BACTERIAL CELLULOSE-CHITOSAN MEMBRANE
GRAFTED WITH THEOPHYLLINE-IMPRINTED COPOLYMER
BY FREE RADICAL COPOLYMERIZATION

MOHD REDZA BIN ABD RAHMAN

UNIVERSITI TEKNOLOGI MALAYSIA
BACTERIAL CELLULOSE-CHITOSAN MEMBRANE
GRAFTED WITH THEOPHYLLINE-IMPRINTED COPOLYMER
BY FREE RADICAL COPOLYMERIZATION

MOHD REDZA BIN ABD RAHMAN

A thesis submitted in fulfilment of the requirements for the award of the degree of Master of Engineering (Bioprocess)

Faculty of Chemical Engineering
Universiti Teknologi Malaysia

FEBRUARY 2012
To those who have inspired me
ACKNOWLEDGEMENTS

First and foremost I offer my deepest gratitude to my respectful supervisor, Assoc. Prof. Dr. Ida Idayu Muhamad, who has supported me throughout my thesis with her patience and knowledge.

My sincerest gratitude goes to my beloved sister and mentor; Che Aishah who has helped me regains some sort of fitness: healthy body, healthy mind. I am grateful to come across several life-long best friends. Thanks Apaziah, Seta, Sa, Hussein and others who have provided assistance at various occasions. My sincerest appreciation also extends to my future wife, Izzaidia for her kindness, encouragement and continued support.

I am grateful to all my friends in Food and Biomaterial Engineering Research Group especially Zuhaili, Mrs. Eraricar, Nozieana and Iryatie for their continued moral support thereafter. I would like to express my acknowledgement to the Department of Bioprocess Engineering, UTM has provided the support and equipment I have needed to produce and complete my thesis and MOSTI has funded this innovative and invaluable research.

Furthermore, I am forever indebted to my parents and family for their understanding, endless patience and encouragement when it was most required.

Last but not least, thanks be to Allah Almighty for my life through all tests in the past three years. You have made my life more bountiful. May your name be exalted, honoured and glorified.
ABSTRACT

In this research, benzyl diethyldithiocarbamate was immobilized on a bacterial cellulose-chitosan membrane via a silane coupler. This treated membrane was grafted with theophylline-imprinted copolymer of methacrylic acid and ethylene glycol dimethacrylate by ultraviolet irradiation. The highest degree of grafting obtained was 0.3334% for \( r \) (weight ratio of monomers to bacterial cellulose-chitosan membrane) equal to 3.244 in mmol/ml. The molecularly imprinted polymer-bacterial cellulose-chitosan membrane was prepared by using 0.5% chitosan solution containing 15.0% polyethylene glycol and evaporating the solution for 2.5 hours after coating at room temperature. The relative flux of 3.69 L/m².h at 12.5 bar was obtained. The average pore diameter was 135 Å in dry state and 404 Å in wet state. Physical properties and morphology of the molecularly imprinted membrane were examined. The chitosan and polyethylene glycol contents in the chitosan solution had a significant effect on porosity of the membrane and the flow rate of water through the membrane. A relatively large flow rate through the membrane with a stable coating of chitosan membrane was observed at optimized evaporation time. The tensile strength provided by the synthesized membrane was larger than the plain bacterial cellulose support, in both wet and dry states.
ABSTRAK

Dalam penyelidikan ini, benzil diethyldithiokarbamat disekat-gerak pada membran selulosa bakteria – kitosan melalui satu silana penggandring. Membran terawat dilekatkan dengan teofilina kopolimer tertera asid metakrilik dan etilena glikol dimetakrilat oleh penyinaran ultralembayung. Kadar cantuman tertinggi yang diperolehi ialah 0.3334% untuk r (nisbah berat monomer-monomer terhadap membran kitosan selulosa bakteria) bersamaan 3.244 dalam mmol / ml. Fluks banding untuk 3.69 L/m².jam pada 12.5 bar diperolehi. Purata diameter pori membran ialah 135 Å pada keadaan kering dan 404 Å pada keadaan basah. Membran molekul polimer-bakteria selulosa-kitosan tercetak yang disediakan dengan menggunakan 0.5% larutan kitosan yang mengandungi 15% polietilena glikol dan larutan itu disejatkan selama 2.5 jam pada tekanan 12.5 bar selepas dilapiskan pada suhu bilik. Sifat fizikal membran diuji dan morfologi membran molekul tercetak diperiksa. Kandungan kitosan dan polietilena glikol di dalam larutan kitosan mempunyai kesan terhadap keporosan membran dan kadar aliran air menembusi membran. Fluks banding yang besar dapat dilihat pada membran di mana salutan kitosan yang stabil disalut pada masa penyegatan yang optimum. Membran molekul tercetak mempunyai kekuatan tegangan lebih besar dalam keadaan kering dan basah berbanding membran selulosa bakteria kosong.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE</td>
<td></td>
<td>i</td>
</tr>
<tr>
<td>DECLARATION</td>
<td></td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td></td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td></td>
<td>iv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td></td>
<td>v</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td></td>
<td>vi</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td></td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td></td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td></td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF SYMBOLS</td>
<td></td>
<td>xviii</td>
</tr>
</tbody>
</table>

1 INTRODUCTION 1
1.1 Research Background 1
1.2 Research Objectives 4
1.3 Research Scopes 4

2 LITERATURE REVIEW 6
2.1 Molecularly Imprinting Polymer 6
   2.1.1 A Brief History of Imprinting 6
   2.1.2 Free Radical Polymerization 7
   2.1.3 Free Radical Copolymerization 9
2.1.4 Cross-linked Polymers  

2.2 MIP Synthesis  
2.2.1 The Basic Strategy  
2.2.2 Template  
2.2.3 Functional Monomer  
2.2.4 Cross Linkers  
2.2.5 Solvents  
2.2.6 Initiators  

2.3 Category of MIP  

2.4 Evaluation of Template–Monomer Interactions  
2.4.1 Fourier Transform Infrared Spectroscopy  
2.4.2 Ultra Violet Spectroscopy  
2.4.3 Computer Simulation  
2.4.4 Surface Area and Porosity  
2.4.5 Spectroscopic Analysis Techniques  
2.4.6 MIP Swelling  

2.5 Application of MIP  
2.5.1 Chemical Sensor  
2.5.2 Robust Food Analysis  
2.5.3 Separation Science  
2.5.4 Controlled Released System  

2.6 Bacterial Cellulose  
2.6.1 Structure of Bacterial Cellulose  
2.6.2 Chemical Analysis and Detection  
2.6.3 Occurrence  
2.6.4 Physiological Function  
2.6.5 Biosynthesis of Bacterial cellulose  
2.6.6 Biotechnological Production  
2.6.7 Properties of Bacterial Cellulose  
2.6.8 Application of Bacterial Cellulose
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7</td>
<td>Chitosan</td>
<td>56</td>
</tr>
<tr>
<td>2.7.1</td>
<td>Membrane Properties</td>
<td>59</td>
</tr>
<tr>
<td>2.7.2</td>
<td>Molecular Weight and Methods of Characterization</td>
<td>60</td>
</tr>
<tr>
<td>2.7.3</td>
<td>Application of Chitosan</td>
<td>61</td>
</tr>
<tr>
<td>2.8</td>
<td>Novel Separation Membranes</td>
<td>63</td>
</tr>
<tr>
<td>2.8.1</td>
<td>Molecularily Imprinted Membrane</td>
<td>64</td>
</tr>
<tr>
<td>2.8.2</td>
<td>Combination of Novel MIP Formats with Membrane Separations</td>
<td>66</td>
</tr>
<tr>
<td>2.9</td>
<td>Bacterial Cellulose-Chitosan Membrane</td>
<td>67</td>
</tr>
<tr>
<td>2.10</td>
<td>Grafting of MIP on Membrane</td>
<td>70</td>
</tr>
</tbody>
</table>

3 METHODOLOGY

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Material</td>
<td>73</td>
</tr>
<tr>
<td>3.2</td>
<td>Membrane Biosynthesis</td>
<td>74</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Chemicals and Reagents</td>
<td>74</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Preparation of the Bacterial Cellulose Membrane</td>
<td>75</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Preparation of the BCC Membrane</td>
<td>75</td>
</tr>
<tr>
<td>3.2.4</td>
<td>Preparation of the MIP-BCC Membrane</td>
<td>76</td>
</tr>
<tr>
<td>3.3</td>
<td>Characterization Methodology</td>
<td>78</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Physical Properties</td>
<td>78</td>
</tr>
<tr>
<td>3.3.1.1</td>
<td>Porosity Measurement</td>
<td>78</td>
</tr>
<tr>
<td>3.3.1.2</td>
<td>Mechanical Properties</td>
<td>79</td>
</tr>
<tr>
<td>3.3.1.3</td>
<td>Surface Morphology and Cross-section Analysis</td>
<td>80</td>
</tr>
<tr>
<td>3.3.1.4</td>
<td>Atomic Force Microscopy</td>
<td>80</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Chemical Properties</td>
<td>81</td>
</tr>
</tbody>
</table>
3.3.2.1 Fourier Transform Infrared Spectroscopy

3.4 Analysis Methodology
3.4.1 Flow Rates of Pure Water Measurement
3.4.2 Optimization of Membrane
3.4.3 The Weight ratio of Monomer
3.4.4 Degree of Grafting
3.4.5 Degree of Swelling
3.4.6 Evaluation of Living Functionality on Synthesized Copolymer
3.4.7 Determination of Membrane
3.4.7.1 Dextran Solutions
3.4.7.2 Size Exclusion Chromatography

4. RESULTS AND DISCUSSION
4.1 Characterization of Membranes
4.1.1 Surface Morphology
4.1.2 Atomic Force Microscopy Analysis
4.1.3 FTIR Analysis
4.1.3.1 Bacterial Cellulose – Chitosan Membrane
4.1.3.2 MIP-Bacterial Cellulose - Chitosan Membrane
4.1.4 Mechanical Property
4.1.5 Porosity
4.2 Optimization of Membranes
4.2.1 Effect of Porogen (PEG) Content in Chitosan solution
4.2.2 Effect of Chitosan Concentration
4.2.3 Effect of Evaporation Time 102
4.3 Characterization of Molecularly Imprinted Membrane
  4.3.1 Living nature of synthesized copolymer 104
  4.3.2 Degree of Grafting 107
  4.3.3 Degree of Swelling 108
4.4 Separation Properties 110
  4.4.1 MIP Membrane Permeability 110
  4.4.2 MIP Membrane Permselectivity 111

5 CONCLUSION AND RECOMMENDATION 113
  5.1 Conclusion 113
  5.2 Recommendations and further works 115

REFERENCES 117
## LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Surface area pose volume and average pose size in MIPs made with EGDMA/MAA monomers using L-phen as template</td>
<td>25</td>
</tr>
<tr>
<td>2.2</td>
<td>Design and application example of molecularly imprinted polymer</td>
<td>35</td>
</tr>
<tr>
<td>2.3</td>
<td>Bacterial cellulose producers</td>
<td>43</td>
</tr>
<tr>
<td>2.4</td>
<td>Properties of bacterial cellulose</td>
<td>52</td>
</tr>
<tr>
<td>2.5</td>
<td>Application of bacterial cellulose</td>
<td>54</td>
</tr>
<tr>
<td>3.1</td>
<td>Materials used in the experiment</td>
<td>76</td>
</tr>
<tr>
<td>3.2</td>
<td>The concentration of dextran solutions</td>
<td>89</td>
</tr>
<tr>
<td>4.1</td>
<td>Average pore size and surface area of the BC, BCC and MIP-BCC analyzed with BET analyzer</td>
<td>102</td>
</tr>
<tr>
<td>4.2</td>
<td>The mass of chitosan coated on the composite membrane</td>
<td>105</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>Conversion of methyl methacrylate monomer by free radical polymerization into poly - (methyl methacrylate)</td>
<td>9</td>
</tr>
<tr>
<td>2.2</td>
<td>Free radical copolymerization of; (a) methyl methacrylate with n-butyl methacrylate, and (b) stilbene and maleic anhydride polymer (a) is a random copolymer whereas polymer (b) is a specially altering copolymer</td>
<td>10</td>
</tr>
<tr>
<td>2.3</td>
<td>Schematic representation showing polymers with different topologies: linear, branched macroscopic, network and microgel</td>
<td>11</td>
</tr>
<tr>
<td>2.4</td>
<td>Schematic representation of the cross-linked polymer network arising from the copolymerization of styrene with p-divinylbenzene</td>
<td>12</td>
</tr>
<tr>
<td>2.5</td>
<td>Schematic representation of the imprinting process</td>
<td>13</td>
</tr>
<tr>
<td>2.6</td>
<td>Structures of templates</td>
<td>14</td>
</tr>
<tr>
<td>2.7</td>
<td>Selection of monomers used in the non-covalent approach</td>
<td>16</td>
</tr>
<tr>
<td>2.8</td>
<td>Selection of cross-linkers used for molecular imprinting</td>
<td>18</td>
</tr>
<tr>
<td>2.9</td>
<td>Chemical structures of selected chemical initiators</td>
<td>20</td>
</tr>
<tr>
<td>2.10</td>
<td>Schematic representation of covalent and non-covalent molecular imprinting procedures</td>
<td>21</td>
</tr>
</tbody>
</table>
2.11 Model of morphology formation that provides the porous network in MIPs 25
2.12 Example of CP/MAS $^{13}$C-NMR Spectra for imprinted polymers formulated an X/M ratio of 4/1, EGDMA/MAA 26
2.13 Schematic representation of the surface imprinting of an enzyme, RNaseA 31
2.14 Schematic model of BC microfibrils (right) drawn in comparison with the ‘fringed micelles’ of PC fibrils 39
2.15 Bacterial cellulose pellicle formed in static culture 40
2.16 Bacterial cellulose pellets in agitated culture 40
2.17 A simplified model for the biosynthetic pathway of cellulose 46
2.18 Assembly of microfibrils by *Acetobacter xylinum* 47
2.19 Production of crude chitosan 59
2.20 Structure of chitin, chitosan and cellulose 60
3.1 Scheme of MIP-grafting onto a bacterial cellulose-chitosan composite membrane by living radical copolymerization 80
3.2 Brunauer-Emmett-Teller (BET) Micromeritics ASAP 2020 surface area analyzer 81
3.3 The INSTRON® 5567 universal testing machine 82
3.4 Schematic diagram showing the operating principles of the AFM in the contact mode 84
3.5 The exploded view of the ultrafiltration apparatus 86
4.1 The FE-SEM of the surface of bacterial cellulose membrane 93
4.2 The FE-SEM of the surface of bacterial cellulose-chitosan membrane 93
4.3 The FE-SEM of the surface of MIP-bacterial cellulose-chitosan membrane 94
4.4 The FE-SEM of the cross-section of bacterial cellulose membrane 94
4.5 The FE-SEM of the cross-section of MIP-bacterial cellulose-chitosan membrane

4.6 The AFM image of MIP-BCC membrane surface

4.7 The AFM image of BCC membrane surface

4.8 The FTIR spectra of BCC membranes in the wave numbers ranging from 2800 to 1200 cm\(^{-1}\)

4.9 The FTIR spectra of BCC membranes in the wave numbers ranging from 1800 to 1500 cm\(^{-1}\)

4.10 The FTIR spectra of the BCC (a) and MIP-BCC (b) membranes

4.11 Tensile strength of the composite membranes in dry and wet states coated with solution of different concentration containing 15% PEG, evaporation time was 2.5 hours.

4.12 Effect of PEG content in chitosan solution on the flow rate of composite membrane. A total of 0.5% chitosan solutions containing different PEG concentration of 15%, 10% and 5%. Evaporation time was 2.5 hours.

4.13 Effect of chitosan concentration on the relationship between flow rate and pressure drop of pure water through the composite membranes. Chitosan solutions contained 15% PEG, evaporation time was 2.5 hours. Chitosan content was 0%, 0.25%, 0.4%, 0.5% and 0.75%.

4.14 Effect of evaporation period on the flow rate. A total of 0.5% chitosan solution contained 15% PEG. Evaporation time (ET) was 1.5, 2.0, 2.5, 3.0 and 4.0 hours.

4.15 The MIP bacterial cellulose-chitosan composite membrane

4.16 The MIP-BCC composite membrane in standard size
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.17</td>
<td>Scheme of MIP-grafting onto a bacterial cellulose - chitosan composite membrane by living radical copolymerization</td>
</tr>
<tr>
<td>4.18</td>
<td>Change in membrane weight by repetition of polymerization (membrane: 5 cm x 5 cm, 20 sheets)</td>
</tr>
<tr>
<td>4.19</td>
<td>The effect of the living radical polymerization on degree of grafting</td>
</tr>
<tr>
<td>4.20</td>
<td>Effect degree of grafting on degree of swelling</td>
</tr>
<tr>
<td>4.21</td>
<td>Effect degree of grafting on water flux</td>
</tr>
<tr>
<td>4.22</td>
<td>Effect of degree of grafting on rejection coefficient of dextran solution. Dextran solutions contained various molecular weights (70, 500 and 2000)</td>
</tr>
</tbody>
</table>
# LIST OF SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>µL</td>
<td>Micro Litre</td>
</tr>
<tr>
<td>µm</td>
<td>Micro Meter</td>
</tr>
<tr>
<td>Å</td>
<td>Angstrom</td>
</tr>
<tr>
<td>A</td>
<td>Area (m²)</td>
</tr>
<tr>
<td>A-BC</td>
<td>Agitated Bacterial Cellulose</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscopy</td>
</tr>
<tr>
<td>ASTM</td>
<td>American Society for Testing and Materials</td>
</tr>
<tr>
<td>BC</td>
<td>Bacterial Cellulose</td>
</tr>
<tr>
<td>BET</td>
<td>Brunauer Emmett Teller</td>
</tr>
<tr>
<td>BSH</td>
<td>Buffered Schamm and Hestrin</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>Calcium Carbonate</td>
</tr>
<tr>
<td>C_b</td>
<td>The Bulk Concentration</td>
</tr>
<tr>
<td>C_p</td>
<td>The Permeate Concentration</td>
</tr>
<tr>
<td>C_feed</td>
<td>The Feed Concentration</td>
</tr>
<tr>
<td>C_filtrate</td>
<td>The Filtrate Concentration</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>Chloride Ion</td>
</tr>
<tr>
<td>C_MAA</td>
<td>The Concentration of Methacrylic Acid Solution</td>
</tr>
<tr>
<td>CBH</td>
<td>Cellbiohydrolase</td>
</tr>
<tr>
<td>CMC</td>
<td>Carboxymethylcellulose</td>
</tr>
<tr>
<td>COOH</td>
<td>Carboxylic Acid Group</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton (g/mol)</td>
</tr>
<tr>
<td>DD</td>
<td>Degree of Deacetylation</td>
</tr>
</tbody>
</table>
DDS - Drug Delivery Systems
DMF - N,N-dimethylformamide
DNA - Deoxyribonucleic Acid
DP - Degree of Polymerization
EC - Endocellulases
EDMA - Ethyleneglycol Dimethacrylate
EDTA - Ethylenediaminetetraacetic Acid
FESEM - Field Emission Scanning Electron Microscopy
FTIR - Fourier Transform Infra Red Spectroscopy
g L⁻¹ - Gram per Litre
g - Gram
g/L - Gram per Liter
GFC - Gel Filtration Chromatography
GPC - Gel Permeation Chromatography
h - Hour
H - Hydrogen
HPLC - High Performance Liquid Chromatography
J - Flux rate (L/m².h)
K - Kelvin
kN - Kilo Newton
kN/m² - Kilo Newton per Area
kV - Kilo Volt
L - Litre
m²/g - Area per Gram
MAA - Methacrylic Acid
MIM - Molecularly Imprinted Membrane
MIP-BCC - Molecularly Imprinted Polymer Bacterial Cellulose Chitosan
ml - Mili Litre
ml/g - Mili Litre per Gram
mm - Mili Meter
mmol/ml - Mili Mol per Mili Litre
MPa - Mega Pascal
MW - Molecular Weight
N - Nitrogen
N₂ - Nitrogen Gas
NaOH - Sodium Hydroxide
NH₂ - Amine Group
nm - Nano Meter
NMR - Nuclear Magnetic Resonance Spectroscopy
O - Oxygen
PC - Plant Cellulose
PEG - Polyethylene Glycol
R - The Ratio of the Heights of the Peaks
r - Weight Ratio of Monomers to the Membrane
RIPP - Recovery, Isolation, Purification and Polishing
RNA - Ribonucleic Acid
RNase A - Ribonuclease A
RNase B - Ribonuclease A
rpm - Revolutions per minute
S-BC - Static Bacterial Cellulose
SDS - Sodium Dodecyl Sulfate
SEC - Size Exclusion Chromatography
SI - System International
SPE - Solid Phase Extraction
UF - Ultrafiltration
UV - Ultraviolet
v/v - Volume per Volume
V_{MAA} - The Volume of MAA Solution
w/v - Weight per Volume
W_d - The Weights of Dried Membranes
W_g - The Weights of Grafted Membrane
W_{membrane} - The weight of bacterial cellulose-chitosan membrane.
W_o - The Weights of Ungrafted Membrane
W_w - The Weights of Wet Membranes
λ_{595} - Wavelength at 595 nm
ρ - Density (kg/m³)
CHAPTER 1

INTRODUCTION

1.1 Research Background

In nature, most biological processes are governed by mechanisms for molecular recognition. These include the immuno response, the ligand–receptor interaction, and enzyme catalysis. They involve such biological hosts as antibodies, enzymes or receptors strongly and specifically binding to a particular molecular structure. A challenge for the contemporary chemists is to develop synthetic receptors with an affinity and specificity approaching that achieved in nature. To this end, many synthetic low molecular weight organic receptors capable of encapsulating reagents have been designed (Hof et al., 2002; Vriezema et al., 2005). The construction of such receptors, however, usually requires complicated multi-step synthesis, which severely limits their large-scale application. Developing other synthetically more accessible receptors is thus highly desirable. Interest in a new class of artificial receptors, molecularly imprinted polymers (MIPs), has increased rapidly in recent years because of their easy preparation, thermal and chemical stability, and highly selective recognition capabilities (Mosbach, 1994; Shea, 1994; Wulff, 2002; Mosbach and Ramstrom, 1996). Nowadays, the molecular imprinting technique has become a straightforward and versatile method for the generation of biomimetic macromolecular receptors. One of the most distinct characteristics of the
molecular imprinting process is its generality, which offers the freedom to prepare receptors for a wide range of templates without appreciably changing the synthetic protocols. It is in this respect, in our opinion, outstanding amongst other non-biological approaches. The binding sites generated during the imprinting process often have an affinity and a selectivity approaching those of antibody antigen systems. MIPs are thus also dubbed “antibody mimics” (Vlatakis et al., 1993). They have much higher chemical and physical stability than such biological entities as antibodies and enzymes. In addition, MIPs show remarkable resistance to extreme pH conditions, organic solvents, metal ions, and autoclave treatment. Such highly appealing physical and chemical characteristics make MIPs very promising candidates for many applications, including chromatographic stationary-phase (Turiel and Martin-Esteban, 2004) and solid-phase separation (Sellergren, 1994; Haginaka, 2004), antibody mimics (biomimetic assays and sensors) (Vlatakis et al., 1993; Kriz et al., 1997; Haupt and Mosbach, 2000; Haupt, 2003), enzymemimics (Ramström and Mosbach, 1999; Wulff, 2002), organic synthesis (Alexander et al., 2003), capillary electrochromatography (Spéigel et al., 2003), and drug delivery (Alvarez-Lorenzo and Concheiro, 2004).

MIPs are applicable in a variety of different configurations. In the past few years molecular imprinting has entered many areas of chemistry, biochemistry and biotechnology. Nowadays polymers imprinted with different templates like drugs, herbicides, sugars, nucleotides, amino acids and protein. MIPs have antibody-like specific binding sites for target molecules (templates). MIPs can be synthesized by conventional radical copolymerization of cross-linking monomers and functional monomers which can form reversible complexes with template molecules (Kempe and Mosbach, 1995). MIPs have been applied in affinity assays, separations and chemical sensors (Kobayashi et al., 2001). In these studies, MIPs are implemented by free radical copolymerization on the bacterial cellulose membrane produced by natural microorganism that has been integrated with chitosan layer and modified with polyethylene glycerol as the porogen.

*Acetobacter xylinum*, a gram-negative bacterium produces cellulose extracellularly. This cellulose is formed as gel-like mass (pellicle) at the surface of
the medium and can be purified by proper chemical treatments. This material has high crystallinity and large surface area and has been attracting attention as a new form of cellulosic material (Shibazaki et al., 1993). When purified pellicle is dried on a flat substrate, a thin translucent cellulose membrane is formed. This membrane is expected to have unique properties because it consists of fine and continuous crystalline microfibrils, not like paper sheets or regenerated cellulose films. One possible application is molecular filtration such as dialysis or ultrafiltration.

Compared with the hydrophobic membranes, cellulose or derived cellulose membranes, hydrophilic in nature, have very low nonspecific binding (Manganaro and Goldberg, 1993). Cellulose fibers are relatively strong, having breaking strengths of up to 1 GN/m² (10 000 MPa). Cellulose membranes have been wide used as dialyzers for hemodialysis and also used as mechanical support of membrane with satisfied mechanical properties for fast protein purification (Hou et al., 1991). On the other hand, regenerated cellulose membranes have been widely used as a dialysis membrane in aqueous systems, where chemical stability and low toxicity of cellulose are preferable properties, especially in applications for labile biological systems (Shibazaki et al., 1993).

However, cellulose membranes offer a poor binding capacity due to crystalline and amorphous regions in their structure; only the hydroxyl groups in the amorphous region and on the surface of the crystalline are available to ligand coupling. Molecularly imprinting polymers is implemented to enhance and improve cellulose’s mechanical and chemical properties.

Recently, chitosan and chitin membranes have been investigated in order to have a high protein binding capacity for protein purification and separation (Zeng et al., 1997). These materials provide an excellent binding capacity because chitosan molecules have both amino and hydroxyl groups that can be used to couple with ligands under mild conditions. But their poor mechanical properties prevented them from being used widely. In order to develop a membrane with good mechanical and chemical properties, these studies propose to make a MIP-bacterial cellulose-chitosan (BCC) membrane which combines the advantages of MIP, cellulose and
chitosan. Both cellulose and Chitosan are biodegradable, natural materials and very abundant on the earth. They also have good blood compatibility (Jia et al., 1999).

1.2 Research Objectives

The objectives of this study are:

i. To develop a membrane of bacterial cellulose-chitosan grafted with theophylline-imprinted copolymer

ii. To characterize its physical and chemical properties of the developed membrane.

1.3 Research Scopes

The scopes of this study include:

i. To evaluate the influence of chitosan, porogen (polyethylene glycerol) contents and evaporation time (ET) on porosity of the bacterial cellulose membrane.

ii. To measure the flux and rejection coefficient of the membrane using pure water and various molecular weights dextran standard solution.

iii. To determine the morphology of the membrane using Field Emission Scanning Electron Microscopy (FESEM), Fourier Transform Infra Red Spectroscopy (FTIR), Atomic Force Microscopy (AFM) and relate it to its performance.
iv. To evaluate weight ratio of monomer, degree of grafting, degree of swelling and living functionality on synthesized copolymer of the developed MIP membrane.
REFERENCES


Baker, R.W. *Membrane Technology and Applications.* England: John Wiley & Sons Ltd. 2004


Hennen, W.J. *Chitosan*. Woodland Publishing. 31; 1996


