

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

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UNIVERSITI TEKNOLOGI MALAYSIA

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To those who have inspired me

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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 pengganding. 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 penyejatan yang optimum. Membran molekul tercetak mempunyai kekuatan tegangan lebih besar dalam keadaan kering dan basah berbanding membran selulosa bakteria kosong.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	TITLE	i
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENTS	iv
	ABSTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLES	xiii
	LIST OF FIGURES	xiv
	LIST OF SYMBOLS	xviii
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	10
2.2	MIP Synthesis	12
2.2.1	The Basic Strategy	12
2.2.2	Template	14
2.2.3	Functional Monomer	15
2.2.4	Cross Linkers	17
2.2.5	Solvents	18
2.2.6	Initiators	19
2.3	Category of MIP	20
2.4	Evaluation of Template–Monomer Interactions	21
2.4.1	Fourier Transform Infrared Spectroscopy	21
2.4.2	Ultra Violet Spectroscopy	22
2.4.3	Computer Simulation	23
2.4.4	Surface Area and Porosity	24
2.4.5	Spectroscopic Analysis Techniques	25
2.4.6	MIP Swelling	27
2.5	Application of MIP	27
2.5.1	Chemical Sensor	32
2.5.2	Robust Food Analysis	33
2.5.3	Separation Science	34
2.5.4	Controlled Released System	35
2.6	Bacterial Cellulose	37
2.6.1	Structure of Bacterial Cellulose	38
2.6.2	Chemical Analysis and Detection	42
2.6.3	Occurrence	43
2.6.4	Physiological Function	44
2.6.5	Biosynthesis of Bacterial cellulose	45
2.6.6	Biotechnological Production	48
2.6.7	Properties of Bacterial Cellulose	48
2.6.8	Application of Bacterial Cellulose	51

2.7	Chitosan	56
2.7.1	Membrane Properties	59
2.7.2	Molecular Weight and Methods of Characterization	60
2.7.3	Application of Chitosan	61
2.8	Novel Separation Membranes	63
2.8.1	Molecularly Imprinted Membrane	64
2.8.2	Combination of Novel MIP Formats with Membrane Separations	66
2.9	Bacterial Cellulose-Chitosan Membrane	67
2.10	Grafting of MIP on Membrane	70
3	METHODOLOGY	73
3.1	Material	73
3.2	Membrane Biosynthesis	74
3.2.1	Chemicals and Reagents	74
3.2.2	Preparation of the Bacterial Cellulose Membrane	75
3.2.3	Preparation of the BCC Membrane	75
3.2.4	Preparation of the MIP-BCC Membrane	76
3.3	Characterization Methodology	78
3.3.1	Physical Properties	78
3.3.1.1	Porosity Measurement	78
3.3.1.2	Mechanical Properties	79
3.3.1.3	Surface Morphology and Cross-section Analysis	80
3.3.1.4	Atomic Force Microscopy	80
3.3.2	Chemical Properties	81

	3.3.2.1 Fourier Transform Infrared Spectroscopy	81
3.4	Analysis Methodology	82
	3.4.1 Flow Rates of Pure Water Measurement	82
	3.4.2 Optimization of Membrane	83
	3.4.3 The Weight ratio of Monomer	84
	3.4.4 Degree of Grafting	84
	3.4.5 Degree of Swelling	85
	3.4.6 Evaluation of Living Functionality on Synthesized Copolymer	85
	3.4.7 Determination of Membrane Permselectivity	86
	3.4.7.1 Dextran Solutions	86
	3.4.7.2 Size Exclusion Chromatography	86
4.	RESULTS AND DISCUSSION	88
4.1	Characterization of Membranes	88
	4.1.1 Surface Morphology	88
	4.1.2 Atomic Force Microscopy Analysis	91
	4.1.3 FTIR Analysis	93
	4.1.3.1 Bacterial Cellulose – Chitosan Membrane	93
	4.1.3.2 MIP-Bacterial Cellulose - Chitosan Membrane	95
	4.1.4 Mechanical Property	96
	4.1.5 Porosity	97
4.2	Optimization of Membranes	99
	4.2.1 Effect of Porogen (PEG) Content in Chitosan solution	99
	4.2.2 Effect of Chitosan Concentration	100

4.2.3	Effect of Evaporation Time	102
4.3	Characterization of Molecularly Imprinted Membrane	104
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 NO.	TITLE	PAGE
2.1	Surface area pore volume and average pore size in MIPs made with EGDMA/MAA monomers using L-phen as template	25
2.2	Design and application example of molecularly imprinted polymer	35
2.3	Bacterial cellulose producers	43
2.4	Properties of bacterial cellulose	52
2.5	Application of bacterial cellulose	54
3.1	Materials used in the experiment	76
3.2	The concentration of dextran solutions	89
4.1	Average pore size and surface area of the BC, BCC and MIP-BCC analyzed with BET analyzer	102
4.2	The mass of chitosan coated on the composite membrane	105

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
2.1	Conversion of methyl methacrylate monomer by free radical polymerization into poly - (methyl methacrylate)	9
2.2	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	10
2.3	Schematic representation showing polymers with different topologies: linear, branched macroscopic, network and microgel	11
2.4	Schematic representation of the cross-linked polymer network arising from the copolymerization of styrene with p-divinylbenzene	12
2.5	Schematic representation of the imprinting process	13
2.6	Structures of templates	14
2.7	Selection of monomers used in the non-covalent approach	16
2.8	Selection of cross-linkers used for molecular imprinting	18
2.9	Chemical structures of selected chemical initiators	20
2.10	Schematic representation of covalent and non-covalent molecular imprinting procedures	21

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 <i>Acetobacter xylinum</i>	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	95
4.6	The AFM image of MIP-BCC membrane surface	96
4.7	The AFM image of BCC membrane surface	96
4.8	The FTIR spectra of BCC membranes in the wave numbers ranging from 2800 to 1200 cm^{-1}	98
4.9	The FTIR spectra of BCC membranes in the wave numbers ranging from 1800 to 1500 cm^{-1}	98
4.10	The FTIR spectra of the BCC (a) and MIP-BCC (b) membranes	99
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.	101
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.	104
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%.	106
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.	107
4.15	The MIP bacterial cellulose-chitosan composite membrane	108
4.16	The MIP-BCC composite membrane in standard size	108

4.17	Scheme of MIP-grafting onto a bacterial cellulose - chitosan composite membrane by living radical copolymerization	110
4.18	Change in membrane weight by repetition of polymerization (membrane: 5 cm x 5cm, 20 sheets)	111
4.19	The effect of the living radical polymerization on degree of grafting	112
4.20	Effect degree of grafting on degree of swelling	113
4.21	Effect degree of grafting on water flux	114
4.22	Effect of degree of grafting on rejection coefficient of dextran solution. Dextran solutions contained various molecular weights (70, 500 and 2000)	116

LIST OF SYMBOLS

%	-	Percentage
°C	-	Degree Celsius
μL	-	Micro Litre
μm	-	Micro Meter
Å	-	Angstrom
A	-	Area (m ²)
A-BC	-	Agitated Bacterial Cellulose
AFM	-	Atomic Force Microscopy
ASTM	-	American Society for Testing and Materials
BC	-	Bacterial Cellulose
BET	-	Brunauer Emmett Teller
BSH	-	Buffered Schamm and Hestrin
C	-	Carbon
CaCO ₃	-	Calcium Carbonate
C_b	-	The Bulk Concentration
C_p	-	The Permeate Concentration
C_{feed}	-	The Feed Concentration
C_{filtrate}	-	The Filtrate Concentration
Cl ⁻	-	Chloride Ion
C_{MAA}	-	The Concentration of Methacrylic Acid Solution
CBH	-	Cellobiohydrolase
CMC	-	Carboxymethylcellulose
COOH	-	Carboxylic Acid Group
Da	-	Dalton (g/mol)
DD	-	Degree of Deacetylation

DDS	-	Drug Delivery Systems
DMF	-	<i>N,N</i> -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 ($\text{L/m}^2 \cdot \text{h}$)
K	-	Kelvin
kN	-	Kilo Newton
kN/m^2	-	Kilo Newton per Area
kV	-	Kilo Volt
L	-	Litre
m^2/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

N_2	-	Nitrogen Gas
NaOH	-	Sodium Hydroxide
NH_2	-	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/m^3)

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 (Spéjel *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.

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