

**DEVELOPMENT OF ALGINATE FIBER WITH ANTIMICROBIAL
PROPERTY BY CHITOSAN DERIVATIVES**

Watcharapong Ariyakriangkrai

A Thesis Submitted in Partial Fulfilment of the Requirements
for the Degree of Master of Science
The Petroleum and Petrochemical College, Chulalongkorn University
in Academic Partnership with
The University of Michigan, The University of Oklahoma,
Case Western Reserve University and Institut Français du Pétrole
2005
ISBN 974-993-730-9

I22243057

Thesis Title: Development of Alginate Fiber with Antimicrobial Property
by Chitosan Derivatives
By: Watcharapong Ariyakriangkrai
Program: Polymer Science
Thesis Advisors: Asst. Prof. Ratana Rujiravanit
Prof. Seiichi Tokura

Accepted by the Petroleum and Petrochemical College, Chulalongkorn University, in partial fulfilment of the requirements for the Degree of Master of Science.

Nantaya Yanumet.
..... College Director
(Assoc. Prof. Nantaya Yanumet)

Thesis Committee:

Ratana Rujiravanit.
.....
(Asst. Prof. Ratana Rujiravanit)

S. Tokura
.....
(Prof. Seiichi Tokura)

Nantaya Yanumet.
.....
(Assoc. Prof. Nantaya Yanumet)

M. Nithitanakul
.....
(Asst. Prof. Manit Nithitanakul)

ABSTRACT

4672035063 : Polymer Science Program
Watcharapong Ariyakriangkrai: Development of Alginate
Fiber with Antimicrobial Property by Chitosan Derivatives
Thesis Advisors: Asst. Prof. Ratana Rujiravanit and
Prof. Seiichi Tokura, 60 pp. ISBN 974-993-730-9

Keywords : Alginate/ Chitosan/ *O*-Carboxymethylated chitosan/
N-Carboxyacylation/ Antibacterial activity

For the purpose of biomedical applications, antimicrobial property was introduced to alginate-based fibers by blending alginate with water-soluble chitosan derivatives, *O*-carboxymethyl chitosan or *N*-(carboxyacyl) chitosan. The antimicrobial activity and mechanical properties of the blend fibers were examined compared to those of alginate fiber. The presence of *O*-carboxymethyl chitosan or *N*-(carboxyacyl) chitosan in alginate-based fibers resulted in a slight decrease in tenacity. The antimicrobial activity of the fibers was determined using the parallel streak method. Alginate fiber showed no antimicrobial property where as both of the blend fibers could inhibit the growth of gram-negative bacteria (*Escherichia coli*, *Pseudomonas auruginosa*), gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus mutans*), and yeast (*Saccharomyces cerevisiae*). However, the clear zone was not observed for the test against *Candida abicans* in alginate and both of the blend fibers. In addition, the alginate-based fibers blended with *N*-(carboxyacyl) chitosan exhibited a larger clear zone than the fibers blended with *O*-carboxymethyl chitosan.

บทคัดย่อ

วัชรพงศ์ อริยเกรียงไกร : การพัฒนาเส้นใยอัลจีเนตที่มีสมบัติต้านจุลชีพโดยใช้อนุพันธ์ของไคโตซาน (Development of Alginate Fiber with Antimicrobial Property by Chitosan Derivatives) อ. ที่ปรึกษา: ผศ. ดร. รัตนา รุจิรวนิช และ ศ. ดร. เซอิจิ โทคุระ 60หน้า ISBN 974-993-730-9

เนื่องจากจุดประสงค์ที่ใช้ในทางการแพทย์ สมบัติต้านจุลชีพจึงถูกนำมาใช้กับเส้นใยอัลจีเนตโดยการผสมกับอนุพันธ์ของไคโตซานสองชนิด คือ คาร์บอกซีเมทิลไคโตซาน (ซีเอ็มไคโตซาน) หรือคาร์บอกซีเอซิลไคโตซาน สมบัติต้านจุลชีพและสมบัติเชิงกลถูกทำการทดสอบโดยเปรียบเทียบกับเส้นใยอัลจีเนต โดยการผสมคาร์บอกซีเมทิลไคโตซานและคาร์บอกซีเอซิลไคโตซานลงในเส้นใยอัลจีเนตนั้นทำให้สมบัติการทนแรงดึงลดลงเพียงเล็กน้อย สมบัติต้านจุลชีพถูกทดสอบด้วยวิธีเพราเรลสทริก โดยที่เส้นใยอัลจีเนตไม่มีสมบัติต้านจุลชีพ ในขณะที่เส้นใยผสมทั้งสองนั้นสามารถยับยั้งจุลชีพได้ทั้งแบคทีเรียชนิดแกรมลบ (เอสเชอริเชียโคไล, ซูโดโมนาสออรูจิโนซา), แบคทีเรียชนิดแกรมบวก (สแตปฟีโลคอคคัสออเรียส, สแตปฟีโลคอคคัสมิวแทน), และยีสต์ (แซคคาโลไมซีสเซเรวิซีอี) โดยสังเกตจากเคลียร์โซน อย่างไรก็ตามเคลียร์โซนเส้นใยผสมทั้งสองไม่ยับยั้งยีสต์ชนิดแคนดิดา อัลบิแคนส์ นอกจากนี้เส้นใยอัลจีเนตที่ผสมคาร์บอกซีเอซิลไคโตซานนั้นมีเคลียร์โซนขนาดใหญ่กว่าเส้นใยอัลจีเนตที่ผสมคาร์บอกซีเมทิลไคโตซาน

ACKNOWLEDGEMENTS

This work would not have been possible without the assistance of the following individuals.

First of all, the author is deeply indebted to the Development and Promotion for Science and Technology Talents Project of Thailand (DPST) for providing the scholarship. This thesis work is partially funded by TRF Master Research Grants and Postgraduate Education and Research Programs in Petroleum and Petrochemical Technology (PPT Consortium). The author would like to express his grateful appreciation to his advisor, Prof. Seiichi Tokura and Asst. Prof. Ratana Rujiravanit, for providing useful recommendations, creative comments, and encouragement throughout the course of his work.

The author would like to thank Assoc. Prof. Nantaya Yanumet and Asst. Prof. Manit Nithitanakul for being on the thesis committee.

Finally, the author would like to take this opportunity to thank all instructors, PPC staffs, PPC Ph.D. students and all his PPC friends for their friendly assistance, cheerfulness, creative suggestions, and encouragement. The author had the most enjoyable time working with all of them. Also, the author is greatly indebted to his parents and his family for their support, love, care and understanding.

TABLE OF CONTENTS

	PAGE
Title Page	i
Abstract (in English)	iii
Abstract (in Thai)	iv
Acknowledgements	v
Table of contents	vi
List of Tables	x
List of Figures	xi

CHAPTER		PAGE
I	INTRODUCTION	1
II	LITERATURE REVIEW	4
	2.1 Classification of Antibacterial	4
	2.2 Antibacterial Action of Chitosan and its Derivatives	7
	2.3 Alginate and Chitosan Fiber	12
III	EXPERIMENTAL	16
	3.1 Materials	16
	3.2 Equipment	16
	3.2.1 Restch Sieving Machine	16
	3.2.2 Capillary Viscometer	16
	3.2.3 Fourier Transform Infrared Spectrophotometer	17
	3.2.4 Elemental Analysis	17
	3.2.5 Wide-angle X-ray analysis	17
	3.2.6 Differential Scanning Calorimeter	17
	3.2.7 Scanning Electron Microscope	17

CHAPTER	PAGE
3.2.8 Atomic Absorption Spectrophotometer	17
3.2.9 Universal Testing Machine	18
3.3 Methodology	18
3.3.1 Preparation of Chitin, Chitosan, <i>O</i> -CM chitosan, <i>N</i> -(carboxyacyl) chitosan	18
3.3.1.1 Preparation of Chitin	18
3.3.1.2 Preparation of Chitosan	18
3.3.1.3 Preparation of <i>O</i> -CM chitosan	19
3.3.1.4 Preparation of <i>N</i> -(carboxyacyl) chitosan	19
3.3.2 Characterization of Chitosan	20
3.3.2.1 Degree of Deacetylation of Chitosan	20
3.3.2.2 Viscosity-Average Molecular Weight of Chitosan	20
3.3.3 Characterization of <i>O</i> -CM chitosan	21
3.3.3.1 The Structure of <i>O</i> -CM chitosan	21
3.3.3.2 Degree of Substitution of <i>O</i> -CM chitosan	22
3.3.4 Characterization of <i>N</i> -(carboxyacyl) chitosan	22
3.3.4.1 The Structure of <i>N</i> -(carboxyacyl) chitosan	22
3.3.4.2 Degree of Substitution of <i>N</i> -(carboxyacyl) chitosan	22
3.3.5 Characterization of Sodium Alginate	22
3.3.5.1 The Structure of Alginate	22
3.3.5.2 Viscosity-Average Molecular Weight of Sodium Alginate	22
3.3.6 Fiber Spinning	23
3.3.6.1 Preparation of Spinning	23
3.3.6.2 Spinning Process	23
3.3.7 Fiber Analysis	24

CHAPTER	PAGE
3.3.7.1 Calcium Content	24
3.3.7.2 Miscibility	24
3.3.7.3 SEM Micrographs	24
3.3.7.4 Linear Density	24
3.3.7.5 Mechanical Properties	24
3.3.7.6 Antimicrobial Property	25
IV RESULTS AND DISCUSSION	26
4.1 Characterization of Chitosan	26
4.1.1 Structural Characterization	26
4.1.2 Degree of Deacetylation	26
4.1.3 Viscosity-Average Molecular weight	27
4.2 Characterization of <i>O</i> -CM chitosan	27
4.2.1 Structural Characterization	27
4.2.2 Degree of Substitution	27
4.3 Characterization of <i>N</i> -(carboxyacyl) chitosan	28
4.3.1 Structural Characterization	28
4.3.2 Degree of Substitution	28
4.4 Characterization of Sodium Alginate	29
4.4.1 Structural Characterization	29
4.4.2 Viscosity-Average Molecular Weight	29
4.5 Characterization of Blend Film	29
4.5.1 Interaction in <i>O</i> -CM/Alginate Blend Film	29
4.5.2 Interaction in <i>N</i> -(carboxyacyl) chitosan (<i>N</i> -CA chitosan)/Alginate Blend Film	31
4.6 Characterization of Blend Fiber	32
4.6.1 SEM Micrographs of <i>O</i> -CM chitosan/Alginate Blend Fiber	32

CHAPTER	PAGE
4.6.2 SEM Micrographs of <i>N</i> -(carboxyacyl) chitosan/ Alginate Blend Fiber	33
4.6.3 DSC analysis of <i>O</i> -CM chitosan/Alginate Blend Fiber	33
4.6.4 DSC analysis of <i>N</i> -(carboxyacyl) chitosan/Alginate Blend Fiber	34
4.6.5 Wide-angle X-ray analysis of <i>O</i> -CM chitosan/ Alginate Blend Fiber	35
4.6.6 Wide-angle X-ray analysis of <i>N</i> -(carboxyacyl) chitosan/ Alginate Blend Fiber	36
4.6.7 Calcium Content in The Blend Fiber	37
4.6.8 Mechanical properties of The Blend Fiber	38
4.6.9 Antimicrobial properties of The Blend Fiber	39
V CONCLUSIONS AND RECOMMENDATIONS	41
5.1 Conclusions	41
5.2 Recommendations	41
REFERENCES	43
APPENDICES	47
Appendix A Test Results of Chitosan	47
Appendix B Test Results of Alginate	48
Appendix C Test Results of The Blend Fibers	49
CURRICULUM VITAE	60

LIST OF TABLES

TABLE	PAGE
2.1 Non-residue-producing antibacterials	4
2.2 Residue-producing antibacterials	5
2.3 Antimicrobial activities of chitosan	8
2.4 The antimicrobial effect of chitosan on <i>E.coli</i>	10
2.5 The antimicrobial effect of chitosan on <i>S.aureus</i>	10
4.1 Calcium content in the blend fiber	38
4.2 Mechanical properties of the blend fiber	39
4.3 Effect of alginate-based antimicrobial fibers on inhibition zone area against microorganisms	40

LIST OF FIGURES

FIGURE	PAGE
2.1	Chemical structure of quaternary ammonium compound 6
2.2	Chemical structure of chitin 7
2.3	Chemical structure of chitosan 7
2.4	Ionic interaction between chitosan and lipid at the cell wall 9
2.5	Ionic interaction between chitosan and DNA 9
2.6	Chemical structure of <i>O</i> -CM chitosan 11
2.7	Chemical structure of <i>N</i> -carboxybutyl chitosan 11
2.8	Chemical structure of <i>N</i> -(carboxyacyl) chitosan 12
2.9	Structure of alginate 13
2.10	Egg-box model 13
2.11	Potential ionic interactions between sodium alginate/alginate acid fibers treated with chitosan. 14
4.1	FTIR spectrum of chitosan 26
4.2	FTIR spectrum of H-form of <i>O</i> -CM chitosan 27
4.3	FTIR spectrum of <i>N</i> -(carboxyacyl) chitosan 28
4.4	FTIR spectrum of alginate 29
4.5	FTIR spectra of (a) alginate film, <i>O</i> -CM chitosan/alginate blend films with (b) 0.5%, (c) 1.0% and (d) 1.5% <i>O</i> -CM chitosan contents, (e) <i>O</i> -CM chitosan film. 30
4.6	FTIR spectra of (a) alginate film, <i>N</i> -CA chitosan/alginate blend films with (b) 0.25%, (c) 0.50% and (d) 0.75% <i>N</i> -CA chitosan contents, (e) <i>N</i> -(carboxyacyl) chitosan film (<i>N</i> -CA chitosan). 31
4.7	SEM micrographs of (a) alginate fiber, (b) 0.5% <i>O</i> -CM chitosan /alginate blend fiber, (c) 1.0% <i>O</i> -CM chitosan/alginate blend fiber and (d) 1.5% <i>O</i> -CM chitosan/alginate blend fiber 32

FIGURE	PAGE
4.8 SEM micrographs of (a) alginate fiber, (b) 0.25% <i>N</i> -(carboxyacyl) chitosan /alginate blend fiber, (c) 0.50% <i>N</i> -(carboxyacyl) chitosan /alginate blend fiber and (d) 0.75% <i>N</i> -(carboxyacyl) chitosan /alginate blend fiber	33
4.9 DSC spectra of (a) <i>O</i> -CM chitosan powder, <i>O</i> -CM chitosan/alginate blend fibers with (b) 0.5%, (c) 1.0% and (d) 1.5% <i>O</i> -CM chitosan contents, (e) alginate fiber.	34
4.10 DSC spectra of (a) <i>N</i> -(carboxyacyl) chitosan powder (<i>N</i> -CA), <i>N</i> -CA chitosan/alginate blend fibers with (b) 0.25%, (c) 0.50% and (d) 0.75% <i>N</i> -CA chitosan contents, (e) alginate fiber.	35
4.11 XRD spectra of (a) <i>O</i> -CM chitosan powder, (b) alginate fiber, <i>O</i> -CM chitosan/alginate blend fibers with (c) 0.5%, (d) 1.0% and (e) 1.5% <i>O</i> -CM chitosan contents.	36
4.12 XRD spectra of (a) <i>N</i> -(carboxyacyl) chitosan powder (<i>N</i> -CA chitosan), (b) alginate fiber, <i>N</i> -CA chitosan/alginate blend fibers with (c) 0.25%, (d) 0.50% and (e) 0.75% <i>N</i> -CA chitosan contents.	37