

# DEVELOPMENT OF MAGNETIC CHITOSAN NANOPARTICLES

Sutima Chatrabhuti

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

2013

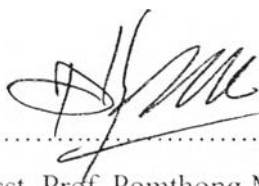
I 28372864

561050

**Thesis Title:** Development of Magnetic Chitosan Nanoparticles  
**By:** Sutima Chatrabhuti  
**Program:** Polymer Science  
**Thesis Advisors:** Prof. Suwabun Chirachanchai

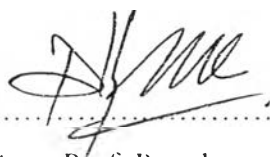
---

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

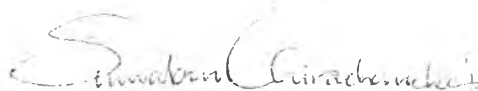


..... Dean  
(Asst. Prof. Pomthong Malakul)

**Thesis Committee:**



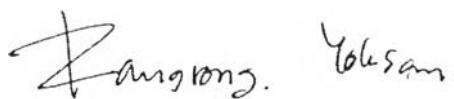
.....  
(Asst. Prof. Pomthong Malakul)



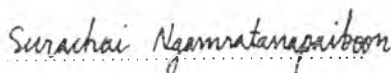
.....  
(Prof. Suwabun Chirachanchai)



.....  
(Asst. Prof. Manit Nithithanakul)



.....  
(Dr. Rangrong Yoksan)



.....  
(Dr. Surachai Ngamratanapaiboon)

## ABSTRACT

5192002063: Polymer Science Program

Sutima Chatrabhuti: Development of Magnetic Chitosan Nanoparticles.

Thesis Advisors: Prof. Suwabun Chirachanchai 92 pages

Keywords: Chitin-chitosan, Magnetic nanoparticles, Surface modification, Silane coupling agent

The present work focuses on preparing the magnetic nanoparticles and chitin- chitosan hybrids materials via secondary forces and covalent bond in water-based system. In the first part, the hybridization via physical interactions and factors related to colloidal stabilization is clarified. The work shows that particle size, surface charge, and morphology can be controlled by pH and polarity of the surrounded environment. The work also extends to implement for genomic DNA isolation. In the second part, the work shows the simple and effective synthesis pathway to fabricate magnetic nanoparticles and chitin- chitosan hybrids materials via covalent bond through simple silane coupling reaction. The epoxy functional group of silane coupling agent is ready to react with hydroxyl group of chitin/ chitosan. In the last part, another potential approach of hydrogel incorporated magnetic nanoparticles is to achieve hydrogel/aerogel responsive to the magnetic field. The high porosity and surface area of aerogel is expected to enhance the ability to adsorb substances.

## บทคัดย่อ

สุติมา ฉัตรภูติ : การพัฒนาแมกเนติกไคโตซานนาโนพาร์ติเคิล (Development of Magnetic Chitosan Nanoparticles) อ. ที่ปรึกษา : ศาสตราจารย์ ดร. สุวบุญ จิราญชัย 92 หน้า

วิทยานิพนธ์ฉบับนี้มุ่งเน้นไปที่การเตรียมของผสมระหว่างแมกเนติกนาโนพาร์ติเคิลกับวัสดุจำพวกไคติน-ไคโตซานโดยแรงอันตรกิริยาแบบอ่อนและพันธะโควาเลนต์ในระบบน้ำ ในส่วนแรก การทำของผสมผ่านแรงอันตรกิริยาแบบอ่อนและปัจจัยที่ส่งผลต่อความเสถียรของคอลลอยด์ได้พิสูจน์ให้กระจ่าง งานนี้แสดงให้เห็นว่าขนาดอนุภาค, ประจุบนพื้นผิว, และมอร์ฟอลอจีสามารถถูกควบคุมได้ด้วยค่าพีเอชและความเป็นขั้วของสารละลายแวลูม งานวิจัยนี้ยังได้ขยายผลไปถึงการนำไปใช้สกัดดีเอ็นเอด้วย ในส่วนที่สอง งานวิจัยแสดงให้เห็นถึงขั้นตอนการเตรียมของผสมระหว่างแมกเนติกนาโนพาร์ติเคิลกับวัสดุจำพวกไคติน-ไคโตซานโดยพันธะโควาเลนต์แบบง่ายและมีประสิทธิภาพผ่านปฏิกิริยาไฮโดรเจนบอนด์ปลิง ซึ่งหมู่ฟังก์ชันไฮดรอกซิลของสารไฮโดรเจนบอนด์เอเจ้นั้นมีความว่องไวในการเกิดปฏิกิริยากับหมู่ไฮดรอกซิลของไคโตซานได้ดี ในงานวิจัยส่วนสุดท้าย การนำไคโตซานไฮโดรเจลที่ถูกเติมแมกเนติกนาโนพาร์ติเคิลเข้าไปสู่การเป็นวัสดุที่สามารถตอบสนองต่อสนามแม่เหล็กจากภายนอกได้ถูกนำเสนอ โดยคาดว่าแอโรเจลที่มีความเป็นรูพรุนและมีพื้นที่ผิวมากจะมีความสามารถในการดูดซับสารต่างๆ ได้ดี

## ACKNOWLEDGEMENTS

The author would like to express her deep appreciation to her advisor, Professor Suwabun Chirachanchai. Without his support, encouragement and inspiration during her valuable years of being a Ph.D. student under his supervision. Thanks for never giving up on her.

She would like to acknowledge the scholarship from Center for Petroleum, Petrochemical and Advanced Materials, the Petroleum and Petrochemical College, Chulalongkorn University. Japan Student Service Organization for her 9 months experience in Japan.

She thanks Dr. Surachai Ngamrattanapaiboon for supporting her work in biochemistry field such as DNA isolation study. This work requires a lot of patience and skill. Without him, the DNA isolation study would not be at this success.

She wishes to thank Professor Mitsuru Akashi for valuable advices and kind support during her short term research in Osaka University.

She thanks all of Suwabun's lab members for being such a good friends, giving her encouragement through the tough moment. She will never forget the wonderful memories with them.

She wishes to thanks her family for never lose hope on her. They always believe that she can pursue her dream for being a good researcher.

## TABLE OF CONTENTS

	<b>PAGE</b>
Title Page	i
Abstract (in English)	iii
Abstract (in Thai)	iv
Acknowledgements	v
Table of Contents	vi
List of Schemes	ix
List of Table	x
List of Figures	xiv
<b>CHAPTER</b>	
<b>I INTRODUCTION</b>	<b>1</b>
<b>II LITERATURE REVIEW</b>	<b>2</b>
2.1 Magnetic Nanoparticles (MAG)	2
2.1.1 Synthesis and General Properties	2
2.1.2 Magnetic Nanoparticles Coating	5
2.1.3 Applications of Magnetic Nanoparticles	10
2.2 Chitin-chitosan	12
2.2.1 Functionalization of Chitin-chitosan	13
2.3 Chitosan as a Coating Material and/ or Stabilizer for MAG	16
2.4 Point of Study	21
<b>III CHITOSAN CORE-CORONA NANOSPHERES: A CONVENIENT MATERIAL TO TAILOR pH AND SOLVENT RESPONSIVE MAGNETIC NANOPARTICLES</b>	<b>22</b>
3.1 Abstract	22
3.2 Introduction	23
3.3 Experimental	25

<b>CHAPTER</b>	<b>PAGE</b>
3.4 Results and discussion	28
3.5 Conclusions	38
3.6 Acknowledgement	38
3.7 References	39
<b>IV SINGLE STEP COUPLING FOR MULTI-RESPONSIVE WATER-BASED CHITIN/CHITOSAN MAGNETIC NANOPARTICLES</b>	<b>43</b>
4.1 Abstract	43
4.2 Introduction	44
4.3 Experimental	46
4.4 Results and discussion	50
4.5 Conclusions	64
4.6 Acknowledgements	64
4.7 References	65
<b>V MAGNETIC -RESPONSIVE CHITOSAN HYDROGELS AND AEROGELS</b>	<b>68</b>
5.1 Abstract	68
5.2 Introduction	69
5.3 Experimental	70
5.4 Results and discussion	71
5.5 Conclusions	79
5.6 Acknowledgements	79
5.7 References	80
<b>VI CONCLUSIONS</b>	<b>81</b>
<b>REFERENCES</b>	<b>82</b>

**CHAPTER**

**PAGE**

**CURRICULUM VITAE**

**91**



## LIST OF SCHEMES

SCHEME	PAGE
<b>CHAPTER II</b>	
2.1 Chemical structures of (a) cellulose, (b) chitin, and (c) chitosan	13
<b>CHAPTER III</b>	
3.1 Synthesis of CSNS	27
<b>CHAPTER IV</b>	
4.1 Preparation of CTWK-MAG and WSCS-MAG.	48

## LIST OF TABLES

TABLE	PAGE
<b>CHAPTER II</b>	
2.1 Physical and chemical properties of hematite, magnetite, and maghemite	5
<b>CHAPTER III</b>	
3.1 Content of CSNS and MAG in CSNS-MAG	37
3.2 <i>E. coli</i> DNA binding content from various type of samples	39
3.3 <i>S. aureus</i> DNA binding content of CSNS-MAG in pH 10 buffer solution from various extraction methods	40

## LIST OF FIGURES

FIGURE	PAGE	
<b>CHAPTER II</b>		
2.1	Types of magnetism: paramagnetism (a), ferromagnetism (b), antiferromagnetism (c), and (d) ferrimagnetism.	3
2.2	Suggested mechanism for sodium dodecyl sulfate adsorption on MAG surface	7
2.3	Co-precipitation of MAG in PEO-PPO grafted copolymer with poly(acrylic acid) solution.	8
2.4	Dendrimer generation on surface of amino silane modified MAG	9
2.5	Ligand exchanging of chemisorbed oleic acid on MAG surface by silanes. Purposed different silanes with wide variety of end groups	10
2.6	MRI cross section of the U87MG tumors implanted in mice: (a) without MAG, and (b) with the injection of 300 $\mu$ g of peptide c(RGDyK)-modified MAG	11
2.7	Preparation procedure of water-in-oil microemulsion	17
2.8	Scheme of amide linkage formation between the carboxylic group on the particle surface and the amino group found in chitosan, via carbodiimide activation	18
2.9	Principle of in situ mineralization of MAG in chitosan hydrogel	20
<b>CHAPTER III</b>		
3.1	Chemical structure, TEM micrographs, and schematic draws of CSNS in water (a); TEM micrographs, and schematic draws of CSNS-MAG (b,d), and CSNS-O-MAG (c,e).	29
3.2	Z-average particle size of CSNS-MAG in toluene ( $\circ$ ), THF ( $\bullet$ ), DMF ( $\square$ ), ethanol ( $\blacksquare$ ), and water ( $\triangle$ ).	30

<b>FIGURE</b>	<b>PAGE</b>
3.3 Photographs (left), TEM micrographs (middle), and schematic draws (right) of CSNS-MAG in water (a, d, g), DMF (b, e, h), and toluene (c, f, i).	32
3.4 Z-average particle sizes of CSNS-MAG in water with various isopropanol contents.	33
3.5 Particle size (a) and zeta potential (b) of CSNS ( $\circ$ ), MAG ( $\square$ ), and CSNS-MAG ( $\triangle$ ) in (pH adjusted) water from pH 2.2 to pH 10; TEM images of CSNS-MAG in (pH adjusted) water pH 3 (c), and pH 6 (d); schematic draws of CSNS-MAG in acidic or basic pHs (e), and neutral pH (f).	36

#### CHAPTER IV

4.1 A) FTIR spectra of (a) S-MAG, (b) CTWK-MAG, and (c) WSCS-MAG; B) curve fitting of (a) S-MAG, (b) CTWK-MAG, and (c) WSCS-MAG; and C) integral ratio of oxirane peak at $900\text{ cm}^{-1}$ to Si-O peak at $1087\text{ cm}^{-1}$ of a) S-MAG, b) CTWK-MAG, and c) WSCS-MAG.	51
4.2 TGA thermograms of (a) O-MAG, (b) S-MAG, (c) CTWK-MAG, and (d) WSCS-MAG.	53
4.3 TEM micrographs of (a) MAG, (b) O-MAG, (c) CTWK, (d) WSCS, (e) CTWK-MAG, (f) WSCS-MAG, and (g) CTWK-MAG under magnetic field. Circles in Figure (e-g) indicate MAG.	55
4.4 A) Photographs of colloidal appearances of (a) MAG, (b) S-MAG, (c) CTWK-MAG, and (d) WSCS-MAG; and B) relative absorbance of MAG ( $\circ$ ), S-MAG ( $\square$ ), CTWK-MAG ( $\triangle$ ), and WSCS-MAG ( $\diamond$ ) in water as a function of time.	57

FIGURE	PAGE
4.5 A) Photographs of CTWK-MAG dispersed in various solvents in relation to the dielectric constant value (k): toluene (k=2.38), chloroform (k=4.81), THF (k=7.58), DMF (k=19.9), ethanol (k=24.5), methanol (k=32.7), DMSO (k=46.7), and water (k=80.1) at 0 min, 30 min, 60 min, 120 min, and 180 min after sonication; B) relative absorbance of CTWK-MAG in various solvents: water (○), DMSO (●), methanol (□), ethanol (■), DMF (△), THF (▲), chloroform (+), and toluene (×); and C) schematic illustration of solvent interactions to CTWK-MAG in (a) water, (b) DMF, and (c) toluene.	59
4.6 <b>Figure 4.6.</b> A) Zeta potential and particle size of (a) MAG, (b) S-MAG, (c) CTWK-MAG, and (d) WSCS-MAG at pH 2.2 to pH 10 in water (pH adjusting by NaOH and HCl); B) schematic illustrations of CTWK-MAG in water at (a) pH 2–4, (b) pH 5–7, and (c) pH 8–10; and C) schematic illustrations of WSCS-MAG in water at (a) pH 2–3, (b) pH 3–7, and (c) pH 8–10.	62

## CHAPTER V

5.1 Appearances of magnetic chitosan hydrogels of; (a) MAG-added, and (b) S-MAG-added in hydrogel with various mole ratio.	72
5.2 ATR-FTIR spectra of; (a) chitosan, (b) chitosan aerogel, (c) chitosan aerogel containing MAG, and (d) chitosan aerogel containing S-MAG.	73
5.3 Magnetization curves for (a) MAG-chitosan aerogel and (b) S-MAG-chitosan aerogel at various MAG and S-MAG content.	74
5.4 XRD patterns of; (a) MAG, (b) chitosan aerogel, (c) MAG-chitosan aerogel, and (d) S-MAG-chitosan aerogel.	75

FIGURE	PAGE
5.5 SEM micrographs at 15 keV of chitosan aerogel (a), MAG-chitosan aerogels (b-e), S-MAG-chitosan aerogels (f-i). Magnetic loading content 0.5% (b, f), 2.5% (c, g), 5.0% (d, h) and 10.0% (e, i). Scale bar, 10 $\mu\text{m}$ .	76
5.6 Swelling ratio of magnetic chitosan hydrogels.	77
5.7 Relative drug release as a function of time of (a) unmodified MAG-OFF magnetic field, (b) unmodified MAG-ON magnetic field, (c) silane-modified MAG-OFF magnetic field, and (d) silane-modified MAG-ON magnetic field. No magnetic nanoparticle added ( $\circ$ ), 0.5% added ( $\square$ ), 5.0% added ( $\Delta$ ), and 10.0% added ( $\times$ ). $C_{\text{inf}}$ represents cumulative mass release at 24 h (N=3). The degrees of crosslinking of all samples were fixed at 50%.	78