

**ORGANO-SOLUBLE AND WATER-SOLUBLE CHITOSANS FOR
EFFECTIVE FUNCTIONALIZATION AND MATERIALIZATION AT
MOLECULAR LEVEL**

Juthathip Fangkangwanwong

A Dissertation Submitted in Partial Fulfilment of the Requirements
for the Degree of Doctor of Philosophy
The Petroleum and Petrochemical College, Chulalongkorn University
in Academic Partnership with
The University of Michigan, The University of Oklahoma,
and Case Western Reserve University

2006

ISBN 974-9990-12-9

Thesis Title: Organo-Soluble and Water-Soluble Chitosans for Effective Functionalization and Materialization at Molecular Level
By: Juthathip Fangkangwanwong
Program: Polymer Science
Thesis Advisors: Assoc. Prof. Suwabun Chirachanchai
 Prof. Mitsuru Akashi

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

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

Thesis Committee:

Nantaya Yanumet

 (Assoc. Prof. Nantaya Yanumet)

Suwabun Chirachanchai

 (Assoc. Prof. Suwabun Chirachanchai)

Mitsuru Akashi

 (Prof. Mitsuru Akashi)

Manit Nithitanakul

 (Asst. Prof. Manit Nithitanakul)

Korbtham Sathirakul

 (Assoc. Prof. Korbtham Sathirakul)

ABSTRACT

4692003063: Polymer Science Program

Juthathip Fangkangwanwong: Organo-Soluble and Water-Soluble Chitosans for Effective Functionalization and Materialization at Molecular Level

Thesis Advisors: Assoc. Prof. Suwabun Chirachanchai, Prof. Mitsuru Akashi, 68 pp. ISBN 974-9990-12-9

Keywords: Chitosan/ Carbodiimide/ Hydrogel/ Homogeneous/ Hydroxyapatite/ Hydroxybenzotriazole/ Organo-Soluble Chitosan/ Water-Soluble Chitosan

The present dissertation proposes the development of organo-soluble and water-soluble chitosans to achieve homogeneous system in functionalization and materialization at molecular level. For organo-soluble chitosan, the present work focuses on *N*-phthaloylchitosan and its chemical homogeneous modification in a system using dimethylformamide (DMF). The introduction of an epoxy group onto chitosan via *N*-phthaloylchitosan and the crosslink network via the ring opening reaction of the epoxy group at room temperature were successful in obtaining chitosan-epoxy gel. The work also shows the mineralization step to grow hydroxyapatite crystals in the chitosan-epoxy gel by alternate soaking with a calcium solution and a phosphate solution. For water-soluble chitosan, the work originally declares that the organo complexation of chitosan and conjugation additives such as 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), *N*-hydroxysuccinimide (HOSu), can be simply formed in water to result in the chitosan water solution. A model conjugation of chitosan using water-soluble carbodiimide (WSC) in water at room temperature via the carboxyl groups of boc-L-phenylalanine, and PEG is demonstrated. The work is also extended to show how the conjugation with a dicarboxyl compound in a water-based system gives hydrogel.

บทคัดย่อ

จุฬาทิพย์ ฟังกังวาลวงศ์ : ไคโตซานที่ละลายในสารละลายอินทรีย์ และละลายในน้ำ เพื่อการเติมกลไกหรือพัฒนาวัสดุอย่างมีประสิทธิภาพในระดับโมเลกุล (Organo-Soluble and Water-Soluble Chitosans for Effective Functionalization and Materialization at Molecular Level) อ. ที่ปรึกษา : รองศาสตราจารย์ ดร. สุวบุญ จิรชาญชัย และ ศาสตราจารย์ ดร. มิชรุ อากาชิ, 68 หน้า ISBN 974-9990-12-9

วิทยานิพนธ์นี้เสนอแนวทางการพัฒนาไคโตซานที่ละลายในสารละลายอินทรีย์ และละลายในน้ำ เพื่อการเติมกลไกหรือพัฒนาวัสดุในระดับโมเลกุล สำหรับไคโตซานที่ละลายในสารละลายอินทรีย์ งานวิจัยนี้ได้เน้นถึงการใช้เอ็น-พทาโลอิวไคโตซาน และนำไปปรับปรุงโครงสร้างทางเคมีในระบบเอกพันธ์ของโคเมททิลฟอมาไมด์ การติดหมู่อีพอกซีกับสายโซ่ไคโตซานผ่านเอ็น-พทาโลอิวไคโตซานและการเชื่อมโครงสร้างผ่านการเปิดวงแหวนอีพอกซีที่อุณหภูมิห้องนำไปสู่การเกิดไคโตซาน-อีพอกซีเจล งานวิจัยนี้ยังแสดงถึงขั้นตอนการเกิดผลึกของไฮดรอกซีอะพาไทต์ในไคโตซาน-อีพอกซีเจลโดยใช้วิธีการจุ่มแบบสลับด้วยสารละลายแคลเซียมและฟอสเฟต สำหรับไคโตซานที่ละลายน้ำ งานวิจัยได้แสดงลักษณะเฉพาะเกี่ยวกับการเกิดสารประกอบอินทรีย์ของไคโตซานกับตัวช่วยในการเกิดปฏิกิริยา เช่น 1-ไฮดรอกซีเบนโซไตรเอโซล 1-ไฮดรอกซี-7-อาซาเบนโซไตรเอโซล เอ็น-ไฮดรอกซีซัคซินิมาย ซึ่งทำให้เกิดสารละลายน้ำไคโตซานได้อย่างง่ายและรวดเร็ว งานวิจัยนี้ยังได้สาธิตแบบจำลองการเกิดปฏิกิริยาของไคโตซานโดยใช้ตัวเชื่อมต่อในรูปของสารละลายน้ำคาร์โบดิอิมไมด์โดยทำปฏิกิริยาที่อุณหภูมิห้องในน้ำ กับหมู่คาร์บอกซิลของ บ็อก-แอล-ฟีนิลอลานีน และพีอีจี งานวิจัยยังขยายไปสู่วิธีการติดสารโคลิการ์บอซิลในระบบน้ำเพื่อการเกิดไฮโดรเจล

ACKNOWLEDGMENTS

The present dissertation would not have been accomplished without the author's Thai supervisor, Associate Professor Suwabun Chirachanchai, who not only originated this work, but also provided her with intensive suggestions, invaluable guidance, constructive criticisms, constant encouragement, inspiration and vital assistance throughout this research. She would also like to acknowledge the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program for the financial support toward her Ph.D. education (Grant No. PHD/0112/2546).

The author would also like to express her thanks to her Japanese co-advisor, Professor Mitsuru Akashi (Department of Applied Chemistry, Graduate School of Engineering, Osaka University, Japan) for the recommendations, strong support, and concerns during her stays in Japan. Deep gratitude is also given to Associate Professor Toshiyuki Kida for his fruitful discussion, worth advice, and warm hospitality during her stay in the Akashi laboratory at Osaka University, Japan. She would like to give sincere thanks to all members in the Akashi laboratory for their help, good time, and good memories throughout her stay in Japan, especially Dr. Michiya Matsusaki for the guidance and help in cell culture work, and Dr. Amornrat Lertworasirikul for her caretaking, lots of help and encouragement during her research period in Japan.

The author is also indebted to Associate Professor Buncha Pulpoka (Department of Chemistry, Faculty of Science, Chulalongkorn University) for his comments and help in the NMR measurement. She extends her appreciation to Seafresh Chitosan (Lab) Company Limited, Thailand, for their support with the chitosan starting materials.

The author is also thankful to the dissertation committee for their suggestions and comments in the reading of the thesis book. She greatly appreciates all Professors who have tendered invaluable knowledge to her at the Petroleum and Petrochemical College, Chulalongkorn University.

Special thanks are given to her seniors, Dr. Rangrong Yoksan and Dr. Sutinun Phongtamrug, for their help, invaluable guidance, suggestions and

encouragement throughout this research. She also would like to thank the college staff members, and all her friends at the Petroleum and Petrochemical College.

Last but not least, she wishes to express her gratitude to her family for their love, understanding, encouragement, limitless sacrifice, and for being a constant source of her inspiration throughout her study.

TABLE OF CONTENTS

	PAGE
Title Page	i
Abstract (in English)	iii
Abstract (in Thai)	iv
Acknowledgments	v
Table of Contents	vii
List of Schemes	ix
List of Tables	x
List of Figures	xi
CHAPTER	
I INTRODUCTION	1
II LITERATURE REVIEW	4
2.1 Chitin-Chitosan: the Specific Structure and Unique Properties	4
2.2 Deveopment of Chitin-Chitosan for Value-Added Applications	6
2.3 Limitations and Strategies to Overcome	8
2.4 Scope of the Present Work	11
III CHITOSAN GEL VIA CHITOSAN-EPOXY AND ITS HYDROXYAPATITE MINERALIZATION	13
Abstract	13
Introduction	14
Experimental	15
Results and Discussion	18
Conclusion	28
Acknowledgments	28
References	29

CHAPTER		PAGE
IV	ONE-POT SYNTHESIS IN AQUEOUS SYSTEM FOR WATER-SOLUBLE CHITOSAN-GRAFT-POLY(ETHYLENE GLYCOL) METHYL ETHER	31
	Abstract	31
	Introduction	32
	Materials and Methods	33
	Results and Discussion	34
	Conclusion	41
	Acknowledgments	41
	References	41
V	CHITOSAN-HYDROXYBENZOTRIAZOLE AQUEOUS SOLUTION: A NOVEL WATER-BASED SYSTEM FOR CHITOSAN FUNCTIONALIZATION	44
	Abstract	44
	Introduction	45
	Experimental	46
	Results and Discussion	49
	Conclusion	57
	Acknowledgements	57
	References	57
VI	CONCLUSIONS AND RECOMMENDATIONS	60
	REFERENCES	62
	CURRICULUM VITAE	67

LIST OF SCHEMES

SCHEME		PAGE
	CHAPTER I	
1		1
	CHAPTER II	
1		4
	CHAPTER III	
1		14
2		18
	CHAPTER IV	
1		33
2		37
	CHAPTER V	
1		47
2		51

LIST OF TABLES

TABLE		PAGE
CHAPTER IV		
1	Degree of substitution and yield of 2a-2f	39
2	Evaluation of solubility in various solvents relating to polarity index	41

LIST OF FIGURES

FIGURE		PAGE
CHAPTER III		
1	FTIR spectra of (a) 1 , (b) 2 , (c) 3 , (d) 4 , and (e) 4 after alternate soaking in $\text{CaCl}_2/\text{Na}_2\text{HPO}_4$ for 5 times	19
2	FTIR spectra of (A) before and (B) after curve fitting of 3 reacted with hydrazine 20 moles equivalent to pyranose ring at room temperature: for (a) 4 minutes, (b) 12 minutes, (c) 20 minutes, (d) 28 minutes; and (C) Gelation time of 4 as quantified by the oxirane peak at 907 cm^{-1} and pyranose ring peak at 1026 cm^{-1}	22
3	X-ray diffractograms of (a) 1 , (b) 4 , and (c) 4 after alternate soaking in $\text{CaCl}_2/\text{Na}_2\text{HPO}_4$ for 5 times	23
4	TGA thermograms of (a) 4 , and 4 after alternate soaking in $\text{CaCl}_2/\text{Na}_2\text{HPO}_4$ for the (b) 1 st cycle, (c) 2 nd cycle, (d) 3 rd cycle, (e) 4 th cycle, and (f) 5 th cycle	24
5	Scanning electron microscopy photographs at 25 kV of (a) 4 with 20 moles of hydrazine ($\times 500$), and 4 after alternate soaking in $\text{CaCl}_2/\text{Na}_2\text{HPO}_4$ at 37°C for (b) the 1 st cycle ($\times 500$), and (c) the 5 th cycle ($\times 2000$)	25
6	(A) Gel strength and water content of 4 under various hydrazine molar ratios, (B) Gel strength of (a) 4 with 20 times of hydrazine and 4 after alternate soaking in $\text{CaCl}_2/\text{Na}_2\text{HPO}_4$ for the (b) 1 st cycle, (c) 2 nd cycle, (d) 3 rd cycle, (e) 4 th cycle, and (f) 5 th cycle	26
7	Micrographs of fibroblast adhesion cultured for 1 day on (a) 4 , and 4 after alternate soaking in $\text{CaCl}_2/\text{Na}_2\text{HPO}_4$ for the (b) 1 st cycle, and (c) 5 th cycle	27
8	Fibroblast adhesion cultured for 1 day on 4 (Gel A), 4 after alternate soaking in $\text{CaCl}_2/\text{Na}_2\text{HPO}_4$ for the 1 st cycle (Gel B),	

FIGURE

PAGE

- the 5th cycle (Gel C), chitosan-glutaraldehyde gel (Gel D),
and chitosan-glutaraldehyde gel/HAp hybrid composite (Gel E) 28

CHAPTER IV

- 1 (A) Appearance of HOBt and chitosan (2:1 molar ratio) in water,
(B) ¹H NMR spectrum of HOBt and chitosan (2:1 molar ratio),
and (C) ¹H NMR spectrum of HOBt in D₂O at room temperature 35
- 2 (A) FTIR spectra of (a) chitosan and (b) **2d**, (B) ¹H NMR
spectrum of **2d** in D₂O at 70°C 38
- 3 (A) FTIR spectra of (a) **2a**, (b) **2b**, (c) **2d**, (d) **2e**, (e) **2f**,
(B) curve fitting of the spectra in (A), and (C) plots of
the integral ratio of ester peak at 1735 cm⁻¹ to amide peak
at 1650 cm⁻¹ relating to %DS 40

CHAPTER V

- 1 ¹H NMR spectra of A) HOBt in D₂O (pH=2.8),
B) chitosan-HOBt with the mole ratios of chitosan:HOBt
for 1:2, C) HOBt with NaOD at pH of: (a) 4.9, (b) 4.4,
(c) 3.8; and D) chitosan-HOBt with the mole ratios of
chitosan:HOBt for: (a) 1:1 at pH 4.9, (b) 1:1.5 at pH 4.3,
(c) 1:2 at pH 4.0; in D₂O at room temperature 50
- 2 ATR-FTIR spectra of (a) chitosan, (b) **1**, (c) **2**, and (d) **4** 53
- 3 ¹H NMR spectra of (a) **2** in CD₃COOD/D₂O at room temperature,
(b) **4** in D₂O at 70°C, and (c) **6** in CD₃COOD/D₂O at 70°C 54
- 4 (a) Gel **6** and (b) viscosity and gelation time during
the gelation of **6** 56