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รายงานวิจัยฉบับสมบูรณ์

ไมโครสเฟียร์แม่เหล็กที่มีสมบัติการตอบสนองต่อสิ่งกระตุ้นหลากหลายของพอลิเอ็นไอโซโพรพิลอะคริลาไมด์/คาร์บอกซีเมทิลไคโตแซน เพื่อใช้ในการควบคุมการปลดปล่อยยา

Multi-responsive magnetic microsphere of poly(*N*-isopropylacrylamide)/carboxymethyl chitosan for drug controlled release

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Abstract

This research discussed about the synthesis of "smart" carboxymethyl-chitosan (CMC) hydrogel microspheres. Multi-responsive composite CMC microspheres were synthesized *via* an *in situ* free radical polymerization of thermo-responsive poly(*N*-isopropylacrylamide) (poly(NIPAAm)) grafted to carboxymethylchitosan (CMC) in the presence of magnetite nanoparticles (MNPs). Formulations of the composite CMC microspheres, such as CMC, NIPAAm and glutaraldehyde crosslinker, were tuned such that spherical microspheres with narrow size distributions were obtained (30.0 ± 1.0 μm in diameter). These microspheres responded to an applied magnetic field and can be separated using a permanent magnet. In addition, they can also respond to the changes of their environmental pH and temperature. Water swelling of the microspheres was enhanced when the solution pH was under basic conditions (pH 11) or the temperature was below its lower critical solution temperature (LCST) of poly(NIPAAm) (32°C). These responsive properties can be used as triggering mechanisms for release of entrapped indomethacin, a model drug, from the microspheres. These "smart" composite CMC microspheres with multi-responsive properties show great potentials for use in controlled release applications.

บทคัดย่อ

งานวิจัยนี้ เป็นการศึกษาการสังเคราะห์ไมโครสเฟียร์“ฉลาด”ของคาร์บอกซี่เมทิลไคโตซาน (CMC) โดยการสังเคราะห์ด้วยปฏิกิริยาพอลิเมอไรเซชันแบบอนุมูลอิสระของพอลิเอ็นไอโซโพรพิลอะคริลาไมด์ (poly(NIPAAm)) ที่กร๊าฟบนโครงสร้างของคาร์บอกซี่เมทิลไคโตซานโดยมีอนุภาคนาโนแมกนีไทท์ร่วมในปฏิกิริยาด้วย โดยในงานวิจัยได้ทำการศึกษาการปรับเปลี่ยนอัตราส่วนการใช้คาร์บอกซี่เมทิลไคโตซาน (CMC) เอ็นไอโซโพรพิลอะคริลาไมด์ (NIPAAm) และสารกลูตารัลดีไฮด์ซึ่งเป็นสารเชื่อมโยงตาข่ายเพื่อให้ได้ไมโครสเฟียร์ที่มีทรงกลมและมีการกระจายของขนาดไมโครสเฟียร์ที่แคบ (ขนาดเส้นผ่าศูนย์กลาง 30.0 ± 1.0 ไมครอน) จากผลการทดลองพบว่าไมโครสเฟียร์ที่สังเคราะห์ได้สามารถตอบสนองต่อสนามแม่เหล็กได้ดีและยังสามารถตอบสนองต่อการเปลี่ยนแปลงสภาวะกรดเบสและอุณหภูมิของสารละลายได้ โดยสมบัติการบวมน้ำของไมโครสเฟียร์พบว่ามีค่ามากขึ้นเมื่ออยู่ในสภาวะสารละลายที่เป็นเบส (pH 11) หรือเมื่ออุณหภูมิของสารละลายต่ำกว่าค่าอุณหภูมิสารละลายวิกฤติล่าง (LCST) ของพอลิเอ็นไอโซโพรพิลอะคริลาไมด์ (poly(NIPAAm)) (32°C) ซึ่งสมบัติการตอบสนองต่อการเปลี่ยนแปลงสภาวะของสิ่งแวดล้อมนี้จะสามารถใช้เป็นกลไกในการกระตุ้นการปลดปล่อยยาที่ถูกกักเก็บไว้จากไมโครสเฟียร์ได้ ดังนั้นไมโครสเฟียร์ฉลาดของคาร์บอกซี่เมทิลไคโตซานชนิดใหม่นี้จึงอาจจะเหมาะสมในการประยุกต์ใช้ในการประยุกต์ใช้ด้านการควบคุมการปลดปล่อยยาได้

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ABBREVIATIONS

NMR	=	Nuclear Magnetic Resonance
FT-IR	=	Fourier Transform Infrared Spectroscopy
VSM	=	Vibrating Sample Magnetometry
SEM	=	Scanning Electron Microscopy
TGA	=	Thermal Gravimetric Analysis
CMC	=	Carboxymethylchitosan
Poly(NIPAAm)	=	Poly(*N*-isopropylacrylamide)
MNPs	=	Magnetite Nanoparticles
APS	=	Diammoniumperoxodisulphate
semi-IPN	=	semi-Interpenetrating Polymer Network
EE	=	Entrapment Efficiencies
DLE	=	Drug Loading Efficiencies
LCST	=	Lower Critical Solution Temperature

CHAPTER I

INTRODUCTION

Rationale of the study

Stimuli-responsive microsphere, also called "intelligent" or "smart" microsphere, has been extensively studied in recent years because it is capable in response to environmental change, e.g. pH, temperature, the presence/absence of chemicals and biological compounds, or applications of external stimuli, such as light, magnetic or electric fields and ionic strength (Ahmad et al., 2014; Sun, Shi, Xu, & Cao, 2013; Klinger & Landfester, 2012; Motornov, Roiter, Tokarev, & Minko, 2010). It has been of particular interest in a wide variety of biomedical or pharmaceutical applications such as controlled drug release and delivery, chemical separation and tissue engineering (Chaleawlert & Pimpha, 2012; Agnihotri & Aminabhavi, 2006; Li, Zhong, Zhou, Ding, & Li, 2011; Li et al., 2011; Varaprasad et al., 2012; Yan et al., 2012). In general, it undergos drastic swelling and shrinkage response to the environmental stimuli at its phase transition.

Weakly ionizable polysaccharide, such as alginate, bean gum, chitosan and its derivative, has also been of great interest as pH-responsive hydrogel due to its excellent antibacterial activity, non-cytotoxicity, excellent biocompatibility and high water solubility (Fan et al., 2006; Zhao, Wang, & Wang, 2003; Don & Chen, 2005). Carboxymethylchitosan (CMC), a water soluble chitosan derivative, is typically prepared *via* a carboxymethylation of chitosan with monochloroacetic acid at some of amino and hydroxyl groups in the glucosamine units (Chen et al., 2007; Tu et al., 2010). Previous works have reported the synthesis of chitosan and CMC in the form of

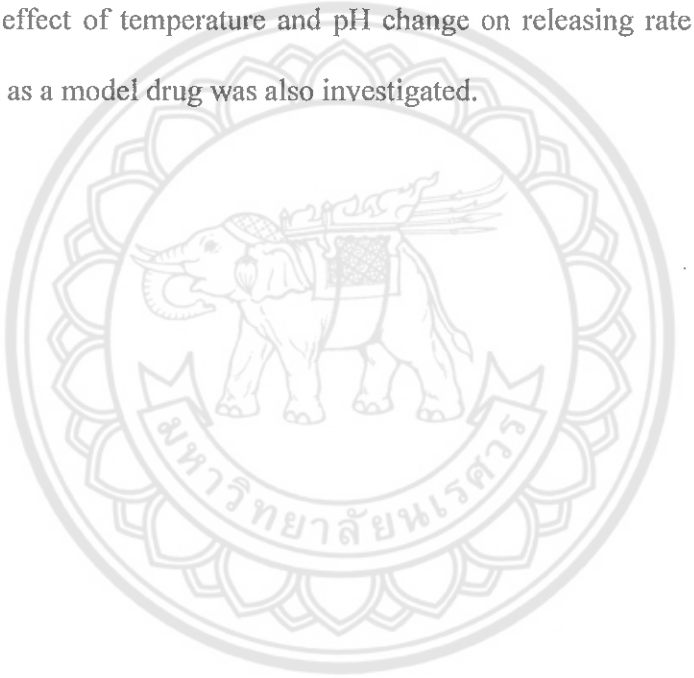
microspheres with great potentials in biomedical applications (Gong, Liu, Zhu, & Zhang, 2012). Also, chitosan- and CMC-based microspheres copolymerized or physically blended with other polymers, compounds or nanoparticles, e.g., poly(ethylene oxide-*g*-acrylamide) (Agnihotri & Aminabhavi, 2006), poly(ε-caprolactone) (Wu et al, 2011), chelerythrine and magnetite nanoparticles (MNPs) (Li, Zhong, Zhou, Ding, & Li, 2011; Li et al., 2011), have been previously studied.

Another type of "smart" materials that has recently gained much attention is thermo-responsive microsphere. Poly(*N*-isopropylacylamide) (poly(NIPAAm)), the best known polymer in this class, undergoes a volume phase transition at the lower critical solution temperature (LCST) around 32°C (Yuan, Venkatasubramanian, Hein, & Misra, 2008; Fundueanu et al., 2013; Mu & Fang, 2008; Glampedaki et al., 2012). At the temperature below its LCST, water molecules act as a good solvent for poly(NIPAAm) chains by forming intermolecular hydrogen bonds with the amide oxygens, resulting in an increase in their solubility in water. When the solution temperature is higher than the LCST, poly(NIPAAm) chains suddenly shrink, resulting in an increase in their hydrophobicity and exclusion of water. As a result, intramolecular hydrogen bonding dominates those between the polymer chains and water molecules. Preparation of poly(NIPAAm) microsphere with other polymers, such as poly(ε-caprolactam) (Reddy et al., 2011), poly(*N*-hydroxymethyl acrylamide)(Constantin, Cristea, Ascenzi, & Fundueanu, 2011), and poly(acrylamide-*co*-aminoethyl acrylamide) (Fundueanu et al., 2013), has been widely reported. However, the study in the synthesis of chitosan- and CMC-based microspheres comprising thermo-responsive poly(NIPAAm) was rather limited (Sun, Shi, Xu, & Cao, 2013; Jiang et al., 2010; Yuan, Venkatasubramanian, Hein, & Misra, 2008).

In addition, the synthesis of polymeric microsphere having magnetic-responsive property is also now gaining growing attention (Lei, Pang, Li, Lin, & Li, 2009). This magnetic polymeric microsphere consisting of one or more magnetic nanoparticles embedded in polymer matrix showed a great potential in biomedical applications, such as drug controlled release and delivery, magnetic resonance imaging, tissue repair and magnetic cell separation (Zhao, Saatchi, & Häfeli, 2009; Yuan et al., 2013; Meng, Xu, Zhou, Wu, & Yang, 2013; Li, Li, & Li, 2011). These applications are based on its advantageous and unique properties, such as high magnetic responsiveness, good stability under physiological conditions and high surface area-to-volume ratio (Chen, Li, Wu, Zhu, Lu, & Du, 2013; Bhatt, Bhat, & Santosh, 2010). Magnetite nanoparticle (MNP) is the best known magnetic nanoparticle due to their facile preparation procedure and good stability against oxidation. MNP can be synthesized *via* various methods such as a co-precipitation of iron salts in an alkaline condition (Meng, Xu, Zhou, Wu, & Yang, 2013; Zhang, Zhang, Wang, & Zeng, 2007; Zhang, Zhang, Wang, & Zeng, 2007), thermal decomposition of iron organic precursors (Maity, Kale, Ghanekar, Xue, & Ding, 2009), micro-emulsion (Ha et al., 2008) and aerosol vapor methods (Laurent et al., 2008), while the first two approaches are the most known techniques for preparing MNP.

In this study, we reported the synthesis and property of a novel multi-responsive poly(NIPAAm)-grafted CMC composite microsphere embedded with MNP (Figure 1.1). Poly(NIPAAm) was synthesized *via* an *in situ* free radical polymerization in the presence of CMC and surface-functionalized MNP and then chemically crosslinked with glutaraldehyde to form water swellable microsphere. They showed

multi-responsive properties due to the presence of thermo-responsive poly(NIPAAm), pH-responsive CMC and magnetic-responsive MNP. The ratio of CMC, NIPAAm, the crosslinker and surfactant was adjusted such that uniform-sized microsphere was obtained. Water swelling property of the microsphere as a function of solution temperature and pH was investigated. Thermogravimetric analysis (TGA) was performed to determine the percentage of MNP in the microsphere and vibrating sample magnetometry (VSM) was conducted to study its magnetic responsiveness. In addition, the effect of temperature and pH change on releasing rate of an entrapped indomethacin as a model drug was also investigated.



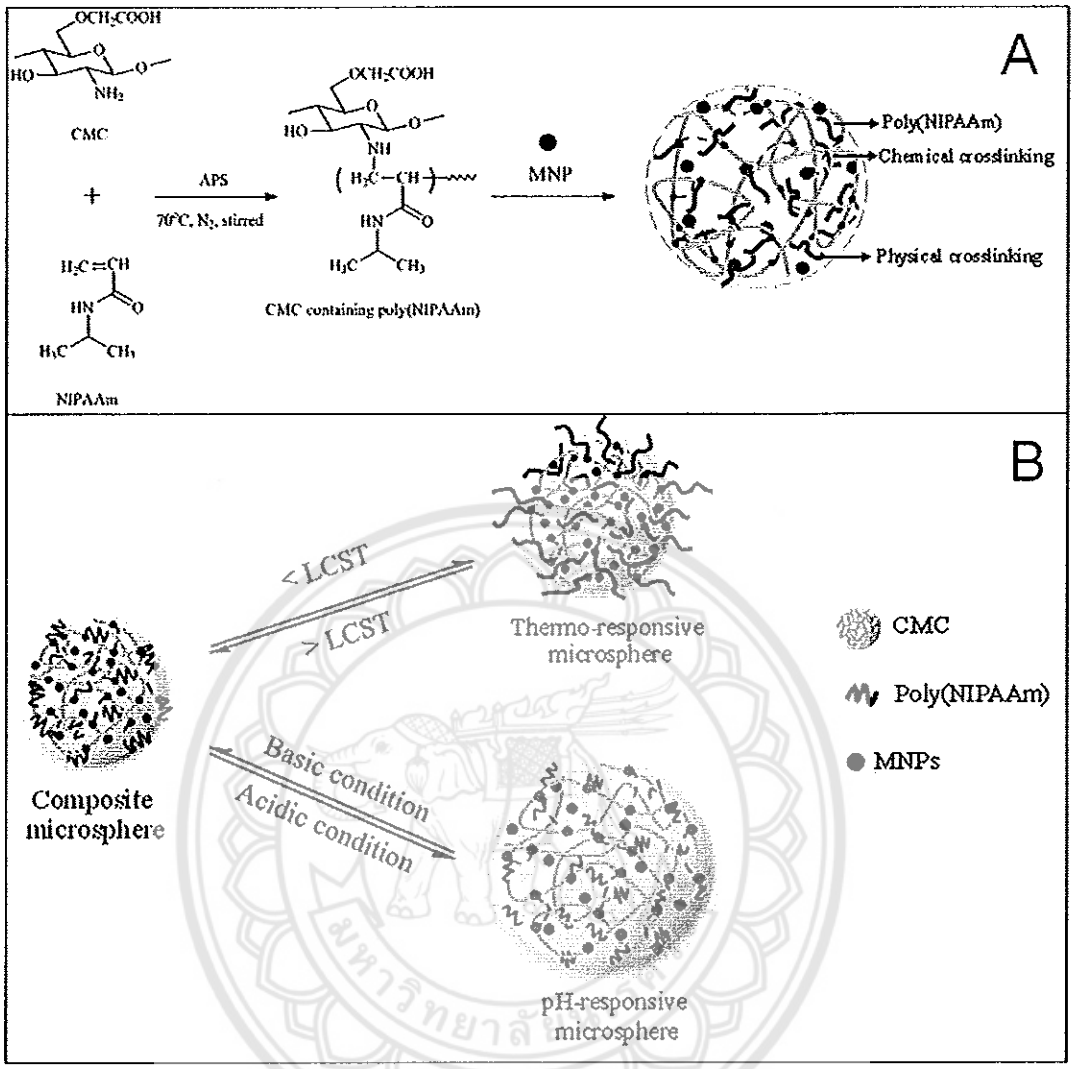


Figure 1.1. (A) Schematic illustration for preparing the composite CMC microsphere containing poly(NIPAAm) and MNP and (B) pH and thermo-responsive behavior of the microsphere

Research objectives

This work highlights on the synthesis of CMC hydrogels in the form of microsphere having multiple responsive properties, such as thermal, pH and magnetic-responsive properties, and also study in the properties of the hydrogels, such as surface morphology, magnetic properties and entrapment efficiency of a model drug. In addition, water swelling and drug release behavior of the hydrogels as a function of solution temperature and pH were also investigated.

CHAPTER II

EXPERIMENTAL

Materials

Unless otherwise stated, all reagents were used as received; chitosan from crab (1.4×105 g/mol) (Taming Enterprise, 98% deacetylation), monochloroacetic acid ($ClCH_2COOH$), 99% (Acros), diammoniumperoxodisulphate (APS) (Carlo Erba, 98%), glutaraldehyde (GA) (Lobachemie, 25% aqueous solution), paraffin liquid (Fisher Chemical), iron (III) chloride anhydrous ($FeCl_3$) (Acros, 98%), iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$) (Acros, 99+%), ammonium hydroxide (J.T. Baker, 28-30%), indomethacin (Sigma, 99% TLC) and Tween-20. *N*-isopropylacrylamide (NIPAAm) (Acros, 99%) was recrystallized in hexane before used to remove radical inhibitors.

Syntheses

1. Synthesis of carboxymethylchitosan (CMC) from chitosan (Fig. 2.1)

Chitosan oligomer from crab (40 g) was immersed in isopropyl alcohol (500 ml) for 24 h and then in a NaOH solution (40.32 g, 1000 mmol in 100 ml H_2O) for another 75 min. Monochloroacetic acid (48 g, 510 mmol in 100 ml H_2O) was added to the swollen chitosan at 60°C with stirring for 5 h (Figure 31). The product was precipitated in an excess of methanol, washed with methanol:H_2O solutions (70:30 and 80:20 v/v, respectively) to remove salts, filtered and dried at 40°C. The final product appeared as a dried yellow powder and was characterized by FTIR (Appendix).

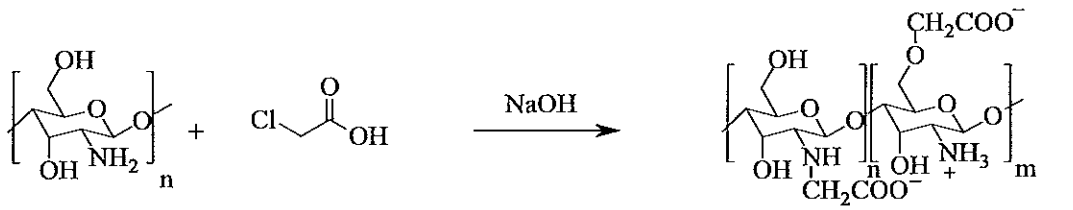


Figure 2.1 Synthesis of carboxymethylchitosan (CMC)

2. Synthesis of magnetite nanoparticles (MNPs)

MNPs (Fe_3O_4) was synthesized *via* a co-precipitation method by mixing aqueous solutions of $FeCl_3$ (1.66 g in 20 ml of deionized water) and $FeCl_2.4H_2O$ (1.00 g in 20 ml deionized water) with stirring, followed by an addition of an NH_4OH solution (25%, 20 ml) to obtain a black precipitant (Figure 2.2). The dispersion was continuously stirred for another 30 min to complete the reaction. The resultant MNPs was then repeatedly washed with deionized water to neutralize the solution.

$$FeCl_3 + FeCl_2.4H_2O \xrightarrow{NH_4OH} Fe_3O_4$$

Figure 2.2 The reaction for the synthesis of MNP

3. Preparation of poly(NIPAAm)-containing composite CMC microspheres loaded with MNPs

An example for the synthesis of TRM4 microsphere was explained herein (Table 2.1). Other samples were prepared in a similar fashion with proper amounts of reagents used. CMC (0.4 g, 1.8 mmol of carboxymethyl glucosamine unit) and

NIPAAm (0.2 g, 1.7 mmol) were dissolved in deionized water (10 ml) and stirred under N_2 atmosphere at room temperature to obtain a homogenous solution. After heating to 60°C, APS (0.03 g, 0.1 mmol), a radical initiator, was added to the solution with stirring for another 30 min, followed by the addition of an MNPs dispersion (0.06 g MNPs in 1 ml water). The mixture was then sonicated at the room temperature for 5 min. The aqueous mixture was then dropped into paraffin oil (30 ml) (mix with tween-20 if needed) with continuous stirring at 300 rpm for 30 min. After the mixture was emulsified, 25% glutaraldehyde solution (6 ml) as a crosslinking agent was added with continuous stirring for 1 h. The final product was separated by centrifugation at 4000 rpm and washed repeatedly with petroleum ether and acetone to completely remove paraffin oil (Figure 2.3).

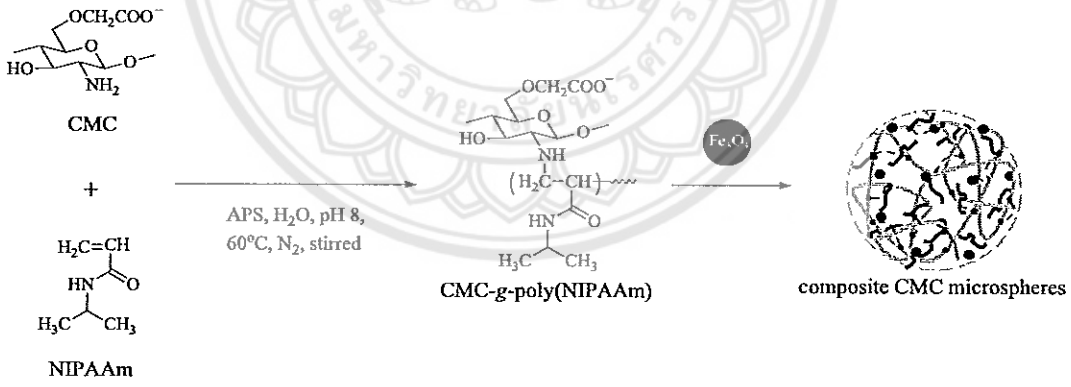


Figure 2.3 Schematic illustrations for the formation of composite CMC microspheres containing poly(NIPAAm) and MNPs

Table 2.1 Formulations of magnetic composite CMC microsphere

Sample[a]	NIPAAm (g)	25% Glutaraldehyde (ml)	Tween-20 (ml)	Appearance[b]
TRM1	0.2	0	0.6	Flake type
TRM2	0.2	4	0.6	Agglomerated microsphere
TRM3	0.2	6	0.6	Agglomerated microsphere
TRM4	-	6	-	Irregular-shape microsphere
TRM5	0.2	6	-	Spherical microsphere
TRM6	0.4	6	-	Spherical microsphere
TRM7	0.8	6	-	Spherical microsphere

[a] 0.4 g of CMC and 0.06 g of MNPs were used in all experiments

[b] SEM and results will be shown and discussed in the result and discussion part

Characterization of composite CMC microsphere

1. Polymers and composite CMC microsphere

FTIR was performed on a Perkin-Elmer Model 1600 series FTIR spectrophotometer using KBr pellets. Morphology of the composite CMC microsphere was performed on LEO 1455 VP SEM with an accelerating voltage of 15 kV. Dried microspheres were adhered to an aluminum stub and coated with gold before each measurement. TGA was performed on TGA/DSC 1 Mettler-Toledo at temperature

ranging between 30 and 800 °C at 20 °C/min heating rate under oxygen atmosphere. Magnetic properties of composite CMC microspheres were measured in the solid state at 300K using a Standard 7403 Series, Lakeshore VSM. The magnetic moment of each sample was investigated over a range of applied magnetic fields from -10,000 to +10,000 G using 30 min sweep time.

2. Determination of water swelling behavior

Dried composite CMC microspheres were precisely weighed and immersed in distilled water at the given temperature (10, 32 and 50°C) and pH (3, 7 and 11) in closed containers. At a predetermined time, the swollen composite CMC microspheres were removed from water and excess water on their surface was carefully wiped off. Equilibrium water content (%EWC) was calculated from the following equation;

$$\%EWC = \frac{W_s - W_d}{W_d} x100 \quad (1)$$

where W_s and W_d are the weights of the swollen and dried samples, respectively.

3. Determination of entrapment efficiencies (%EE) and drug loading efficiencies (%DLE)

Indomethacin was used as a model drug for drug loading and releasing experiments. Dried composite CMC microspheres were immersed in an indomethacin solution (0.10 g of indomethacin in 5 ml of ethanol) at 10 °C for 2 days to fully swell

the microspheres and obtain maximum drug uptake. The difference of the weights of indomethacin in the solution before and after the swelling experiments, reflecting the entrapped drug in the microspheres, was determined *via* UV spectrophotometry at wavelength of 320 nm. %EE and %DLE were calculated according to the following equations;

$$\%EE = \frac{\text{Weight of the entrapped drug in the microsphere}}{\text{Weight of the loaded drug}} \times 100 \qquad (2)$$

$$\%DLE = \frac{\text{Weight of the entrapped drug in the microsphere}}{\text{Weight of the dried microsphere}} \times 100 \qquad (3)$$

4. Studies in the in vitro drug release behavior

Indomethacin release behavior from composite CMC microspheres was studied as a function of solution temperature and pH. The drug-loaded microspheres were immersed in a phosphate buffered saline (PBS) solution (pH 7.4) at various temperatures (10, 32 or 50 °C) to study the effect of solution temperature on drug releasing behavior. Similarly, the drug-loaded microspheres were immersed in the solutions having various pHs (3, 7 or 11 buffer solutions) at 25 °C to investigate the effect of solution pH on drug releasing properties. The concentrations of the released drug were measured *via* UV spectrophotometry at wavelength of 320 nm. The releasing percentages of indomethacin were calculated using the following equation:

$$\%\text{Drug release} = \frac{\text{weight of released drug at a given time}}{\text{the total absorbed drug in microsphere}} \times 100 \qquad (4)$$

CHAPTER III

RESULTS AND DISCCUSION

The originality of this work is that we here demonstrate a facile and efficient synthesis of novel CMC-based microspheres that have multi-responsive properties toward external stimuli; magnetic field gradient, solution pH and temperature changes. Carboxyl and amino groups in CMC played a role in pH-responsive properties, while poly(NIPAAm) grafted in the microspheres provided thermal-sensitive behavior. In addition, MNPs embedded in the structure provided magnetic field-responsive properties to the microspheres. The reactions between poly(NIPAAm) and CMC were prepared *via* an APS-initiated free radical *in situ* polymerization in the presence of MNPs with the use of glutaraldehyde as a crosslinker (Appendix). These composite microspheres were synthesized *via* a water-in-oil emulsion with or without a surfactant (Tween-20), followed by an emulsion crosslinking technique. Effect of the concentrations of NIPAAm, the crosslinkers and the surfactant on microsphere formation was also investigated.

To form CMC microspheres, ungrafted poly(NIPAAm) homopolymers might also exist in their structure due to the polymerization initiated from ammonium sulfate free radicals in the solution. To simplify the microsphere preparation procedure, these homopolymers were left in the solutions without extraction. It was thus envisioned that they were physically locked in CMC microspheres when chemically crosslinked with glutaraldehyde. Figure 1.1 shows schematic illustration for the formation of

composite CMC microspheres in the presence of MNPs and free poly(NIPAAm) homopolymers locked in the structure.

In addition, poly(NIPAAm) covalently grafted on CMC chains might also exist in the structure. Amine radicals (•NH-) can be formed on CMC chains, resulting in free radical initiating sites for poly(NIPAAm). To confirm this assumption, polymerization of poly(NIPAAm) in the presence of CMC was performed, followed by repeated extractions of unbound poly(NIPAAm) and unreacted monomers from CMC without a crosslinking reaction step. The presence of poly(NIPAAm) bound to CMC chains was evidenced by ^{1}H NMR spectroscopy. ^{1}H NMR spectrum of the product shows distinctive signals of poly(NIPAAm); 1.11 ppm of methyl protons of NIPAAm ($CHCH_3$) and 1.3-2.1 ppm of methylene and methine protons in poly(NIPAAm) backbones (Appendix).

Characterization of composite CMC microspheres containing poly(NIPAAm) and MNPs

Figure 3.1 shows FTIR spectra of unmodified CMC, poly(NIPAAm) homopolymer, bare MNPs and composite CMC microsphere (TRM6). Poly(NIPAAm) homopolymers were separately synthesized for the analysis of their functional groups (Figure 3.1B). FTIR spectrum of TRM6 (Figure 3.1D) exhibited the characteristic absorption signals of amide at 1,625 cm^{-1} (NH-CO- stretching) and magnetite core at 574 cm^{-1} (Fe-O stretching), indicating the presence of poly(NIPAAm) and MNPs in composite CMC microspheres.

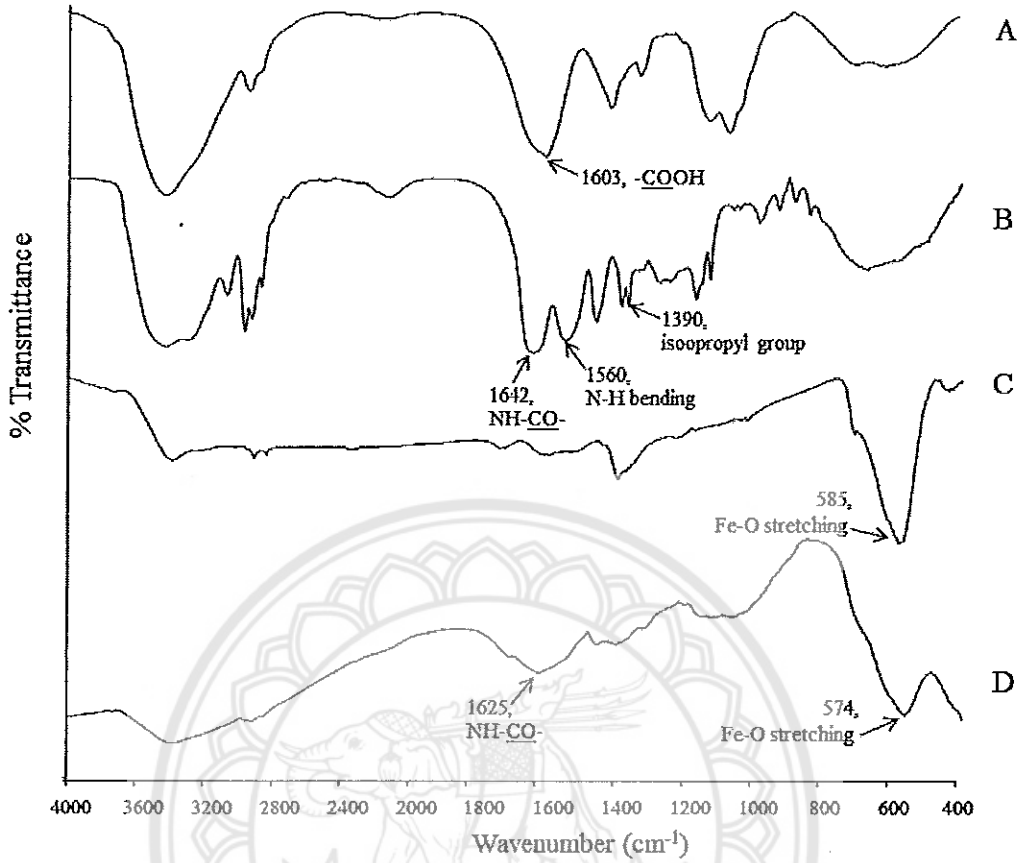


Figure 3.1 FTIR spectra of (A) CMC, (B) poly(NIPAAm), (C) MNPs (D) composite CMC microsphere (TRM6 microsphere)

Morphological studies of composite CMC microspheres

Because ratios of CMC, NIPAAm, glutaraldehyde and paraffin oil were important factors that affect shape of the microspheres, tuning the ratios of these compositions in the emulsion reaction to obtain uniform-sized and spherical microspheres were investigated. Formulations of composite CMC microsphere are shown in Table 2.1. Figure 3.2 shows SEM images of composite CMC microspheres after *in situ* emulsion polymerizations using fixed amounts of Tween-20 emulsifier and NIPAAm monomers with various amounts of glutaraldehyde crosslinker in the presence of MNPs (the average size of MNPs ranged between 8 and 12 nm in

diameter). Without the use of glutaraldehyde crosslinker, flake type morphologies without any microspheres were observed (Figure 3.2A). Addition of 4-6 ml of glutaraldehyde solutions resulted in the formation of agglomerated microspheres (Figure 3.2B and 3.2C). The formation of these microspheres was attributed to crosslinking reactions of glutaraldehyde and thus shaping up CMC microspherical structure. However, optimization of the ratio of all components was still necessary to lessen the formation of agglomerated microspheres.

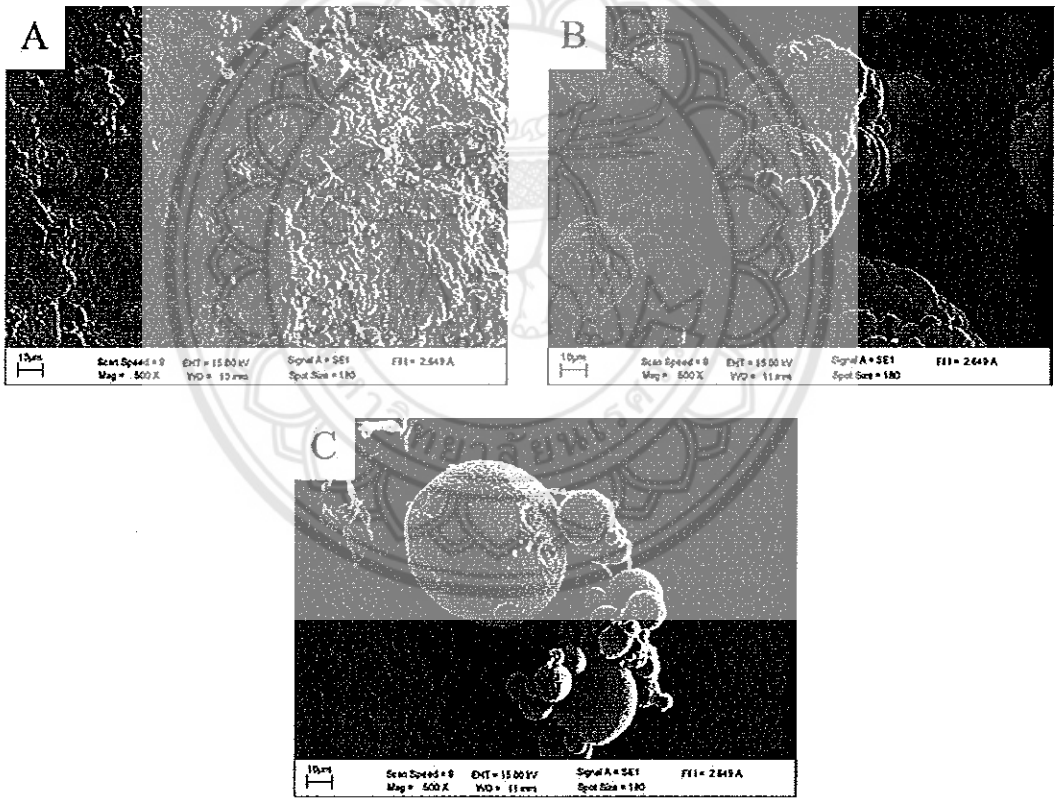


Figure 3.2 SEM images of (A) TRM1, (B) TRM2 and (C) TRM3 microspheres

Figure 3.3 shows SEM images of composite CMC microspheres having various amounts of NIPAAm monomers with fixed amounts of glutaraldehyde crosslinker and MNPs in the absence of Tween-20 emulsifier. It should be mentioned that the ones without poly(NIPAAm)(TRM4) showed irregular-shape microspheres. This indicated that poly(NIPAAm) played a role in the formation of the spherical shape (Figure 3.3A). Increasing NIPAAm concentrations in the reactions seemed to promote the formation of the microspheres with spherical shapes (Figure 3.3B, 3.3C, 3.3D). However, the presence of high poly(NIPAAm) concentrations in the reactions (TRM7) influenced the formation of large microspheres with broad size distributions. The sizes of TRM7 microspheres ranged between 30 and 60 μm with the average diameter (Dave) of 45.0 ± 3.8 μm, while those of well discrete microspheres (TRM5 and TRM6) ranged between 15 and 30 μm with narrow size distributions (Dave = 30.0 ± 1.0 μm).

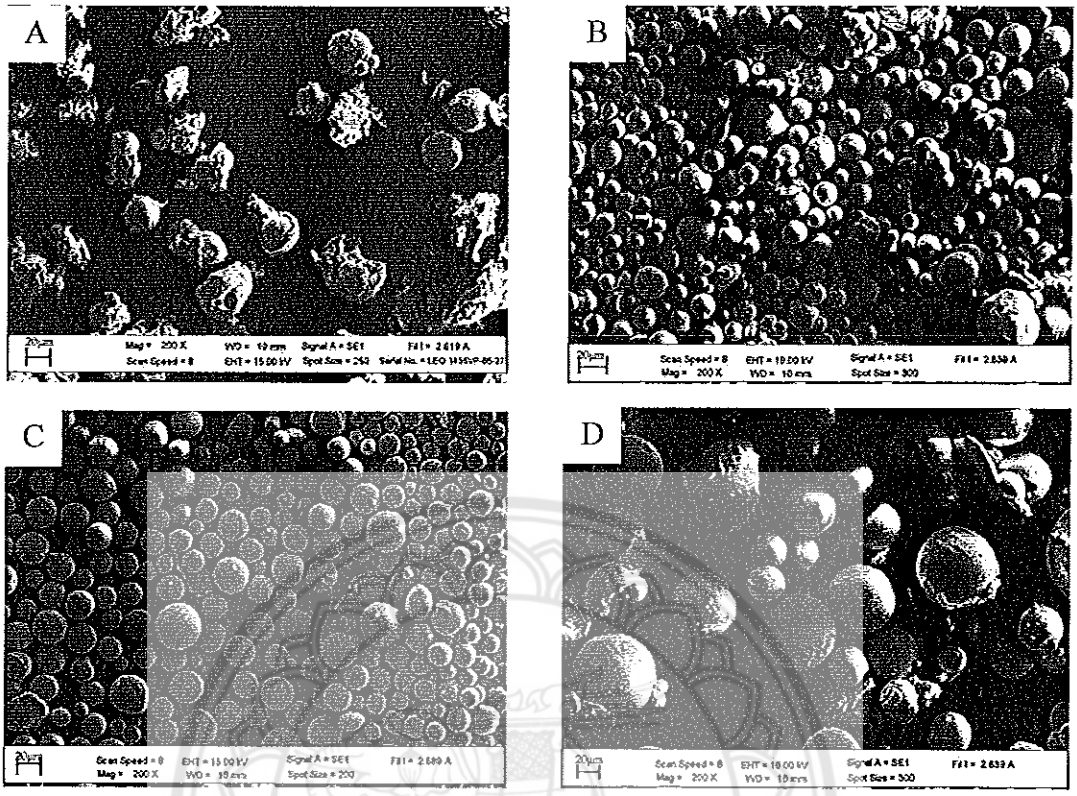


Figure 3.3 SEM images of (A) TRM4, (B) TRM5, (C) TRM6 and (D) TRM7 microspheres

Water swelling properties of composite CMC microspheres as a function of solution temperature and pH

Figure 3.4A shows effect of solution temperature on water swelling behavior of composite CMC microspheres. The experiments were performed in three solution temperatures (10, 30 and 50 °C) based on the hypothesis that poly(NIPAAm) can swell in solutions at temperatures below LCST of poly(NIPAAm) (32°C) and de-swell at those above the LCST. TRM4 as a control sample showed no temperature dependence due to the absence of thermo-responsive poly(NIPAAm) in its structure. The microspheres containing poly(NIPAAm) (TRM5, TRM6 and TRM7) showed thermo-

responsive behavior as indicated by a drop of %EWC when solution temperatures were increased from 10°C to 50°C. This behavior corresponded to the phase transition from swollen state to shrinkage state of poly(NIPAAm) in the microspheres when the solution temperature passed its LCST (32°C). In addition, incorporation of poly(NIPAAm) to the microspheres seemed to increase their water swellability; %EWC significantly increased from 150% in the samples without poly(NIPAAm) (TRM4) up to 236% in the samples with poly(NIPAAm) (TRM7). This was attributed to the presence of poly(NIPAAm) grafted in the microspheres, which thus promoted their water swellability.

Water swelling behavior of composite CMC microspheres as a function of solution pH is shown in Figure 3.4B. The experiments were carried out in three solution pHs (3, 7 and 11) to investigate %EWC of the microspheres in acidic, neutral and basic pHs. Similar to the discussion above, increasing the poly(NIPAAm) contents in the microspheres seemed to increase their water swellability. In addition, these microspheres also exhibited pH-responsive properties due to the existence of COOH in CMC structure. Water swellability of the microspheres at pH 11 was better than those at pH 7 and pH 3. This was attributed to the change from COOH in pH 3 to COO^- in pH 11 in CMC chains. At pH 3, most of carboxylic groups should exist in the form of COOH because the pK_a of CMC was about 6-7 (Mourya, Inamdar, & Tiwari, 2010; Tungtong, Okonogi, Chowwanapoonpohn, Phutdhawong, & Yotsawimonwat, 2012). The hydrogen bonds among COOH in CMC chains led to the polymer-polymer interactions, which dominated polymer-water interactions, therefore %EWC of the microspheres was low. At pH 11, the carboxylic groups became ionized and existed in the form of carboxylate anions (COO^-). Therefore, electrostatic repulsion among COO^-

promoted water swellability of the microspheres. In addition, hydrogen bonds between COO^- and H_2O were easily formed and thus enhanced %EWC of the microspheres.

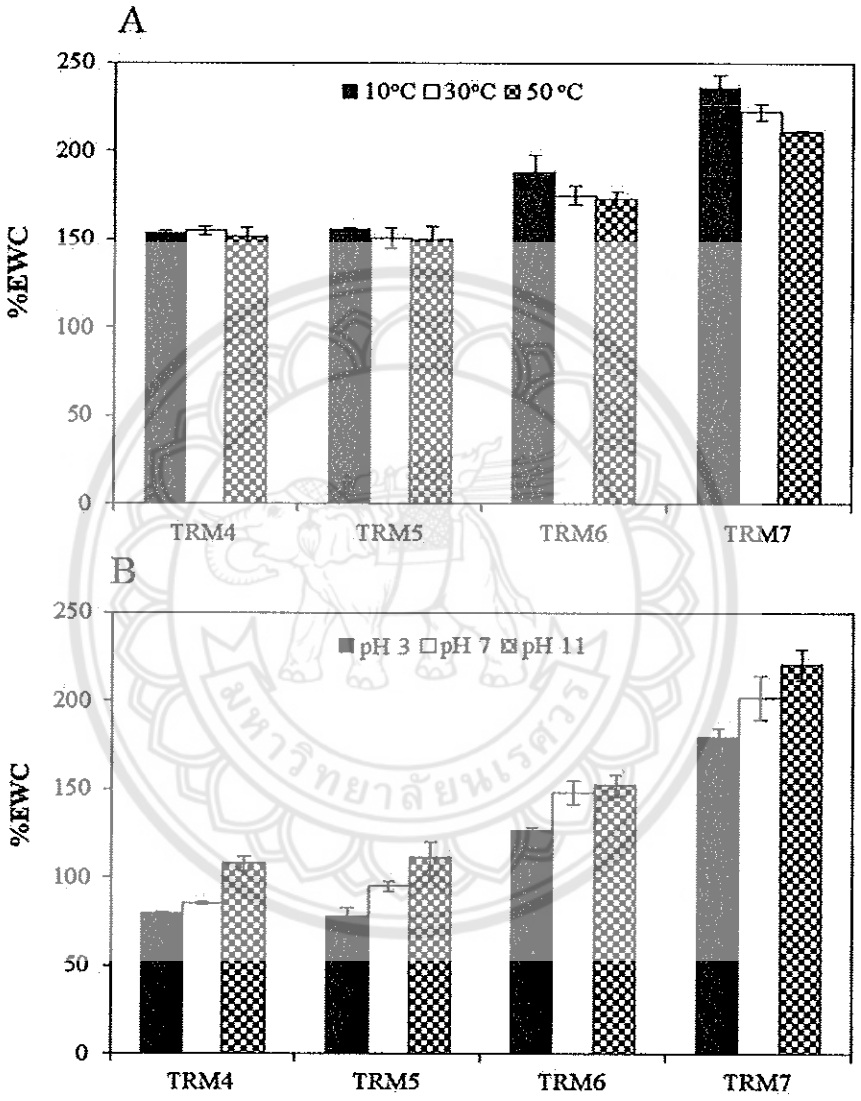


Figure 3.4 Equilibrium water content (%EWC) of composite CMC microspheres, (A) as a function of temperatures (10°C, 30°C and 50°C) in a pH 7 solution and (B) as a function of solution pHs (pH 3, pH 7 and pH 11) at 25°C

Thermogravimetric analysis of composite CMC microspheres

TGA curves of MNPs and all composite CMC microspheres (TRM4, TRM5, TRM6 and TRM7) are shown in Figure 38. The weight loss of MNPs (Figure. 3.5A) was 12 % and this was probably due to volatilization from their surface. The weight loss of composite CMC microspheres (TRM4, TRM5, TRM6, and TRM7) ranged between 83 % and 87 % (Figure 3.5B-3.5E). The drastic drops of their weights were contributed to the decomposition of organic components, including CMC and poly(NIPAAm), in the microspheres. When taking the initial weight loss of MNPs into account, there was about 71-75% of organic polymers and 25-29% of MNPs embedded in the microspheres.

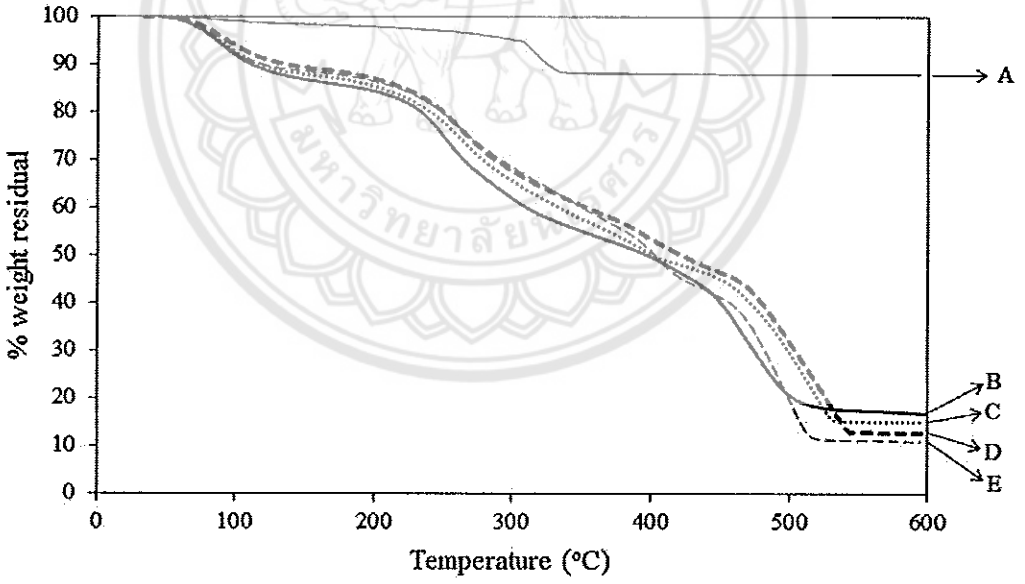


Figure 3.5 TGA thermograms of (A) MNPs, (B) TRM4, (C) TRM5, (D) TRM6 and (E) TRM7

Magnetic properties of composite CMC microspheres

Magnetic properties of composite CMC microspheres were investigated by VSM technique. As shown in Figure 3.6, saturation magnetization (M_s) of the microspheres (TRM4, TRM5, TRM6 and TRM7) ranged between 2.1 and 3.6 emu/g sample, while that of MNPs was 54.5 emu/g. The low M_s values of the microspheres as opposed to MNPs were attributed to the low MNPs contents due to the existence of diamagnetic CMC and poly(NIPAAm) in their structure. This was in good agreement with TGA results, indicating high organic components (>70%) and low MNPs contents (<20%) in the microspheres (Figure 3.5). Despite a significant decrease of M_s values as compared to MNPs, these microspheres could be separated from aqueous dispersions with an assistance of a magnet. The capability in magnetic-assisted separation of these microspheres is shown in the inset in Figure 3.6. Moreover, all composite CMC microspheres displayed superparamagnetic behavior as indicated by the absence of remanence and coercivity upon removal of the applied magnetic field.

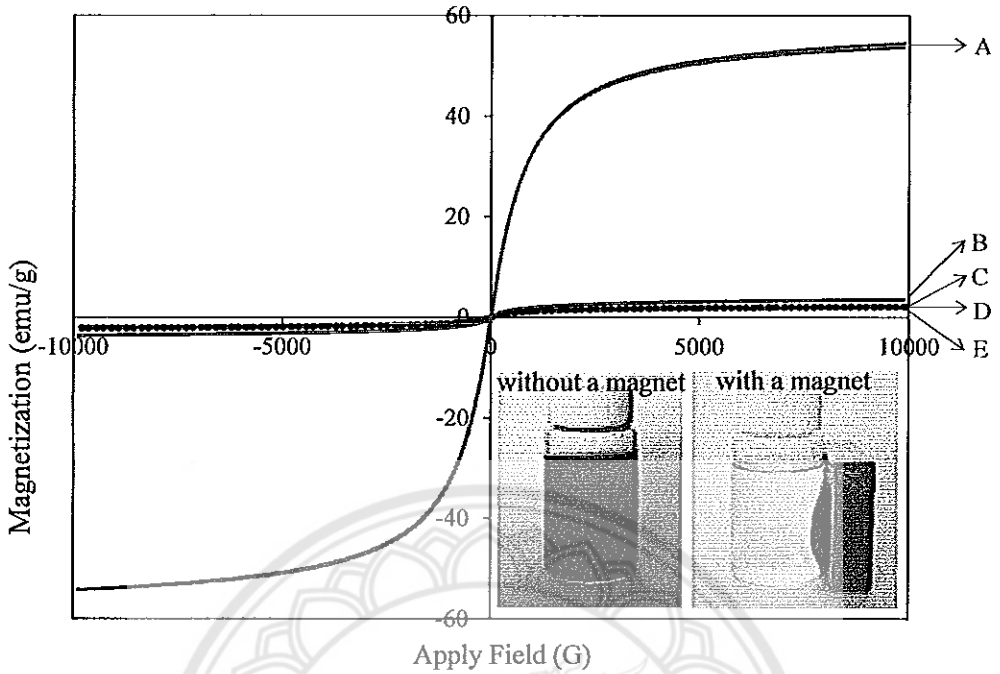


Figure 3.6 *M-H* curves of A) MNPs, B) TRM4, C) TRM5, D) TRM6 and E) TRM7 microspheres. The inset shows a magnetic-assisted separation of TRM6 microspheres in an aqueous dispersion

Effect of temperature and pH changes on releasing rate of indomethacin

Indomethacin was used a model drug for the controlled release studies with varying solution temperatures and pHs. TRM6 microspheres were selected as a representative for these studies because they showed good spherical morphology and narrow size distribution (Figure 3.3). It was found that %EE and %DLE of TMR6 microspheres were 95.5% and 2.7%, respectively. The indomethacin releasing studies of the microspheres were carried out in phosphate buffer saline (PBS) solution with pH 7.4 with varying temperatures (10, 30 and 50 ^{o}C) and in buffer solutions with varying pHs (pH 3, 7 and 11) (Figure 3.7). The release of indomethacin was measured after 48 h observation. 87% Indomethacin was released at the temperature above its

LCST (50 °C), compared to 70% indomethacin released when the temperature was below its LSCT (10 °C). The higher percentage drug release at the temperature above its LCST was attributed to the squeezing mechanism of the shrinking poly(NIPAAm) in the microspheres at 50 °C (Figure 3.0A), resulting in an increased amount of indomethacin being released.

Figure 3.7B presents the drug release profiles of TRM6 microspheres in buffer solutions with pH 3, 7 and 11 at room temperature. It was hypothesized that the drug releasing behavior of the microspheres was also dependent on the solution pH due to the presence of pH-responsive carboxylate groups in CMC chains. After 48 h observation, a high percentage of drug release was observed in the basic pH solution (26%), while those in neutral and acidic pH solutions (18% and 8%, respectively) were significant lower. This was attributed to the increase in volume of swollen TRM6 microspheres in basic solutions due to negative charged repulsion of carboxylate (COO^-) among CMC chains and promoting hydrogen bonding with surrounding water. Therefore, external water molecules could diffuse into the microspheres and the drug could be dissolved and released because of the osmotic pressure. In pH 3, TRM6 microspheres barely swelled in the aqueous solutions, therefore the amounts of released drug were lower than those at pH 11. This explanation agreed well with good water swelling properties of the microspheres in pH 11 solutions and low %EWC in pH 3 solutions (Figure 3.4B).

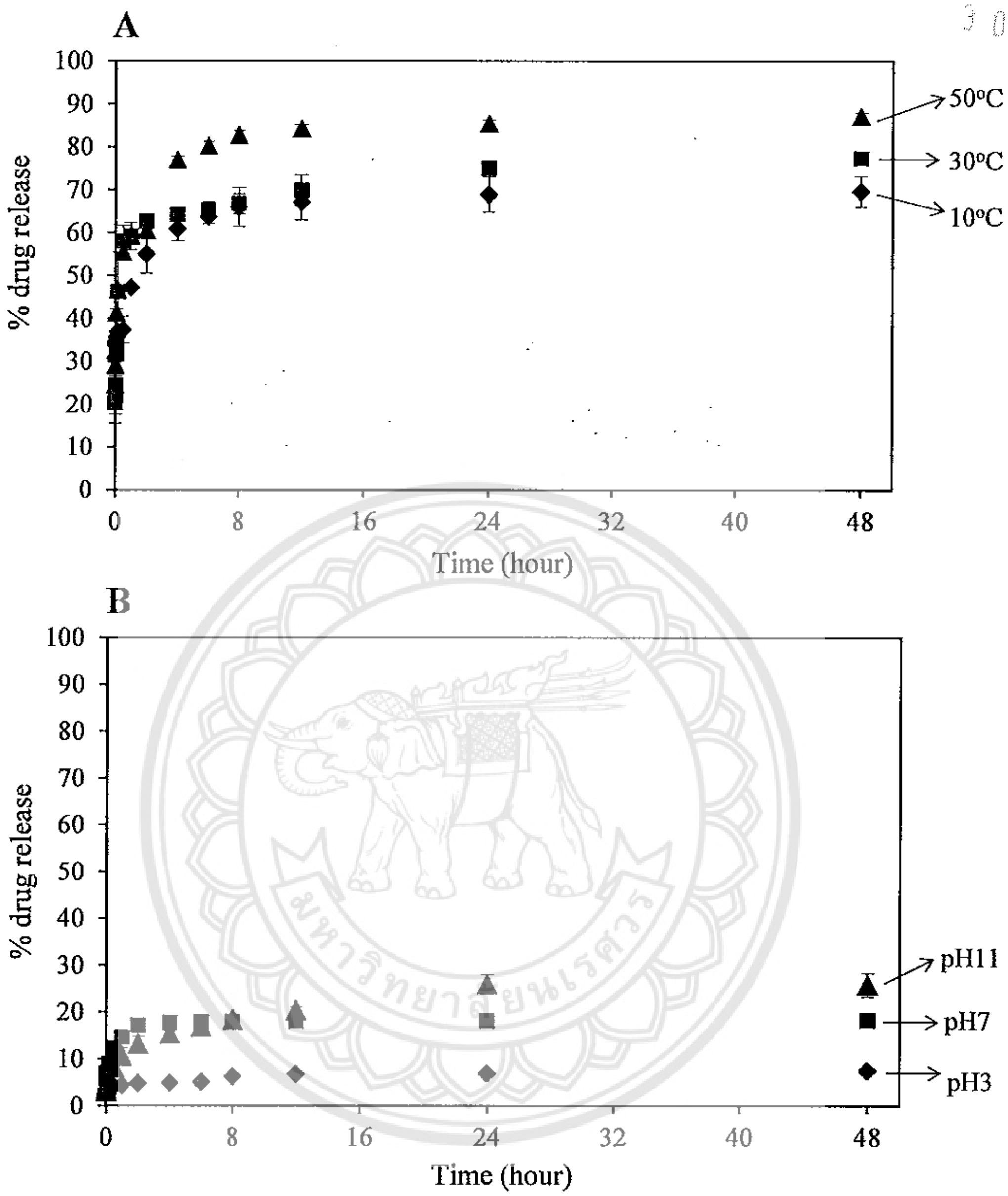


Figure 3.7 Release behavior of indomethacin of TRM6 micropheres in different (A) temperatures and (B) pHs

CHAPTER IV

CONCLUSIONS

Poly(NIPAAm)-grafted composite CMC microspheres embedded with MNPs were synthesized *via* an *in situ* free radical polymerization and an emulsion crosslinking technique to form multi-responsive microspheres with good water swellability. The composite CMC microspheres exhibited good responses to applied magnetic fields and the changes in their solution temperature and pH. Magnetic-responsive properties allowed them to be facilely separated with the use of a permanent magnet, while their temperature and pH-responsive properties can be used as triggering mechanisms for controlled releases of entrapped therapeutic drugs.

REFERENCES

Ahmad, H., Nurunnabi, M., Rahman, M. M., Kumar, K., Tauer, K., Minami, H., & Gafur, M. A. (2014). Magnetically doped multi stimuli-responsive hydrogel microspheres with IPN structure and application in dye removal. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *459*, 39-47.

Agnihotri, S. A., & Aminabhavi, T. M. (2006). Novel interpenetrating network chitosan- poly(ethylene oxide-*g*-acrylamide) hydrogel microspheres for the controlled release of capecitabine. *International Journal of Pharmaceutics*, *324*, 103-115.

Bhatt, A. S., Bhat, D. K., & Santosh, M. S. (2010). Electrical and magnetic properties of chitosan-magnetite nanocomposites. *Physica B: Condensed Matter, 405*, 2078-2082.

Chaleawlert, S., & Pimpha, N. (2012). Preparation of magnetic polymer microspheres with reactive epoxide functional groups for direct immobilization of antibody. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *414*, 66-74.

Chen, D., Li, W., Wu, Y., Zhu, Q., Lu, Zh., & Du, G. (2013). Preparation and characterization of chitosan/montmorillonite magnetic microspheres and its application for the removal of Cr (VI). *Chemical Engineering Journal, 221*, 8-15.

Chen, J., Sun, J., Yang, L., Zhang, Q., Zhu, H., & Wu, H., et al. (2007). Preparation and characterization of a novel IPN hydrogel membrane of poly(*N*-isopropylacrylamide)/carboxymethyl chitosan (PNIPAAM/CMCS). *Radiation Physics and Chemistry, 76*, 1425-1429.

Constantin, M., Cristea, M., Ascenzi, P., & Fundueanu, G. (2011). Lower critical solution temperature versus volume phase transition temperature in

thermoresponsive drug delivery systems. *eXPRESS Polymer Letters, 5*(10), 839-848.

Delmar, K. & Bianco-Peled, H. (2016) Composite chitosan hydrogels for extended release of hydrophobic drugs. *Carbohydrate Polymers, 136*, 570–580.

Don, T. M., & Chen, H. R. (2005). Synthesis and characterization of AB-crosslinked graft copolymers based on maleilated chitosan and *N*-isopropylacrylamide. *Carbohydrate Polymers, 61*, 334-347.

Fan, L., Du, Y., Zhang, B., Yang, J., Zhou, J., & Kennedy, J. F. (2006). Preparation and properties of alginate/carboxymethyl chitosan blend fibers. *Carbohydrate Polymers, 65*, 447-452.

Fundueanu, G., Constantin, M., Asmarandei, I., Bucatariu, S., Harabagiu, V., Ascenzi, P., & Simionescu, B. C. (2013). Poly(*N*-isopropylacrylamide-co-hydroxyethylacryl-amide) thermosensitive microspheres: the size of microgels dictates the pulsatile release mechanism. *European Journal of Pharmaceutics and Biopharmaceutics, 85*, 614-623.

Fundueanu, G., Constantin, M., Asmarandei, I., Harabagiu, V., Ascenzi, P., & Simionescu, B. C. (2013). The thermosensitivity of pH/thermoresponsive microspheres activated by the electrostatic interaction of pH-sensitive units with a bioactive compound. *Journal of Biomedical Materials Research Part A, 101*(6), 1661-1669.

Glampedaki, P., Krägel, J., Petzold, G., Dutschk, V., Miller, R., & Warmoeskerken, M. M. C. G. (2012). Polyester textile functionalisation through incorporation of pH/thermo-responsive microgels. Part I: Microgel preparation and

characterization. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 47 (5)*, 2078-2087.

Gong, Y., Liu, Q. L., Zhu, A. M., & Zhang, Q. G. (2012). One-pot synthesis of poly(*N*-isopropylacrylamide)/chitosan composite microspheres *via* microemulsion. *Carbohydrate Polymers, 90*, 690-695.

Guo, F., Zhang, Q., Wang, W., Zhang, H. & Sun, J. (2011). Preparation of pH-responsive Fe_3O_4/Poly (acrylic acid-stat-methyl methacrylate-block-(2-dimethylamino) ethyl methacrylate) magnetic composite microspheres and its application in controlled release of drug. *Materials Science and Engineering C, 31*, 938–944.

Guo, F., Zhang, Q., Zhang, B., Zhang, H. & Zhang, L. (2009). Preparation and characterization of magnetic composite microspheres using a free radical polymerization system consisting of DPE. *Polymer, 50*, 1887–1894.

Ha, Ng. Th., Hai, Ng. H., Luong, Ng. H., Chau, Ng., & Chinh, Y. D. (2008). Effects of the conditions of the microemulsion preparation on the properties of Fe_3O_4 nanoparticles. *VNU Journal of Science, Natural Sciences and Technology, 24*, 9-15.

Jiang, T., Nukavarapu, S. P., Deng, M., Jabbarzadeh, E., Kofron, M. D., Doty, S.B., et. al. (2010). Chitosan-poly(lactide-*co*-glycolide) microsphere-based scaffolds for bone tissue engineering: In vitro degradation and in vivo bone regeneration studies. *Acta Biomaterialia, 6*, 3457-3470.

Klinger, D., & Landfester, K. (2012). Stimuli-responsive microgels for the loading and release of functional compounds: Fundamental concepts and applications. *Polymer*, *53*(23), 5209-5231.

Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., & Elst, L. V., et. al. (2008). Magnetic iron oxide nanoparticles: Synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Chemical Reviews, 108*(6), 2064-2110.

Lei, Zh., Pang, X., Li, N., Lin, L., & Li, Y. (2009). A novel two-step modifying process for preparation of chitosan-coated Fe_3O_4/SiO_2 microspheres. *Journal of materials processing technology, 209*, 3218-3225.

Li, F., Li, X., & Li, B. (2011). Preparation of magnetic polylactic acid microspheres and investigation of its releasing property for loading curcumin. *Journal of Magnetism and Magnetic Materials, 323*, 2770-2775.

Li, G. Y., Zhong, M., Zhou, Z. D., Ding, P., & Li, Y. J. (2011). Novel Carboxymethyl chitosan microspheres for controlled delivery of chelerythrine. *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, *48*, 904-911.

Li, G. Y., Zhong, M., Zhou, Z. D., Zhong. Y. D., Ding, P., & Huang, Y. (2011). Formulation optimization of chelerythrine loaded O-carboxymethylchitosan microspheres using response surface methodology. *International Journal of Biological Macromolecules*, *49*, 970-978.

Maity, D., Kale, S. N., Ghanekar, R. K., Xue, J. X., & Ding, J. (2009). Studies of magnetite nanoparticles synthesized by thermal decomposition of iron (III) acetylacetonate in tri(ethylene glycol). *Journal of Magnetism and Magnetic Materials, 321*(19), 3093-3098.

Meng, X., Xu, G., Zhou, Q. L., Wu, J. P., & Yang, L. R. (2013). Improvements of lipase performance in high-viscosity system by immobilization onto a novel kind of poly(methylmethacrylate-*co*-divinylbenzene) encapsulated porous magnetic microsphere carrier. *Journal of Molecular Catalysis B: Enzymatic, 89*, 86-92.

Motornov, M., Roiter, Y., Tokarev, I., & Minko, S. (2010). Stimuli-responsive nanoparticles, nanogels and capsules for integrated multifunctional intelligent systems. *Progress in Polymer Science, 35*, 174-211.

Mourya, V. K., Inamdar, N. N., & Tiwari, A. (2010). Carboxymethyl chitosan and its applications. *Advanced Materials Letters, 1*(1), 11-33.

Mu, Q., & Fang, Y. (2008). Preparation of thermosensitive chitosan with poly(*N*-isopropylacrylamide) side at hydroxyl group *via O*-maleoyl-*N*-phthaloyl-chitosan (MPCS). *Carbohydrate Polymers, 72*, 308-314.

Reddy, C. L. N., Swamy, B. Y., Prasad, C. V., Aswini, C., Mamatha, P., Subha, M. C. S., & Rao, K. C. (2011). Synthesis and characterization of poly(NIPAM-*co*-caprolactam) thermoresponsive microspheres for controlled release of acebutolol hydrochloride. *International Journal of Pharmacy and Pharmaceutical Sciences, 3*(1), 215-221.

Sun, X., Shi, J., Xu, X., & Cao, Shaokui. (2013). Chitosan coated alginate/poly(N-isopropylacrylamide) beads for dual responsive drug delivery. *International Journal of Biological Macromolecules, 59*, 273-281.

Tu, H., Qu, Y., Hu, X., Yin, Y., Zheng, H., & Xu, P., et al. (2010). Study of the Sigmoidal swelling kinetics of carboxymethylchitosan-*g*-poly(acrylic acid)

hydrogels intended for colon-specific drug delivery. *Carbohydrate Polymers, 82*, 440-445.

Tungtong, S., Okonogi, S., Chowwanapoonpohn, S., Phutdhawong, W., & Yotsawimonwat, S. (2012). Solubility, viscosity and rheological properties of water-soluble chitosan derivatives. *Maejo International Journal of Science and Technology, 6*(02), 315-322.

Varaprasad, K., Vimala, K., Ravindra, S., Reddy, N. N., Reddy, G. S. M., Raju, K. M. (2012). Biodegradable chitosan hydrogels for In Vitro drug release studies of 5-flurouracil an anticancer drug. *Journal of Polymers and the Environment, 20*, 573-582.

Wu, J., Liao, Ch., Wang, Zh., Cheng, W., Zhou, N., Wang, Sh., & Wan, Y. (2011). Chitosan-polycaprolactone microspheres as carriers for delivering glial cell line-derived neurotrophic factor. *Reactive & Functional Polymers, 71*, 925-932.

Yan, H., Yang, L., Yang, Z., Yang, H., Li, A., & Cheng, R. (2012). Preparation of chitosan/poly(acrylic acid) magnetic composite microspheres and applications in the removal of copper(II) ions from aqueous solutions. *Journal of Hazardous Materials, 229*(230), 371-380.

Yuan, Q., Venkatasubramanian, R., Hein, S., & Misra, R. D. K. (2008). A stimulus-responsive magnetic nanoparticle drug carrier: Magnetite encapsulated by chitosan-grafted-copolymer. *Acta Biomaterialia, 4*, 1024-1037.

Yuan, Q., Li, N., Chi, Y., Gen, W., Yan, W., Zhao, Y., et. al. (2013). Effect of large pore size of multifunctional mesoporous microsphere on removal of heavy metal ions. *Journal of Hazardous Materials, 254-255*, 157-165.

Zhang, J., Zhang, S., Wang, Y., & Zeng, J. (2007). Preparation and characterization of composite magnetic microspheres. *Journal of Composite Materials, 41*(14), 1703-1712.

Zhang, J., Zhang, S., Wang, Y., & Zeng, J. (2007). Composite magnetic microspheres: Preparation and characterization. *Journal of Magnetism and Magnetic Materials. 309*, 197-201.

Zhao, H., Saatchi, K., & Häfeli, U. (2009). Preparation of biodegradable magnetic microspheres with poly(lacticacid)-coated magnetite. *Journal of Magnetism and Magnetic Materials, 321*, 1356-1363.

Zhao, Z. P., Wang, Z., & Wang, S. C. (2003). Formation, charged characteristic and BSA adsorption behavior of carboxymethyl chitosan/PES composite MF membrane. *Journal of Membrane Science, 217*, 151-158.

Zhang, H., Zhang, Q., Zhang, B. & Guo, F. (2009). Preparation of magnetic composite microspheres by surfactant free controlled radical polymerization: Preparation and characteristics, *Journal of Magnetism and Magnetic Materials*, *321*, 3921–3925.

APPENDIX

Supporting information

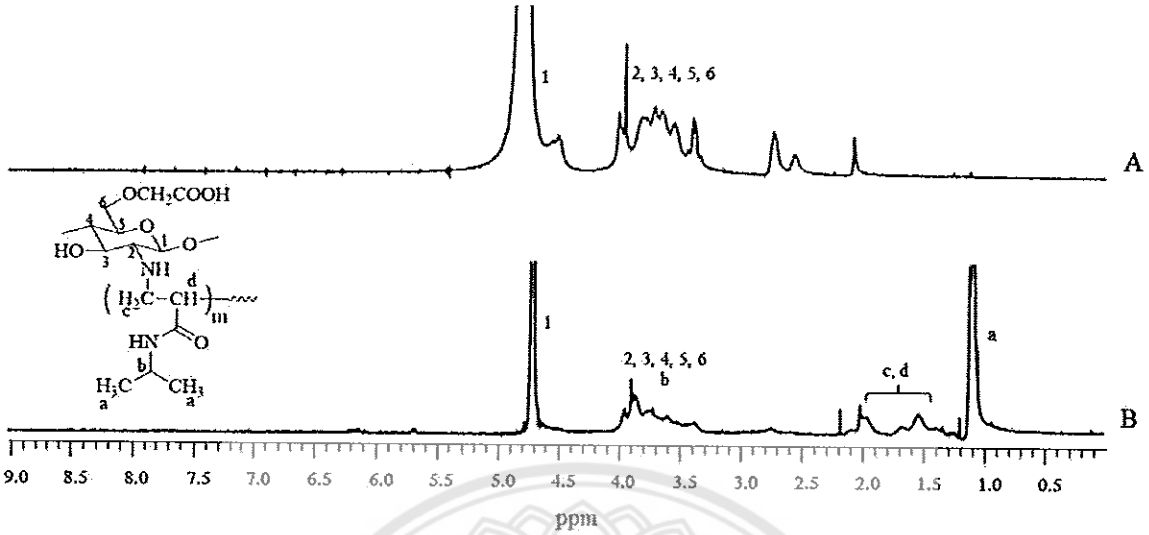


Figure 1S. ^{1}H NMR spectra of A) CMC and B) poly(NIPAAm)-grafted CMC without a crosslinking reaction (solvent: D_2O)

Table 1S Formulations of the composite CMC microsphere to study the effect of the amount of paraffin oil used in the reactions on the formation of the microsphere

Sample[a]	NIPAAm (g)	25% Glutaraldehyde (ml)	Tween-20 (ml)	Paraffin oil (ml)	Appearance[b]
TRM6	0.4	6	-	30	Spherical microsphere
TRM8	0.4	6	-	20	Irregular-shape microsphere
TRM9	0.4	6	-	10	Irregular-shape microsphere

[a] 0.4 g of CMC and 0.06 g of MNP were used in all experiments, [b] Observed from SEM

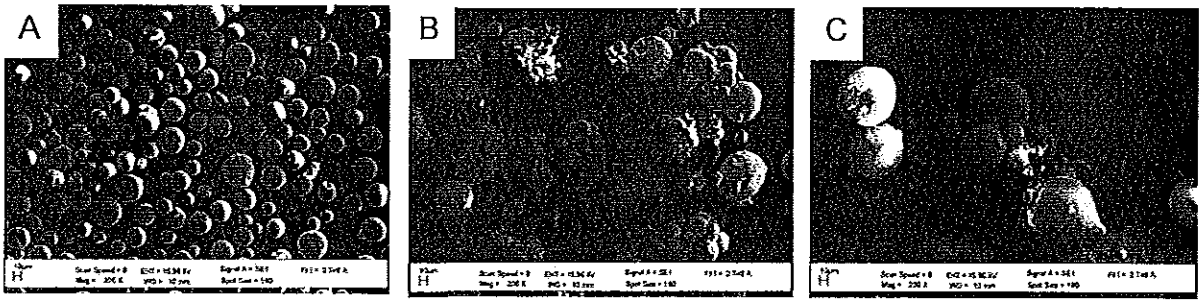


Figure 2S The effect of the amount of paraffin oil used in the reactions on the formation of the composite CMC microspheres, (A) TRM6, (B) TRM8 and (C) TRM9.

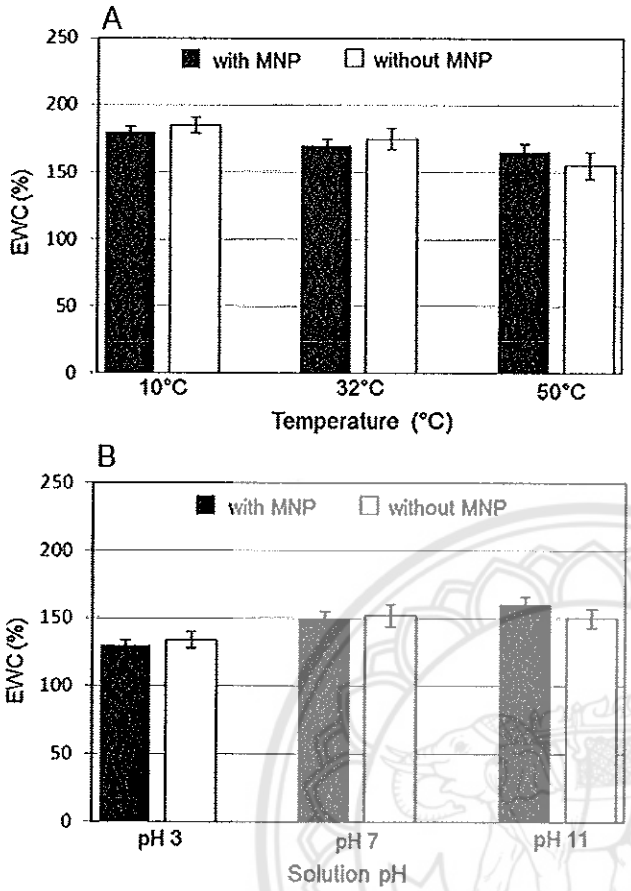


Figure 3S The effect of MNP in the microsphere on EWC as a function of (A) temperature and (B) solution pH

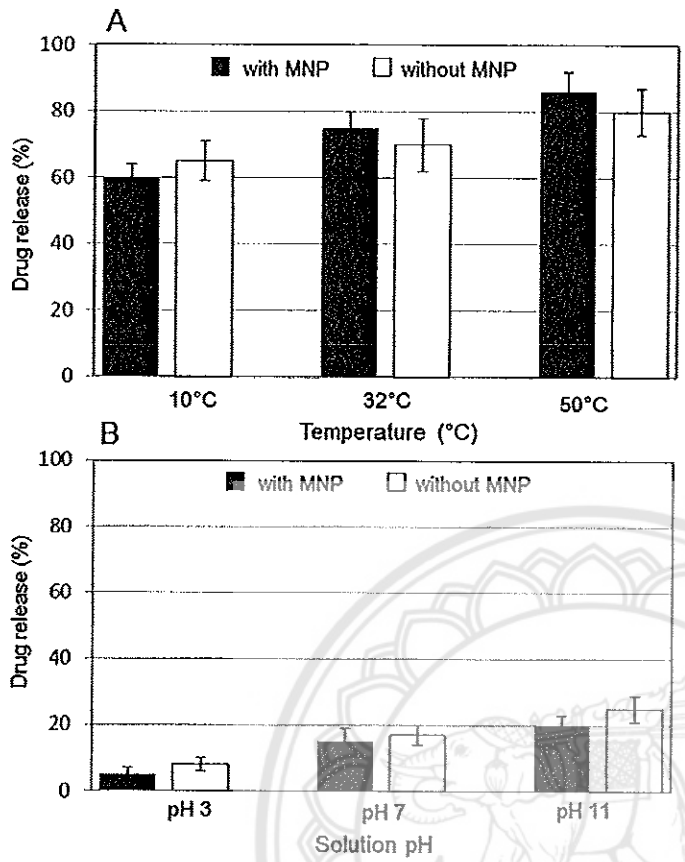


Figure 4S The effect of MNP in the microsphere on drug release properties as a function of (A) temperature and (B) solution pH

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Multi-responsive magnetic microsphere of poly(*N*-isopropylacrylamide)/carboxymethylchitosan hydrogel for drug controlled release

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ABSTRACT

Multi-responsive composite microspheres were synthesized *via* an *in situ* free radical polymerization of thermo-responsive poly(*N*-isopropylacrylamide) (poly(NIPAAm)) in the presence of carboxymethylchitosan (CMC) and magnetite nanoparticles (MNPs) followed by glutaraldehyde crosslinking. Formulation conditions of the composite microspheres were tuned such that spherical microspheres with narrow size distributions were obtained (30.0 ± 1.0 μm in diameter). They responded well to an applied magnetic field and showed water swelling responses to the change in solution pH and temperature. The release of an entrapped indomethacin model drug was accelerated when the solution temperature was above its lower critical solution temperature (LCST) (50 °C) or when the solution pH was in basic conditions (pH 11). These responsive properties can be used as triggering mechanisms for releases of the entrapped drugs from the microspheres, indicating their great potentials for use in controlled release applications.

1. Introduction

Stimuli-responsive microsphere, also called "intelligent" or "smart" microsphere, has been extensively studied in recent years because it is capable in response to environmental change, *e.g.* pH, temperature, the presence/absence of chemicals and biological compounds, or applications of external stimuli, such as light, magnetic or electric fields and ionic strength (Ahmad et al., 2014; Klinger & Landfester, 2012; Motornov, Roiter, Tokarev, & Minko, 2010; Sun, Shi, Xu, & Cao, 2013). It has been of particular interest in a wide variety of biomedical or pharmaceutical applications such as controlled drug release and delivery, chemical separation and tissue engineering (Agnihotri and Aminabhavi, 2006; Chaleawlert & Pimpha, 2012; Li, Zhong, Zhou, Ding, & Li, 2011; Li, Zhong, Zhou, Zhong et al., 2011; Varaprasad et al., 2012; Yan et al., 2012). In general, it undergos drastic swelling and shrinkage response to the environmental stimuli at its phase transition.

Weakly ionizable polysaccharide, such as alginate, bean gum, chitosan and its derivative, has also been of great interest as pH-responsive hydrogel due to its excellent antibacterial activity, non-cytotoxicity, excellent biocompatibility and high water solubility (Fan et al., 2006; Zhao, Wang, & Wang, 2003; Don and Chen, 2005). Carboxymethylchitosan (CMC), a water soluble chitosan derivative, is typically prepared *via* a carboxymethylation of chitosan with monochloroacetic acid at some of amino and hydroxyl groups in the glucosamine units (Chen et al., 2007; Tu et al., 2010). Previous works have reported the synthesis of chitosan and CMC in the form of microspheres with great potentials in biomedical applications (Gong, Liu, Zhu, & Zhang, 2012). Also, chitosan- and CMC-based microspheres copolymerized or physically blended with other polymers, compounds or nanoparticles, *e.g.*, poly(ethylene oxide-*g*-acrylamide) (Agnihotri and Aminabhavi, 2006), poly(ε-caprolactone) (Wu et al., 2011), chelerythrine and magnetite nanoparticles (MNPs) (Li, Zhong, Zhou, Ding et al., 2011; Li, Zhong, Zhou, Zhong et al., 2011), have been previously studied.

Another type of "smart" materials that has recently gained much attention is thermo-responsive microsphere. Poly(*N*-isopropylacylamide) (poly(NIPAAm)), the best known polymer in this class, undergoes a volume phase transition at the lower critical solution temperature (LCST) around 32 °C (Fundueanu, Constantin, Asmarandei, Bucatariu et al., 2013; Fundueanu, Constantin, Asmarandei, Harabagiu et al., 2013; Glampedaki et al., 2012; Mu and Fang, 2008; Yuan, Venkatasubramanian, Hein, & Misra, 2008). At the temperature below its LCST, water molecules

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