<td>38</td>
</tr>
<tr>
<td>3.5</td>
<td>Flow chart to measure the contact angle of a droplet of fluid</td>
<td>39</td>
</tr>
<tr>
<td>3.6</td>
<td>Open pushbutton in MATLAB GUI</td>
<td>39</td>
</tr>
<tr>
<td>3.7</td>
<td>Command for OPEN pushbutton</td>
<td>40</td>
</tr>
<tr>
<td>3.8</td>
<td>Distilled water interacts with PDMS</td>
<td>40</td>
</tr>
<tr>
<td>3.9</td>
<td>Command for SNAP pushbutton</td>
<td>41</td>
</tr>
<tr>
<td>3.10</td>
<td>Command for SAVE pushbutton</td>
<td>41</td>
</tr>
<tr>
<td>3.11</td>
<td>Image processing command including Browse function</td>
<td>41</td>
</tr>
<tr>
<td>3.12</td>
<td>An enlarged image of the selected point of a Acetone on PDMS surface</td>
<td>42</td>
</tr>
<tr>
<td>3.13</td>
<td>Tools in MATLAB GUI</td>
<td>43</td>
</tr>
<tr>
<td>3.14</td>
<td>Command for search critical surface tension</td>
<td>43</td>
</tr>
<tr>
<td>3.15</td>
<td>Push button in GUI for Fox Zisman graph</td>
<td>44</td>
</tr>
</tbody>
</table>
3.16 Cholesteryl ester liquid crystal formulation
3.17 Mixture of cholesteryl ester liquid crystal
3.18 CELC in cholesteric phase at room temperature
3.19 Differential pressure sensor ASP1400 (SENSIRION)
3.20 A schematic diagram of ASP 1400 pressure sensor with RS 232
3.21 Experimental setup for measuring surface tension using bubble pressure method
3.22 Sample drop at the end of capillary tip
3.23 CELC sample in DSC pan
3.24 Q20 Differential scanning calorimeter
3.25 Experimental setup using OES
3.26 Ocean Optics HR4000 spectrophotometer
3.27 CELC substrate immersed in cell culture media
3.28 Sample placed on the sample holder for analysis
3.29 CELC sample were placed on top of ATR crystal
3.30 Medium for cell harvesting
3.31 HaCaT cells were sub-cultured and were plated in each petri dish
3.32 HaCaT were sub-culture without and with liquid crystal
3.33 Image of silicon cantilever from the AFM system over the HaCaT cell sample
3.34 Glass coverslips with gold sputter coater
4.1 The prototype models of a designed contact angle measurement system
4.2 A DC motor controller circuit connected to Arduino UNO
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td>Enlarged image for angles value at the intersection point a droplet of Acetone with solid surface.</td>
</tr>
<tr>
<td>4.4</td>
<td>Enlarge image of contact angles using angle tool shows intersection point and contact angle.</td>
</tr>
<tr>
<td>4.5</td>
<td>Eight probe liquids droplets on PDMS and Polyimide surface.</td>
</tr>
<tr>
<td>4.6</td>
<td>Fluid surface tension of the eight probe liquid generated in excel for PDMS and Polyimide.</td>
</tr>
<tr>
<td>4.7</td>
<td>CELC in cholesteric form</td>
</tr>
<tr>
<td>4.8</td>
<td>Image of sessile drops in contact with CELC coated glass slides and their contact angles.</td>
</tr>
<tr>
<td>4.9</td>
<td>A Fox-Zisman plot for cholesteryl ester liquid crystal.</td>
</tr>
<tr>
<td>4.10</td>
<td>Hardware consists of ASP1400 pressure sensor.</td>
</tr>
<tr>
<td>4.11</td>
<td>CELC in 0.4 second, 0.8 second and 1 second before the bubble burst</td>
</tr>
<tr>
<td>4.12</td>
<td>Enlarged image of hemisphere</td>
</tr>
<tr>
<td>4.13</td>
<td>Samples reading of surface tension using bubble pressure.</td>
</tr>
<tr>
<td>4.14</td>
<td>Image of pendant drop digitized drop and edge detection.</td>
</tr>
<tr>
<td>4.15</td>
<td>DSC shows heat flow as a function of temperature of cholesteryl liquid crystals. No exothermic or endothermic activities were observed at 37 °C.</td>
</tr>
<tr>
<td>4.16</td>
<td>Intensity versus wavelength for CELC</td>
</tr>
<tr>
<td>4.17</td>
<td>Percentage of transmission versus temperature</td>
</tr>
</tbody>
</table>
Image comparison using phase contrast and cross-polarizing optical microscopy.

A cross-polarizing micrograph of cholesteric based lyotropic liquid crystals shows the wide band streaks and focal conic textures.

The surface of CELC after immersion in cell culture media for 24 hours, 48 hours and 72 hours.

XRD for CELC and lyotropic layer of CELC.

The FTIR spectrum of cholesteryl ester liquid crystals.

HaCaT cells morphology on glass substrate and for 24, 48 and 72 hours.

AFM images for keratinocytes cultured on a plain glass and liquid crystal substrates.

Image of HaCaT cell plated on the glass substrate and liquid crystal substrate after 24, 48 and 72 hours.

Thickness of HaCaTs membrane cultured with and without the presence of liquid crystal as the substrate.

Line profiles of cells cultured on plain glass and liquid crystal substrate.

Human keratinocyte cell morphology cultured on glass on FE-SEM.

Human keratinocyte cell morphology cultured on cholesteryl ester liquid crystal on FE-SEM.
<table>
<thead>
<tr>
<th>SYMBOL</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )</td>
<td>- Radius of curvature at apex drop</td>
</tr>
<tr>
<td>( \text{Å} )</td>
<td>- Angstrom</td>
</tr>
<tr>
<td>B</td>
<td>- Drop shape factor</td>
</tr>
<tr>
<td>C</td>
<td>- Carbon</td>
</tr>
<tr>
<td>cm</td>
<td>- Centimetre</td>
</tr>
<tr>
<td>cm-1</td>
<td>- Wavenumber</td>
</tr>
<tr>
<td>( ^\circ \text{C} )</td>
<td>- Degree celcius</td>
</tr>
<tr>
<td>( d )</td>
<td>- Interplanar spacing</td>
</tr>
<tr>
<td>( \gamma ) or ( \sigma )</td>
<td>- Surface tension</td>
</tr>
<tr>
<td>( \Delta \rho )</td>
<td>- Difference between densities</td>
</tr>
<tr>
<td>( \Phi )</td>
<td>- Coordinate</td>
</tr>
<tr>
<td>( \theta )</td>
<td>- angle</td>
</tr>
<tr>
<td>( F )</td>
<td>- Free energy</td>
</tr>
<tr>
<td>( H )</td>
<td>- Hidrogen</td>
</tr>
<tr>
<td>( I )</td>
<td>- Intensity</td>
</tr>
<tr>
<td>( l )</td>
<td>- litre</td>
</tr>
<tr>
<td>( g )</td>
<td>- Gravity</td>
</tr>
<tr>
<td>( N )</td>
<td>- Nitrogen</td>
</tr>
<tr>
<td>( % )</td>
<td>- Percentage</td>
</tr>
<tr>
<td>( O )</td>
<td>- Oxygen</td>
</tr>
<tr>
<td>( P ) or ( p )</td>
<td>- Pressure</td>
</tr>
<tr>
<td>( R )</td>
<td>- Radius</td>
</tr>
<tr>
<td>( T )</td>
<td>- Transmission</td>
</tr>
<tr>
<td>( T_g )</td>
<td>- Glass Transition</td>
</tr>
<tr>
<td>( T_m )</td>
<td>- Melting points</td>
</tr>
<tr>
<td>( \lambda )</td>
<td>- Wavelength</td>
</tr>
<tr>
<td>( m )</td>
<td>- metre</td>
</tr>
<tr>
<td>Symbol</td>
<td>Unit</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>mA</td>
<td>Mili Amp</td>
</tr>
<tr>
<td>ml</td>
<td>Mili litre</td>
</tr>
<tr>
<td>mg</td>
<td>Mili gram</td>
</tr>
<tr>
<td>mN/m</td>
<td>Mili Newton per metre</td>
</tr>
<tr>
<td>mM</td>
<td>Micro molar</td>
</tr>
<tr>
<td>kW</td>
<td>Kilo Watt</td>
</tr>
<tr>
<td>kV</td>
<td>Kilo Volt</td>
</tr>
<tr>
<td>µ</td>
<td>micro</td>
</tr>
<tr>
<td>SYMBOL</td>
<td>DESCRIPTION</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>5CB</td>
<td>Pentylycyanobiphenyl</td>
</tr>
<tr>
<td>8CB</td>
<td>4′-n-octyl-4-cyano-biphenyl</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated Total Reflectance</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscopy</td>
</tr>
<tr>
<td>CELC</td>
<td>Cholesteryl Ester Liquid Crystal</td>
</tr>
<tr>
<td>CST</td>
<td>Critical Surface Tension</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagles’s Medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl Sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimeter</td>
</tr>
<tr>
<td>DSCG</td>
<td>Disodium Cromoglycate</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular Matrix</td>
</tr>
<tr>
<td>EG</td>
<td>Ethylene Glycol</td>
</tr>
<tr>
<td>FESEM</td>
<td>Field Emission Scanning Electron Microscope</td>
</tr>
<tr>
<td>Fmoc</td>
<td>Fluorenylmethyloxycarbonyl</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
</tr>
<tr>
<td>GND</td>
<td>Ground</td>
</tr>
<tr>
<td>GUI</td>
<td>Graphic User Interface</td>
</tr>
<tr>
<td>HaCaTs</td>
<td>Human Keratinocytes</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hanks Balance Salt Solution</td>
</tr>
<tr>
<td>IPA</td>
<td>Isopropyl alcohol</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid Crystal</td>
</tr>
<tr>
<td>LCD</td>
<td>Liquid Crystal Display</td>
</tr>
<tr>
<td>LCPA</td>
<td>Liquid Crystal Pixel Array</td>
</tr>
<tr>
<td>LED</td>
<td>Light Emitting Diode</td>
</tr>
<tr>
<td>LLC</td>
<td>Lyotropic Liquid Crystal</td>
</tr>
<tr>
<td>MBBA</td>
<td>4-methoxybenzylidene-4′-n-butylaniline</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MMA</td>
<td>Methylmethacrylate</td>
</tr>
<tr>
<td>MTT</td>
<td>3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide</td>
</tr>
<tr>
<td>OES</td>
<td>Optical Emission Spectroscopy</td>
</tr>
<tr>
<td>PEGA</td>
<td>Thermolysinpolyethylene Glycol Acrylamide</td>
</tr>
<tr>
<td>POM</td>
<td>Polarizing Optical Microscopy</td>
</tr>
<tr>
<td>PSPD</td>
<td>Position Sensitive Photo Diode</td>
</tr>
<tr>
<td>PWM</td>
<td>Pulse Width Modulation</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>TV</td>
<td>Television</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>UTHM</td>
<td>Universiti Tun Hussein Onn Malaysia</td>
</tr>
<tr>
<td>V</td>
<td>Volt</td>
</tr>
<tr>
<td>Vis</td>
<td>Visible</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray Diffraction</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Project background

Liquid crystal (LC) is a new class of biomaterial that gained attention for usage in biomedical engineering. They provide means for label free observation of biological phenomenon such as non-toxic thermotropic liquid crystal overlaid with matrigel that had been used in recognizing restructuring of mammalian cells [1, 2]. Recently, a research found that [3], cholesteryl ester liquid crystals (CELC) containing cholesteryl moieties may have suitable biological affinity and also enable cell adhesion and proliferation without the need for pre-treatment with extracellular matrix molecules.

Due to the compact molecular arrangement, liquid crystal was shown to have high resolution on sensing ability in detecting localized cell exerted force and could be used for cell traction force sensor [4]. This is an important property that may reveal the potential of liquid crystal in a biomaterial to function on inert surface. Cell-compatible materials are very important in many biomedical applications [5-7]. Moreover, the cholesterol derivative in the liquid crystal was a well known mesogenic nature with their potential to self order into liquid crystalline substances.

However, the use of liquid crystal in biological study has not been fully explained. In 1857, Mettenheimer used a polarising light microscope and observed double refractility and black formed cross image composed of cholesterol from blood. Researchers proved that, occurrence of anisotropic myelin-like bodies in fatty streaks of the atheromatous lesions of the aorta of man [8]. The anisotropic basic structure has been interpreted as that of liquid crystal. In our living system, liquid crystalline order of molecule occurs in degenerating tissues of liver, spleen, lung and thyroid in the form of small spheres which appear under a conventional light
microscope as highly retractile and with double contours at their edges having the inner ring brighter than the outer ring [8].

This type of liquid crystal contains cholesteryl moieties which have affinity for cell membranes and have the abilities to change their properties. Cholesteryl moieties homeostasis is an important element in cellular membrane traffic and cell survival [9]. Therefore, this type of biomaterial is attractive as substrates because it could provide universally fundamental molecule to mammalian cells. Cholesteryl ester are considered to be easily biodegradable thus making the system suitable for the preparation of tissue engineering that sustain cell proliferation and migration [10]. The advantage of using liquid crystals as the biomaterial is it being non toxic and sensitive to biological interactions [11].

In addition, in order to study the biocompatibility of a biomaterial, surface energy property and thermal stability of a biomaterial are important factors in attracting the adhesion of cells and may affects cell surface interface [12]. Hence, finding surface tension of CELC was an important factor in this study. Moreover, biomaterials are majorly reliant on the surface energy [13] and stable over certain temperature. However, from the perspective of biophysical compatibility, little publication has been studied on the physical properties of liquid crystal. Through this study, synthesis and characterisation of surface properties CELC to interact with human keratinocytes (HaCaT) cells were reported. Furthermore, surface properties of CELC materials that promoted cell affinity were presented in this thesis.

1.2 Problem statement

In liquid crystal based biosensor development, an exposure to cell culture media is unavoidable and this may alter the surface properties of the liquid crystals over time. After immersion in cell culture media, the hydrophilic head and hydrophilic tails of the liquid crystal molecules would reoriented to interface with the water at the surface of the bulk liquid crystal as reported in [4]. However, the wettability of the surface or the surface tension of these liquid crystals in attracting the adhesion of cells have not been clearly identified and understood. Furthermore, the chemical properties of the CELC used in cell culture remain unknown. Although new applications of liquid crystals have been revealed [4], little is known about the
expression of surface proteins and effects onto morphological changes due to the interactions with the liquid crystals. Driven by the positive reports of CELC in supporting cell adhesion, it is necessary to further investigate the physical properties of CELC and characteristics of cells after interaction with the CELC. Thus, this study reveals the application of CELC that could be extended as a new class of bio-physical relevant cells adhesion substrate which does not require pre-conditioning with ligands.

1.3 Objectives

The main purpose of this research work was to investigate the surface properties of CELC. To achieve the goal of this research, the following objectives had been achieved. The objectives for this project were:

a) to develop contact angle and bubble pressured measurement systems that enabled analysis of the surface tension of the CELC surface.

b) to investigate the physical properties of the CELC that enabled human keratinocytes cell attachment.

c) to determine the characteristic of the human keratinocytes interaction with the CELC.

1.4 Scopes of project

This project was divided into six scopes which involve:

a) Motorised contact angle measurement system was developed using Arduino UNO microcontroller, L293D motor driver and MATLAB GUI.

b) Bubble pressure method used ASP1400 pressure sensor to find the surface tension of the CELC.

c) Investigating the surface properties of the liquid crystal using a Differential Scanning Calorimeter (DSC), Optical Emission Spectroscopy (OES), Polarising Optical Microscopy (POM), X-ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR).
d) Use human Keratinocytes (HaCaTs) cell lines for cell interaction study.
e) Studying the cell attachment to the liquid crystal using Atomic Force Microscopy (AFM) and Field Emission Scanning Electron Microscope (FESEM).

1.5 Thesis organisation

This thesis is divided into five chapters. Chapter 1 provides an overview of this project and the objectives, scopes and problem statement of the research. Chapter 2 reveals on the CELC background and its application in biosensing and generative medicine. Chapter 3 outlines the experiment procedure of this project including the background information on the software used for the contact angle measurement and bubble pressure method system, preparing sample for physical properties and cells interaction on CELC analysis. Chapter 4 presents the results and discussion of the project. Chapter 5 was devoted for the conclusions and recommendations for future work.

1.6 Thesis contribution

In this thesis, the experimental finding of physical properties of CELC and cell interactions with CELC revealed a new understanding in biomaterial for biosensing application. The contributions of this study are as listed below:

a) Fox Zisman graph automated generated using Matlab GUI

Using measured angle value, Matlab GUI can automatically generate Fox Zisman graph thus provides the critical surface tension (CST) value of materials.

b) Surface tension of CELC

The value of surface tension of CELC were identified and clearly explain the understanding of wettablility of the surface of these liquid crystals in attracting the adhesion of cells.
c) New class of biological relevant material

The physical expression of cell adhered on CELC showed biocompatibility of CELC exploring new potentials of liquid crystals in bioengineering and biosensing.
CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to liquid crystal

Liquid crystal is a state of matter in addition to the solid crystalline and liquid phases. Liquid crystal exhibits intermediate phases in which the molecules flow like liquids yet possess some physical properties characteristic of crystal [14]. In other word, it is known as mesophases. The classification of the mesophases is based on the positional and orientation order of the molecules. The molecules of the liquid crystals are position in a lattice and orientated in a specific directions. One can classify liquid crystal in accordance with the physical parameters controlling the existence of the liquid crystalline phases.

The first liquid crystal was discovered in 1888 by an Austrian botanical physiologist Fredrich Reinitzer (1865-1927) that accidentally observed that cholesteryl benzoate changed into a cloudy liquid at 145.5 °C and melted again into a clear liquid at 178.5 °C. This material has two melting points and it is quite different from the three states of matter (solid, liquid and gas) recognised at that time. After identifying this phenomenon, Reinitzer asked help from a physicist, Otto Lehmann for further investigation. Lehmann examined the intermediate cloudy fluid and reported seeing crystallites and named the new substance as ‘liquid crystal’ (LC). Liquid crystal molecules are often aromatic and it is attached to two substituent groups. Liquid crystals that contain benzene rings are referred to as benzene derivatives [15].

Figure 2.1 shows the basic structure of a liquid crystal molecule of 4-methoxybenzylidene-4’-n-butylaniline (MBBA) which can be an example of liquid crystal molecule that contain two benzene rings as the basic structure [15].
2.2 Types of liquid crystal

Considering the geometrical structure of the molecules, the liquid crystal can be grouped into several types. There are two types of liquid crystal; the thermotropic and lyotropic liquid crystals. These materials exhibit liquid crystalline properties as a function of different physical parameters and environments. Different types of liquid crystals have different physical properties and application. There were two processes to enable the transition of the mesophases; one could be altered by thermal processes and the other by the influence of solvent [17]. For example, thermotropic liquid crystal change phases with temperature and are often composed of a single type of molecule whereas lyotropic liquid crystals are obtained when appropriate concentration of a material is dissolved in a solvent such as water.

2.2.1 Thermotropic liquid crystal

This type of liquid crystal changes its orientation and positional as a function of temperature. From the form of anisotropic phase, the crystal changes into isotropic phases gradually as the temperature increase. In isotropic phase, the physical properties are the same in all directions, while the physical properties for anisotropic phase are different between one direction and another. By definition, anisotropic means the physical properties of the phases are different and depending on the direction and orientation of the component molecules. Each molecule in thermotropic liquid crystal is oriented in a long range order. Thermotropic liquid crystal can be divided into three mesophases; nematic, cholesteric and smectic phases.
2.2.1.1 Nematic phases

The simplest and least-ordered phase of liquid crystals is the nematic phase (N) where rod-like molecules directed on average in a particular direction which is known as the director, \( n \) [17]. The molecules possess a high degree of long-range order with their long axes approximately parallel, but without the distinct layers of the smectic crystal [15]. The molecules in the nematic phase are arranged parallel to each other and slide past each other.

2.2.1.2 Cholesteric phases

Cholesterol derivatives are the most common chemical compounds that exhibit the cholesteric and it is also known as chiral nematic phases, this is because of the first thermotropic liquid crystalline materials exhibiting this phase were cholesterol derivative.

![Figure 2.2: A graphical illustration of cholesteric phase [18].](image)

Chiral means the molecule cannot be transformed into their mirror image by rotations or translations [18]. Chiral cholesteric phase has a helical structure in which the director is twisted along the z-axis normal to the x-axis as shown in Figure 2.2. The cholesteric phase is similar to nematic phase but chiral molecule cause a twist in
the nematic structure which is normal to the long axis. The strong twists in the molecule cause cholesteric have different optical properties from nematic phase. Figure 2.2 shows the structure of cholesteric phase where p is the pitch of helix. Colourful textures are observed due to the selective colour reflection from the mesogens that are lying in planes of helical structures with different pitch length. The planar and homeotropic orientations of the cholesteric liquid crystal mesogens portray different textures. The colour of the textures often appears dark with very low light reflectivity in the crossed polariser when the mesogens oriented close to the homeotropic alignment.

Figure 2.3: Types of cholesteric derivatives (a) Cholesteryl pelargonate, (b) Cholesteryl chloride, and (c) Cholesteryl oleyl carbonate at 10× magnification. (Scale bar: 25 µm)

Some common cholesteric liquid crystals are cholesteryl chloride, cholesteryl pelargonate and cholesteryl oleyl carbonate. These cholesteric liquid crystals reversibly change colour as the temperature changes. Figure 2.3 shows the birefringence properties of the cholesteric liquid crystal.
2.2.1.3 Smectic phase

The second common liquid crystal phase is the smectic phase, where the molecules form single layer of thickness approximate one molecular length as shown in Figure 2.4 [17]. As the temperature increase, the liquid crystal from crystal phase change to smectic C, smectic A, nematic, and isotropic phase. The smectic C phase (SmC) where the director, n, at some angle with respect to the layer normal, z. The smectic A phase (SmA) has the directors n orientates in the same direction with respect to the layer normal, z.

![Smectic phases](image)

Figure 2.4: Standard thermotropic liquid crystal phases arranged from left to right in order of increasing temperature [17].

Smectic and nematic liquid crystal phases are subjected to changes in temperature which they can change their form and their light transmission properties splitting a beam of light into two polarised components (ordinary and extraordinary) to produce the phenomenon of double refraction [15]. Due to the ability of the liquid crystal in responding to magnetic field they are found useful in liquid crystal display (LCD) which is one of the applications of thermotropic liquid crystal [15].

2.2.2 Lyotropic liquid crystal

Lyotropic liquid crystals (LLC) are forms from dissolution surfactants in a solvent such as water [19]. An example of the LLC systems is a fluid formed by water and amphiphilic molecules whose constituent molecules are formed from polar head
group which attracts water and a nonpolar chain that avoids water [18]. The hydrophilic group interacts strongly with water and the hydrophobic group is water insoluble such as soaps and detergents [17]. The phase changes of lyotropic liquid crystal are determined by the concentration of the solute material in a solvent.

![Diagram showing the aggregation of amphiphiles into micelles and then into lyotropic liquid phases as a function of amphiphile concentration and of temperature producing micellar cubic phase (l1), hexagonal phase (H1), bi-continuous cubic phases (V1) and lamellar phases (Lα) [20].](image)

Lyotropic liquid crystal has an axial ratio of less than 15 and made up of molecules larger than the thermotropic liquid crystal [21]. This type of liquid crystal are mostly found in soap, food and living things such as cell membranes [14]. Figure 2.5 shows the relationship between the liquid crystalline phases of surfactants (Surface Active Agents) and its composition concentration is expressed in a phase diagram as a function of temperature. At 30-40wt% of amphiphile concentration, the micellar cubic phase (l1) will be formed. Lyotropic liquid crystal turns into a cubic lattice when the concentration of amphiphiles get to 60% while remaining in the isotropic phase. As the amphiphile concentration increase, cylindrical shaped that is normal hexagonal phase (H1) was formed from the micelles in the cubic lattice. A complex bicontinuous (V1) was formed at a very narrow range of concentrations.
Following by high concentration of amphiphiles, the lamellar phase (Lα) in which the amphiphile molecules are arranged in bilayers and each bilayer is separated by water molecules.

2.3 Application of liquid crystal in biosensing

In recent years, liquid crystals were discovered with their potential in biosensing. Liquid crystals were applied as biosensors because they provide label free observation of biological phenomenon. Among the applications of liquid crystal in biosensing include the detection of distortion in bulk of lyotropic liquid crystals due to the presence of immune complexes [22]. A study also found that a liquid crystal can be used to discriminate fluorescence signals on a biosensor array using liquid crystal pixel array (LCPA) [23]. Moreover, nematic liquid crystal were found to detect and amplifies the presence of immune complexes [24] and used as protein and deoxyribonucleic acid (DNA) separators [25].

The LCD based detection of protease for example trypsin, elastase and thermolysin that cleaved off the Fluorenylemthylxycarbonyl (Fmoc) peptide backing by a thermolysinpolyethylene glycol acrylamide (PEGA) hydrogel support and specific detection or protease function [26]. Previous study also found that lyotropic liquid crystal was used as biosensor for the compatibility of lyotropic liquid crystal with virus. Most of the technique exploited the optical properties of the liquid crystal in sensing changes in biological interactions [27]. Furthermore, bacterial detection also one of the application use liquid crystal as biosensor. Research found that, use of liquid crystal provided infectious disease diagnostics in clinical specimen [28].

Application of liquid crystal by using 4-cyano-4’-pentylbiphenyl (5CB) in biosensor also had been developed for urea detection in human body [29] and glucose biosensor [30]. In addition, by using nematic liquid crystal, 5CB, a novel highly-sensitive liquid crystal (LC) biosensing approach based on target-triggering DNA dendrimers (branched molecules) was developed for the detection of p53 mutation gene segment at the LC aqueous interface [31]. Recently, cholesteryl ester liquid crystal (CELC) was discovered for its new application in detecting single cell traction forces [4]. Table 2.1 shows the application of liquid crystals as biosensors.
CELC was found to support cell adhesion and response locally to cell exerted forces because of its compact molecular arrangements. As a biosensor, liquid crystals are very often used in the presence of cell culture media or interact with a fluidic environment. In this environment, amphillic molecules of liquid crystals could

<table>
<thead>
<tr>
<th>Types of Liquid Crystal</th>
<th>Compound</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyotropic liquid crystal</td>
<td>Disodium cromoglycate (DSCG), water</td>
<td>Detecting distortion in bulk of lyotropic liquid crystals due to the presence of immune complexes.</td>
<td>[22]</td>
</tr>
<tr>
<td>Liquid crystal pixel array (LCPA)</td>
<td>LCPA device from Ferroelectric liquid crystal (FLQ devices (part c LV-1300P) and the FLC driver (part LV-1300P)</td>
<td>Discriminate fluorescence signals on a biosensor array.</td>
<td>[23]</td>
</tr>
<tr>
<td>Nematic liquid crystal</td>
<td>Disodium cromoglycate (DSCG)</td>
<td>Detect and amplifies the presence of immune complexes.</td>
<td>[24]</td>
</tr>
<tr>
<td>Nematic liquid crystal</td>
<td>5CB</td>
<td>Monitor the presence of a targeted biological species for rapid screening of solution conditions leading to crystallization of proteins at interfaces.</td>
<td>[26]</td>
</tr>
<tr>
<td>Lyotropic liquid crystal</td>
<td>Tetradecyldimethyl-amineoxide C_{14}AO, Decanol, water.</td>
<td>The compatibility of LLC with virus.</td>
<td>[32]</td>
</tr>
<tr>
<td>Nematic liquid crystal</td>
<td>5CB</td>
<td>Virus detection.</td>
<td>[27]</td>
</tr>
<tr>
<td>Nematic liquid crystal</td>
<td>5CB</td>
<td>Screening the urea level in the human body.</td>
<td>[29]</td>
</tr>
<tr>
<td>Nematic liquid crystal</td>
<td>5CB</td>
<td>Screening glucose in the human body.</td>
<td>[30]</td>
</tr>
<tr>
<td>Nematic liquid crystal</td>
<td>5CB</td>
<td>Signal-enhanced LC biosensing method based on target-triggering DNA dendrimers for the label-free and sensitive detection of p53 mutation gene segment.</td>
<td>[31]</td>
</tr>
<tr>
<td>Cholesteric ester liquid crystal</td>
<td>Cholesteryl pelargonate C_{36}H_{62}O_2, Cholesteryl chloride C_{27}H_{45}Cl, Cholesteryl oleyl carbonate C_{46}H_{80}O_3.</td>
<td>Detecting single cell traction forces.</td>
<td>[4]</td>
</tr>
</tbody>
</table>
reoriented due to the interaction with the water molecules. Therefore, it is important to investigate the surface tension of the CELC.

2.4 Human keratinocytes (HaCaTs)

Human keratinocytes (HaCaTs) cells were used in this project because of long term aim such as for pharmacology study.

Figure 2.6: Layer of skin at epidermis consists of keratinocytes.

Figure 2.6 shows the layer of skin consist of keratinocytes. This cell is one of the major cell types from the epidermis layer of a skin which is at outermost layer of skin. At the epidermis layer, stratum corneum is the outermost layer of the skin serves as waterproof barriers of the epidermis protects from dirt. Selected keratinocytes is at stratum garnulosum. Stratum spinosum is the layer where keratinocytes become larger, flatter and contain less water as they travel to the surface of the skin. These cells ultimately flatten and lose their nuclei, thereby forming the stratum corneum and eventually replacing select cells that migrate to the skin surface and die [33].
2.5 Cellular adhesion

Multicellular organisms such as keratinocytes require adhesion for cells to adhere to each other and the extracellular matrix. The binding of a cell to an inanimate surface, cell-cell or the extracellular matrix are called cellular adhesions [34]. Adhesion occurs from the action of proteins such as integrins which interact with ligands including extracellular matrix. This process participates in cell adhesions in a large number of physiologically important processes such as wound healing [35]. Figure 2.7 shows schematic diagram of cell adhesion in order to understand the biological role of adhesion junctions.

Figure 2.7: A schematic diagram of cellular adhesion.

Cell adhesion is mediated by cell junctions such as desmosomes, adherens, tight and gap junctions. Desmosomes (bond) is the intermediate filaments anchor at the extracellular junctions, whilst hemidesmosome (half) have a different function which is to connect the intermediate filaments of a cell to the extracellular matrix that is a substances secreted by cells lying outside the cell membrane [36]. They can be found in skin such as muscle cells. Adherens junctions are microfilaments anchor the plaque that occurs under the membrane of each cell. It is a complex proteins
found at the sites of cell-cell adhesion [37]. Transmembrane proteins, calcium
dependent, mediate cell to cell attachment at the adherens junctions.

Tight junctions or zonula occludens are formed from membrane proteins such
as occluding, claudins and e-cadherin that strongly couple the adjacent cell
membranes [37]. Gap junctions are formed from membrane spanning proteins called
connexins [38]. Each gap junction formed from six connexins molecules. It allows
direct communication between cells such as open and closed, formed from the
channel that passes through the membrane of both cells. They are found in heart
muscle, smooth muscle electrical and chemical integration as a single functional unit
and also in embryonic development. Focal adhesions are the link at the outside of the
extracellular matrix through transmembrane proteins (integrins) with the cell
cytoskeleton (actin microfilaments). They mechanically bind the cell membrane to
the extracellular matrix via specific transmembrane receptors.

2.6 Surface tension measuring technique

The study of surface tension has wide application in applied surface science.
Adhesion and morphological of cells mostly depend on the wettability or surface
energy of a biomaterial [39].

Surface tension is related to the surface energy of a surface. Surface tension,
γ, is the force, $F$, per unit length, $L$, (equation (2.1)) that must be applied parallel to
the surface so as to counterbalance the net inward pull and has the units of dyne/cm
or mN/m.

$$\gamma = \frac{F}{L}$$  (2.1)

Greater surface tension reflects higher intermolecular force of attraction, thus
increase in hydrogen bonds induced increase in surface tension. Many techniques
used in finding surface tension of biomaterials have been produced. In this work,
surface tension of CELC was studied by using different techniques such as contact
angle, pendant drop and maximum bubble pressure methods.
2.6.1 Contact angle measurement

Contact angle measurement is often used to evaluate the surface and liquid cleanliness and the effects of surface treatments developed as a part of fundamental research in surface science as well as for industrial applications [40]. A contact angle is the macroscopic representation of microscopic phenomena such as surface roughness, surface energies of the materials involved and surface coatings play a role in the wettability of a material. By definition, a contact angle is the interior angle formed by the substrate being used and the tangent to the drop interface at the apparent intersection of all three interfaces which is called contact line. A static contact angle on a flat surface is defined by Young’s equation, which is related to interfacial surface tension between solid, vapour and liquid.

![Diagram of contact angle measurement](image)

**Figure 2.8:** Contact angle measurement for a droplet of liquid.

The Young’s equation is essentially a force which balanced in the horizontal direction. The contact angle may also be directly determined by the ratio of interfacial surface tension of the unknown interfacial surface tensions using equation (2.2).

$$\cos \theta = \frac{\gamma_{sv} - \gamma_{sl}}{\gamma_{lv}}$$  \hspace{1cm} (2.2)

where, $\gamma_{sv}$, $\gamma_{sl}$ and $\gamma_{lv}$ are the interfacial force of surface and vapour, interfacial force of liquid and surface, interfacial force of liquid and vapour, respectively. $\gamma_{lv}$ and $\cos \theta$ can be determined from the experiment. $\theta$ is the contact angle formed by
the substrate being used and the tangent to the drop interface at the apparent
intersection of the three phase boundary (solid, liquid and vapour). This intersection
is called contact line. Fox-Zisman method was applied to determine the critical surface
energy ($\gamma_c$). According to Zisman, the value of $\gamma_c$ of a solid is equal to the value of $\gamma_l$ of
a liquid being in contact with this solid and for which the contact angle is zero [41].
Based on the theory, $\gamma_c$ value consists of the contact angle measurements for the
studied solid and liquids of a homologous series of compounds.

In Figure 2.8, it shows the contact angle measurement where a liquid was
placed on top of a surface of a solid. It is assumed that the liquid does not react with
the solid and that the solid surface is smooth. In contact angle measurement,
selecting the suitable probe liquids to interface with a surface is an important part.
One must ensure that the liquid does not react or swell with the surfaces. Hence, it
can be considered a viable probe liquid for a dynamic contact angle. Different probe
liquids formed different contact angles.

Using the value of the $\cos \theta$ and the known interfacial tension of each probe
liquid, Fox-Zisman graph can be plotted corresponding to the cosine values of the
contact angle at the y-axis and surface tension of different probe liquids at the x-axis.
The intersection point at which the best fit linear regression line of the data set
intercept with $\cos \theta = 1$ indicates the critical surface tension [42]. Selecting several
suitable probe liquids to interface with a surface is an important decision for contact
angle measurement. Fox-Zisman theory suggested the use of at least four probe
liquids in order to obtain enough data points for plotting the Fox-zisman graph
[43]. A liquid can be considered a viable probe liquid for a dynamic contact angle
measurement if it does not swell or react with the solid surface.

Table 2.2: Probe liquids and their interfacial tension.

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Interfacial tension (mN/m)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropyl alcohol (IPA)</td>
<td>21.7</td>
<td>[44]</td>
</tr>
<tr>
<td>Acetone</td>
<td>23.0</td>
<td>[44]</td>
</tr>
<tr>
<td>Methyl-methacrylate (MMA)</td>
<td>30.6</td>
<td>[45]</td>
</tr>
<tr>
<td>Mineral Oil</td>
<td>35</td>
<td>[46]</td>
</tr>
<tr>
<td>Dimethyl sulfoxide (DMSO)</td>
<td>42.90</td>
<td>[47]</td>
</tr>
<tr>
<td>Ethylene glycol (EG)</td>
<td>47.3</td>
<td>[47]</td>
</tr>
<tr>
<td>Glycerol</td>
<td>64</td>
<td>[44]</td>
</tr>
<tr>
<td>Distilled water</td>
<td>72.8</td>
<td>[44]</td>
</tr>
</tbody>
</table>
For selecting the probe liquid, using a syringe, a drop of known volume of liquid is dropped to a test surface and observe what happened to the droplet. If it is absorbed into the solid or completely spreads out across the surface, the probe liquid is not suitable to be used. This is to make sure that no additional forces and thus affects the contact angle calculation. Polar liquid is selected as probe liquid for this test. Some standard test liquid that can be used as probe liquids and their interfacial tensions are shown in Table 2.2. They are selected in such a way that linear regression line on the cos θ versus surface tension graph can be obtained over a wider range of surface tension.

### 2.6.2 Bubble pressure

Another technique for determining surface tension is bubble pressure method. A simple experiment apparatus from [48] was propose in finding surface tension using bubble method based on the Laplace Young equation [49]. While, in [50], bubble pressure method was used to measure the surface tension of smectic liquid crystal. Figure 2.9 shows the apparatus for measuring surface tension using bubble pressure method.

![Apparatus for surface tension measurement using bubble pressure](image)

*Figure 2.9: Apparatus for surface tension measurement using bubble pressure [50].*
From Figure 2.9, two capillaries with same diameter were used in order to reduce the errors in pressure measurement with differing surface tension using different materials drawn on both openings. $R_1$ and $R_2$ were referred to the radius of two inflate materials respectively. After that, the materials were deflected to a bubble by injecting the syringe. Images were recorded by a video camera perpendicular to the capillary axes. A sensitive pressure gauge was used to determine the pressure differences. However, this method also can be done using single capillary.

Surface tension can be measured using the straightforward Young Laplace as in equation (2.3) [48]:

$$p = \frac{4\sigma}{R}$$  \hspace{1cm} (2.3)

where $p$ is inner excess pressure, $R$ is the radius of a spherical bubble and $\sigma$ is the surface tension [51]. This method provides a direct simple access to $\sigma$ and also suitable for study of small surface tension differences.

### 2.6.3 Pendant drop

The apparatus for finding surface or interfacial tension by pendant drop image analysis through video imaging digitisation and numerical curve-fitting, VCA Optima, developed from AST Products, INC is as shown in Figure 2.10.

![Figure 2.10: A pendant drop system from AST Products, INC.](image)
This system applies Laplace’s equation of capillarity in order to find the value of surface tension. This pendant drop apparatus consist of illuminating system for improving image quality, viewing system to visualise the drop image and data acquisition system to find the interfacial tension from the pendant drop profile. From the image taken, extraction of the drop contour to determine the radius of curvature at the apex is necessary for the calculation of interfacial tension. Next, shape comparison between the theoretical and experimental drop to get interfacial tension value. The shape of the drop hanging on the needle is determined from the balance of force which include the surface tension of that liquid. The values of surface tension of unknown samples are automatically generated.

Based from Laplace’s equation, the equation of Bashforth and Adam [52] relates the drop profile to the interfacial tension through a nonlinear differential equation which is given in equation (2.4) and (2.5).

\[
\frac{1}{R_1} + \frac{\sin \Phi}{x} = -\beta \frac{z}{a} + 2 \tag{2.4}
\]

where \(\beta\), the shape factor of the drop is given by

\[
\beta = \frac{a^2 g \Delta \rho}{\gamma} \tag{2.5}
\]

where \(\Delta \rho\) is the difference between the densities of the two liquids in contact. For example, the density difference between air and liquid. While, \(g\) is the gravitational constant, \(\gamma\) is the surface or interfacial tension, \(a\) is the radius of curvature at the apex of the drop, \(x, z\) and \(\Phi\) are the defines coordinates and \(R_1\) is the radius of curvature at the point with coordinates \((x,z)\), as shown in Figure 2.11.
2.6.4 Surface tension of liquid crystal

The research by [50] used bubble pressure method to measure the surface tension of smectic liquid crystal (8CB) at room temperature, 25 °C. The surface tension determined was 27.8 mN/m. While, from [53], surface tension measured for 5CB liquid crystal using pendant drop method at temperature dependence is 16.22 mN/m. Table 2.3 summarises the surface tensions value of 5CB and 8CB liquid crystals using these two methods.

Table 2.3: Surface tension values for liquid crystal.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Types of liquid crystal</th>
<th>Temperature, °C</th>
<th>Surface tension, mN/m</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bubble pressure</td>
<td>8CB</td>
<td>25</td>
<td>27.8</td>
<td>[50]</td>
</tr>
<tr>
<td>Pendant drop</td>
<td>5CB</td>
<td>-15 to 10</td>
<td>16.22</td>
<td>[53]</td>
</tr>
</tbody>
</table>
2.7 Spectrophotometer

Spectrum was measured using a spectrometer. From the spectrophotometer, a graph of intensity as a function of wavelength was shown. Optical Emission Spectroscopy (OES) is one of the spectrometers that shows the intensity of light.

![Diagram of light transmission and absorption](image)

Figure 2.12: Light transmitted or reflected through a sample.

Figure 2.12 shows the block diagram of a spectrophotometer where incident light beam can be absorbed, transmitted, and reflected. Many compounds absorb ultraviolet (UV) or visible (Vis) light. Based on Beer’s Law, the transmittance of any sample at wave number is given by the ratio of the radiant power emerging from the rear face of the sample at the wave number, I, to the power of the radiation at the front face of the sample I_o [54].

Using Beer’s Law, the percentage of light transmitted can be measured using equation (2.6):

\[
\%T = \frac{I}{I_o} \times 100 \tag{2.6}
\]

Absorbance provides value of intensity of a light. Thus, if all the light passes through a sample without any absorption, then the absorbance is zero and gives 100% of transmittance.
2.8 Differential scanning calorimetry (DSC)

A Differential Scanning Calorimetry (DSC) measures the difference in heat flow rate between a sample and inert reference as a function of time and temperature. DSC provides information about the physical and chemical changes that involve endothermic (absorption of heat) or exothermic (release of heat) activities from a sample [55].

![Schematic DSC heating curve of a semi-crystalline polymer.](image)

Figure 2.13: Schematic DSC heating curve of a semi-crystalline polymer.

The results of glass transition (Tg), melting and boiling points (Tm), and evaporation temperature can be found from endothermic heat flows into the sample. While results of exothermic heat flow out of the sample provides, crystallization time and temperature, percent crystallinity, rate and degree of cure, and oxidation or thermal stability. Figure 2.13 shows typical DSC heating curve of a semi-crystalline polymer with glass transition, exothermic recrystallization, melting endothermic and exothermic decomposition.

2.9 X-ray diffractometer (XRD)

The laboratory used in this project is equipped with a PANalytical X’Pert Powder X-Ray Diffraction (XRD) machine (Figure 2.14). This machine operates with a power of 3kW at 40 kV and 40 mA, with a cooper X-ray tube, which has a characteristic
REFERENCES


