CHAPTER V

MAGNETIC-RESPONSIVE CHITOSAN HYDROGELS AND AEROGELS

5.1 Abstract

Magnetic-responsive materials are known for their external magnetic field responsive functions. Recently many studies have shown that hydrogels and/ or aerogels incorporated magnetic nanoparticles are another potential approach to achieve functional materials response to the magnetic field. Due to high surface area of the aerogels, the high adsorption of any substances (e.g. heavy metal, DNA) is expected. In the present work, we prepared magnetic responsive chitosan hydrogel/ aerogel by dispersing magnetic nanoparticles in chitosan aqueous solution followed by dicarboxylic acid terminated poly(ethylene glycol) conjugating reaction. The porous network of chitosan hydrogel with well-dispersed magnetic nanoparticles is obtained. SEM-EDX reveals the presence of magnetic nanoparticles on chitosan matrix even the gel is in fully swollen stage. Surface area analysis and DNA isolating study suggested that the highly porous chitosan aerogel shows high amount of extracted DNA.

Keywords: Magnetic Responsiveness, External Stimuli, Chitosan Hydrogel, Chitosan Aerogel

5.2 Introduction

Chitosan is a derivative of chitin, the second most abundant naturally occurring polysaccharide, obtained from chitin deacetylation. As chitosan is lacking of the water solubility, including organic solvent insolubility, based on its strong inter and intra-molecular hydrogen-bonded networks. Up to present, the use of acid solvent is the most practical way to materialize chitosan [1].

Hydrogels are three-dimensional networks with covalent bond or ionic crosslink which are able to retain water molecules more than 99% without dissolving. Hydrogels have received much attention for preparing biomedical materials as drug delivery and tissue engineering due to their significant water uptake [2]. Hydrogels provide the flexible texture as much as natural tissues. Thus, the use as implants can be expected for good compatibility with less irritation to the surrounding tissues. For chitosan gel, the easiest way is to dissolve chitosan in aqueous acetic acid and crosslink it by using dialdehyde. However, the gel obtained has disadvantages in terms of acid odor, toxicity (based on aldehyde and acid used) resulting in the non-biocompatibility and the risks for the use in biomedical fields. Chitosan hydrogels have known for a variety of biomedical application such as drug delivery, and tissue engineering. Fabricating chitosan hydrogel in water-based system by effective conjugation of chitosan and dicarboxylated poly(ethylene glycol) was purposed to avoid the problem of using acid or organic solvent [3].

Recently, many studies have shown that hydrogel incorporated magnetic nanoparticles is another potential approach to achieve hydrogel responsive to magnetic field which the controlled release can be expected [4].

In this work, we propose a simple chitosan gelation by using water soluble conjugating agent and dicarboxylated poly(ethylene glycol) to obtain chitosan hydrogel. The chitosan hydrogel and magnetic nanoparticles hybrid hydrogel and aerogel were fabricated via a simple mixing method. The surface area related to the DNA isolating property is studied.

5.3 Experimental

5.3.1 Materials

Chitosan (95% DD, M_w of 7.0 × 10^5) was supplied by Seafresh Chitosan (Lab) Co., Ltd., Thailand. 1-Hydroxybenzotriazole monohydrate ($HOBt{\cdot}H_2O$) and 1-ethyl-3-(3-dimethylaminopropyl-carbodiimide) hydrochloride (EDC·HCl) was purchased from Tokyo Chemical Industry Co., Ltd., Japan. Poly(ethylene glycol) (PEG) (M_n 1450 Dalton) was purchased from Sigma-Aldrich, Inc., USA. Carboxy-terminated poly(ethylene glycol) (COOH-PEG-COOH was prepared as reported previously [3]. Iron (II) sulfate hexahydrate (99%) and Iron (III) chloride heptahydrate (98%) was purchased from BDH chemicals. Oleic acid was purchased from Sigma Aldrich Inc., USA. 3-glycidoxypropyltrimethoxy silane was obtained from Dow Corning Toray Co., Ltd., Japan. Sodium hydroxide platelet was bought from Carlo Erba Reagent, Italy. Tetrahydrofuran (THF), and ethanol were purchased from LabScan, Ireland. All chemicals were used without further purification.

5.3.2 Preparation of magnetic nanoparticles, MAG

Magnetic nanoparticles (MAG) were prepared by co-precipitation of Fe^{2+} and Fe^{3+} ions (1:2 molar ratios) by 2M NaOH. The obtained black magnetic nanoparticles were then washed several times by distilled, de-ionized, and de-oxygen water until pH of washing water was decreased from ~11 to ~8. The magnetic nanoparticles were kept in distilled, de-ionized, and de-oxygen water with the known concentration (around 0.04 mg/mL) in the refrigerator to prevent oxidation reaction.

5.3.3 Magnetic chitosan hydrogel preparation

Chitosan hydrogel was prepared follow previous work described by our group [3]. In brief, chitosan (0.1g, 0.61 mmol) was mixed with $HOBt{\cdot}H_2O$ (0.094g, 0.61 mmol) in 10 mL of deionized water at ambient temperature until the clear solution was obtained. PEG (M_n 1450, 20.00 g, 13.8 mmol) was reacted with succinic anhydride (2.762 g, 27.6 mmol) in the presence of pyridine at 65 °C for 24 h. The crude product was purified by reprecipitating in diethyl ether, washing for

several times and drying in vacuum to obtain carboxyl terminated poly(ethylene glycol) (COOH-PEG-COOH). In case of magnetic chitosan hybrid hydrogel, MAG were mixed well in water-soluble chitosan or so called chitosan-hydroxy benzotriazole (CS-HOBt) solution at a concentration of chitosan 1 wt% before adding COOH-PEG-COOH. The hydrogels were formed at ambient temperature after mixing 15 min. The obtained hydrogels were then cut into a cube shape of 10 mm length and washed in DI water for 3 days to remove excess HOBt before freeze drying to get aerogel products for further studying.

5.3.4 Genomic DNA isolation from bacterial cells

The procedure for genomic DNA isolation from bacterial cells was carried out follow procedure in Chapter IV.

5.3.5 Characterizations

Fourier transform infrared spectrophotometer (FTIR) spectrum was carried out by using a Nicolet/Nexus 670 equipped with an attenuated total reflection (ATR) accessory. The analysis was carried out with 32 scans at a resolution of 4 cm^{-1} in a frequency range of 4000–650 cm^{-1}. Wide angle X-ray diffraction (WAXD) was studied by a RIGAKU RINT 2000 with CuKα as an X-ray source (λ=0.154178 nm). The scanning range was 2–90 degree with scanning rate of 2 degree/min. Scanning electron microscopy (SEM) observations were performed using a JEOL JSM-6701F at a working voltage of 15 keV. The static magnetization curves of different magnetic chitosan aerogels have been determined at room temperature. The magnetic measurements were carried out by a Lakeshore model 7404 vibrating sampling magnetometer (VSM). Swelling ratio can be calculated by weight of hydrogel in a fully swollen state (after 3 days of immersing in water at room temperature) divided by dried weight before soaking.

5.4 Results and Discussion

Magnetic chitosan hydrogels are obtained by using dicarboxylated poly(ethylene glycol) as a cross-linker and MAG as an inorganic modifier. All

reactions were carried out in water at room temperature to obtain chitosan hydrogel. The MAG and S-MAG ratios were varied to clarify the degree of responsiveness in a magnetic field.

5.4.1 Structural Characterization and Morphological Study

Figure 5.1 shows the appearance of magnetic chitosan hydrogels. The dark colors are depending on the ratio of MAG or S-MAG added.

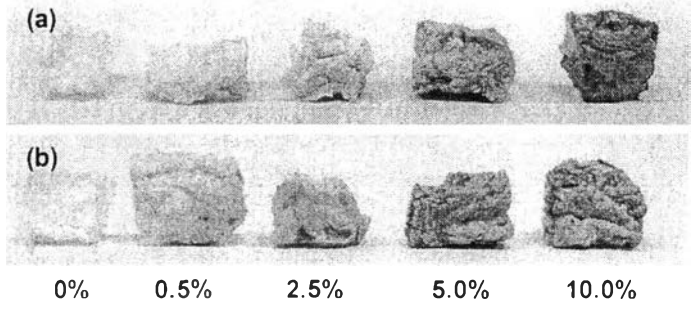

Figure 5.1 Appearances of magnetic chitosan hydrogels of; (a) MAG-added, and (b) S-MAG-added in hydrogel with various mole ratio.

Chitosan hydrogels were dialyzed and freeze-dried before analyzing by ATR-FTIR. Chitosan-HOBt shows a peak at 1647 cm^{-1} (amide I) (Figure 5.2 (a)), whereas the chitosan aerogels (curve (b) – (d)) show peaks at 1730 cm^{-1} and 1650 cm^{-1} corresponding to C=O ester and amide I (Figure 5.2 (b) – (d)). Peak at 2890 cm^{-1} in Figure 5.2 (b) – (d) belongs to C–H stretching.

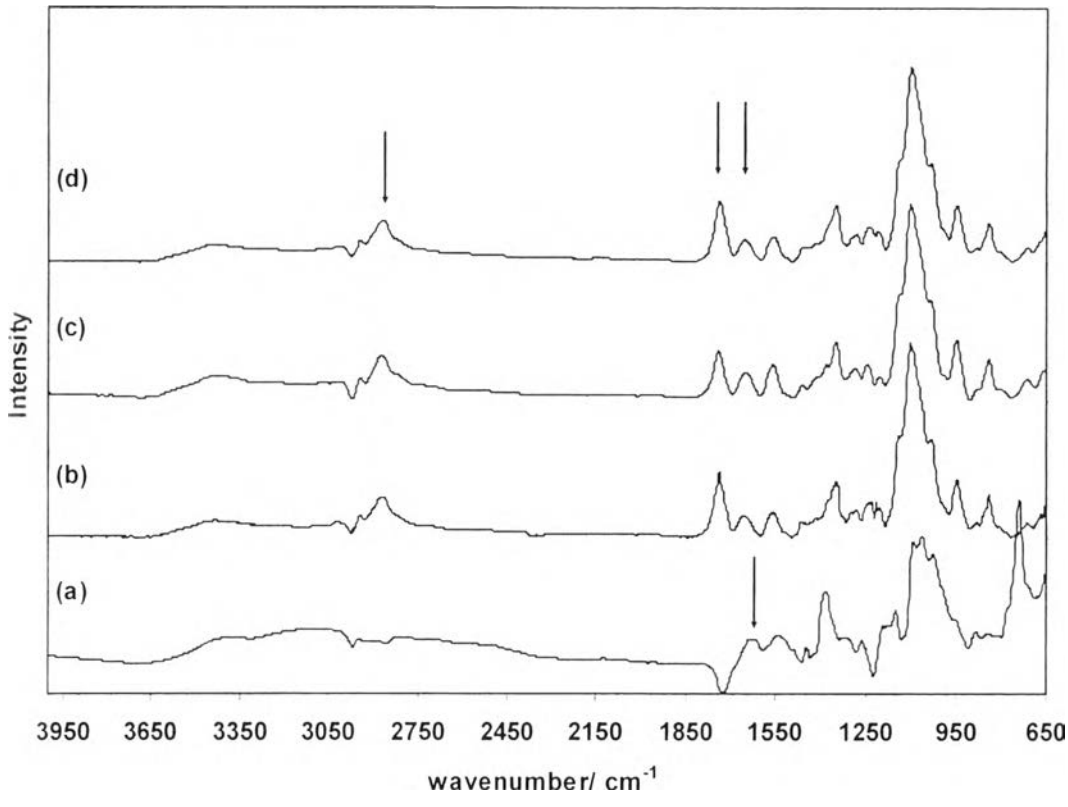

Figure 5.2 ATR-FTIR spectra of; (a) chitosan, (b) chitosan aerogel, (c) chitosan aerogel containing MAG, and (d) chitosan aerogel containing S-MAG.

The samples were analyzed by VSM and showed superparamagnetic behavior with different level of magnetic saturation (Figure 5.3). The increasing in magnetic saturation as the MAG or S-MAG content increase can be attributed to the increase of weight and volume of MAG and S-MAG. Moreover, S-MAG-chitosan aerogels show lower magnetic saturation than MAG-chitosan aerogels.

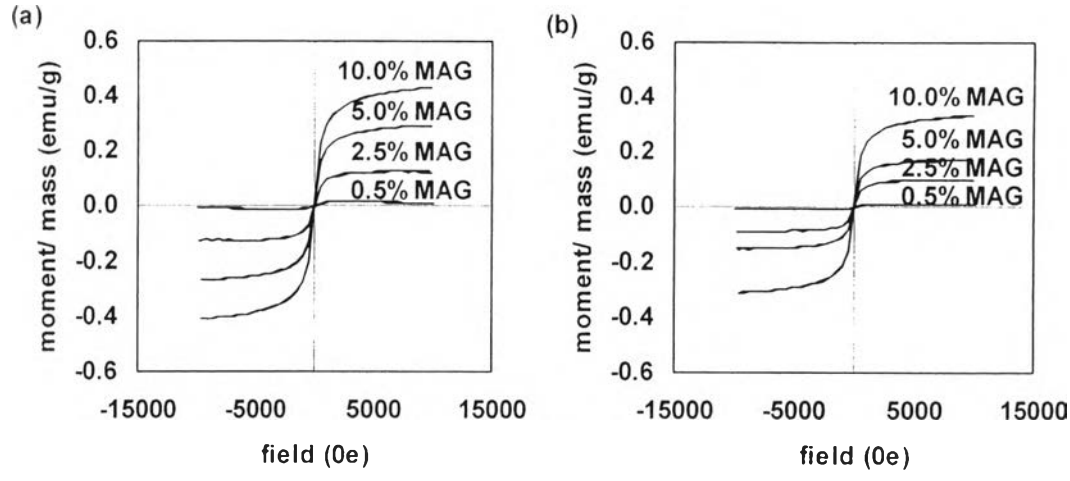

Figure 5.3 Magnetization curves for (a) MAG-chitosan aerogel and (b) S-MAG-chitosan aerogel at various MAG and S-MAG content.

Figure 5.4 shows XRD patterns of pure MAG, chitosan aerogel, MAG-chitosan aerogel, and S-MAG chitosan aerogel. MAG (Figure 5.4 (a)) shows obvious characteristic diffraction patterns at 30° (220), 35° (211), 43° (400), 53° (422), 58° (511), and 63° (440) which indicates crystalline cubic spinal structure [5]. Chitosan aerogel (Figure 5.4 (b)) shows characteristic peaks of chitosan at 9°, 19°, and 25°. In the case of MAG-chitosan aerogel and S-MAG-chitosan aerogel (Figure 5.4 (c) and Figure 5.4 (d)), materials show both characteristic peaks of MAG and chitosan. This indicates that mixing MAG or S-MAG with chitosan solution does not affect the crystal structure of MAG. Moreover, the sizes of MAG and S-MAG are too small to disturb the crystal structure of chitosan.

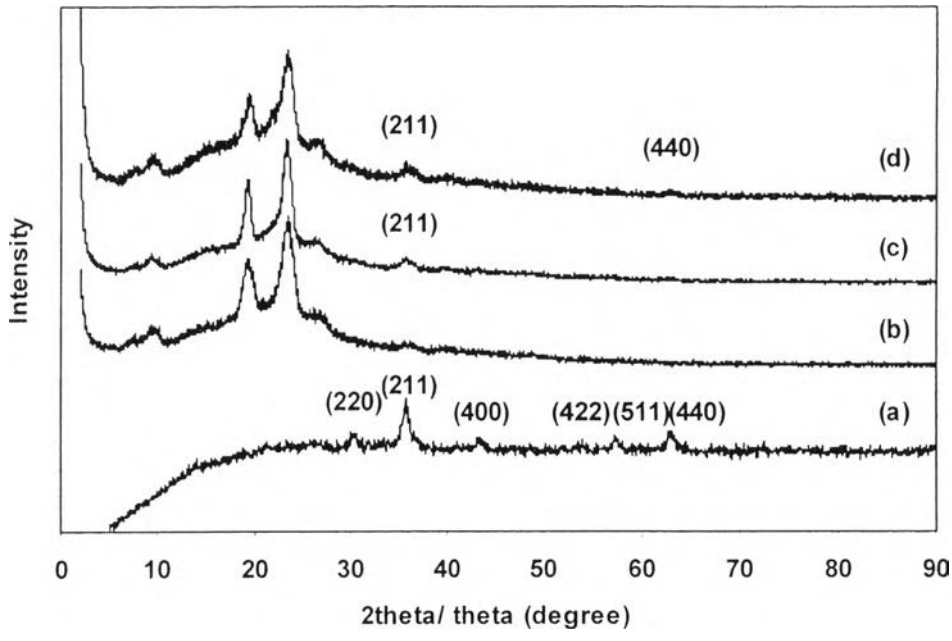

Figure 5.4 XRD patterns of; (a) MAG, (b) chitosan aerogel, (c) MAG-chitosan aerogel, and (d) S-MAG-chitosan aerogel.

Figure 5.5 shows morphology of obtained hydrogels after freeze-drying. The porous structure was observed by SEM in all samples. The roughness of the aerogel texture increases as the magnetic content increases.

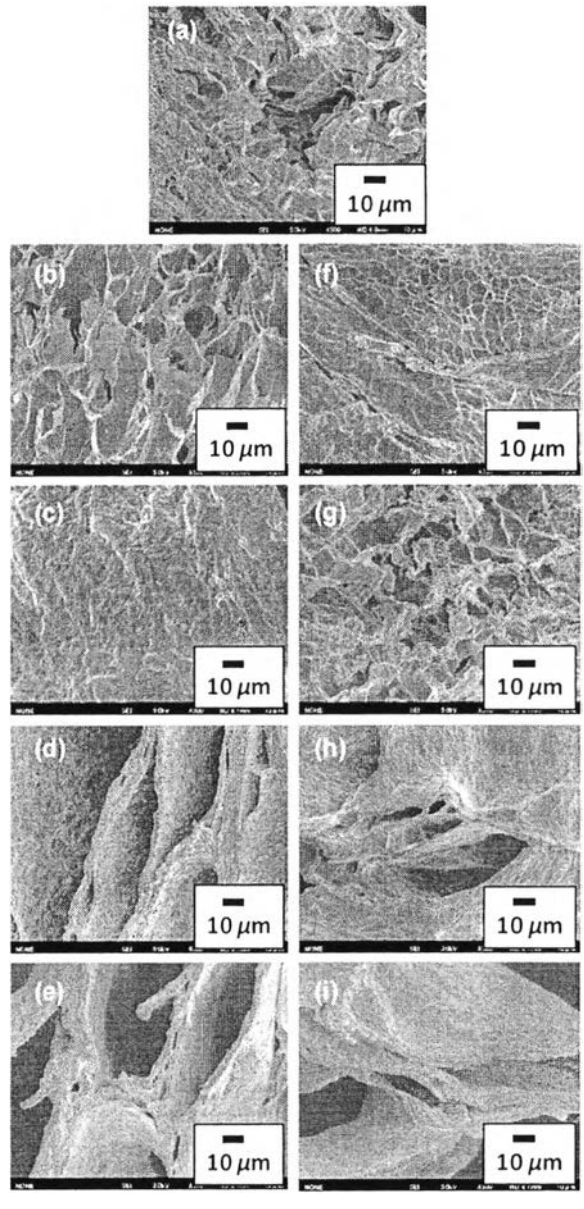

Figure 5.5 SEM micrographs at 15 keV of chitosan aerogel (a), MAG-chitosan aerogels (b-e), S-MAG-chitosan aerogels (f-i). Magnetic loading content 0.5% (b, f), 2.5% (c, g), 5.0% (d, h) and 10.0% (e, i). Scale bar, 10 μm.

Figure 5.6 shows how MAG and S-MAG content affect the swelling ratio of hydrogels. Correspond with the presence of MAG and S-MAG, the aerogels shows significantly lower in swelling ratio compared to aerogel without MAG or S-MAG added. However, MAG and S-MAG chitosan aerogels show no difference in swelling ratios. Furthermore, applying an external magnetic field does not decrease

swelling ratio as we expected. This might be due to an incomplete closed structure of magnetic chitosan hydrogel under a magnetic field causes water to penetrate throughout the porous gel structures.

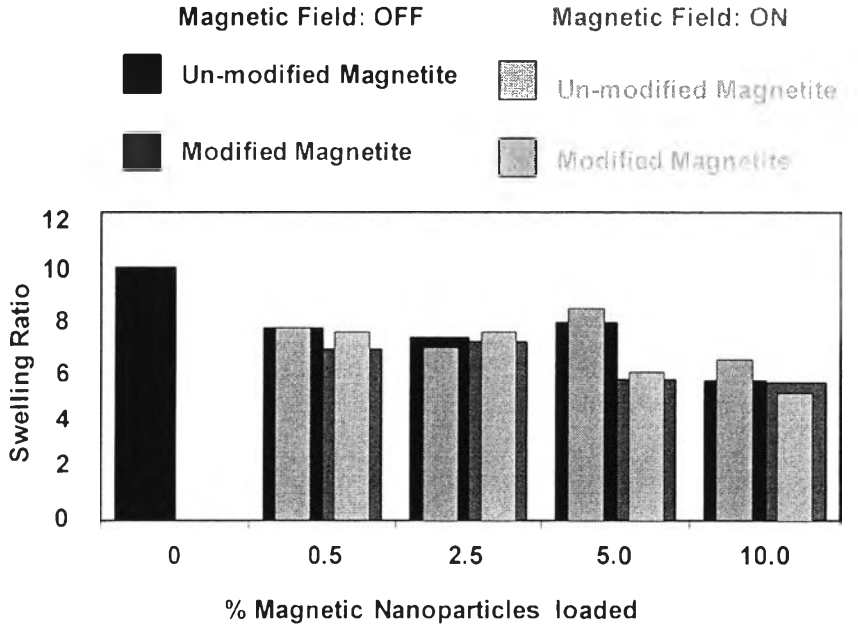

Figure 5.6 Swelling ratio of magnetic chitosan hydrogels.

5.4.2 Effect of magnetic content and external magnetic field on drug release

In order to study effect of magnetic content, samples were compared with various MAG loading content (from blank sample to 10.0% MAG loading) at the same condition of surface modification and external magnetic field application. Figure 5.7 (a)-(d) show the decrease in drug release profile after magnetic nanoparticles added.

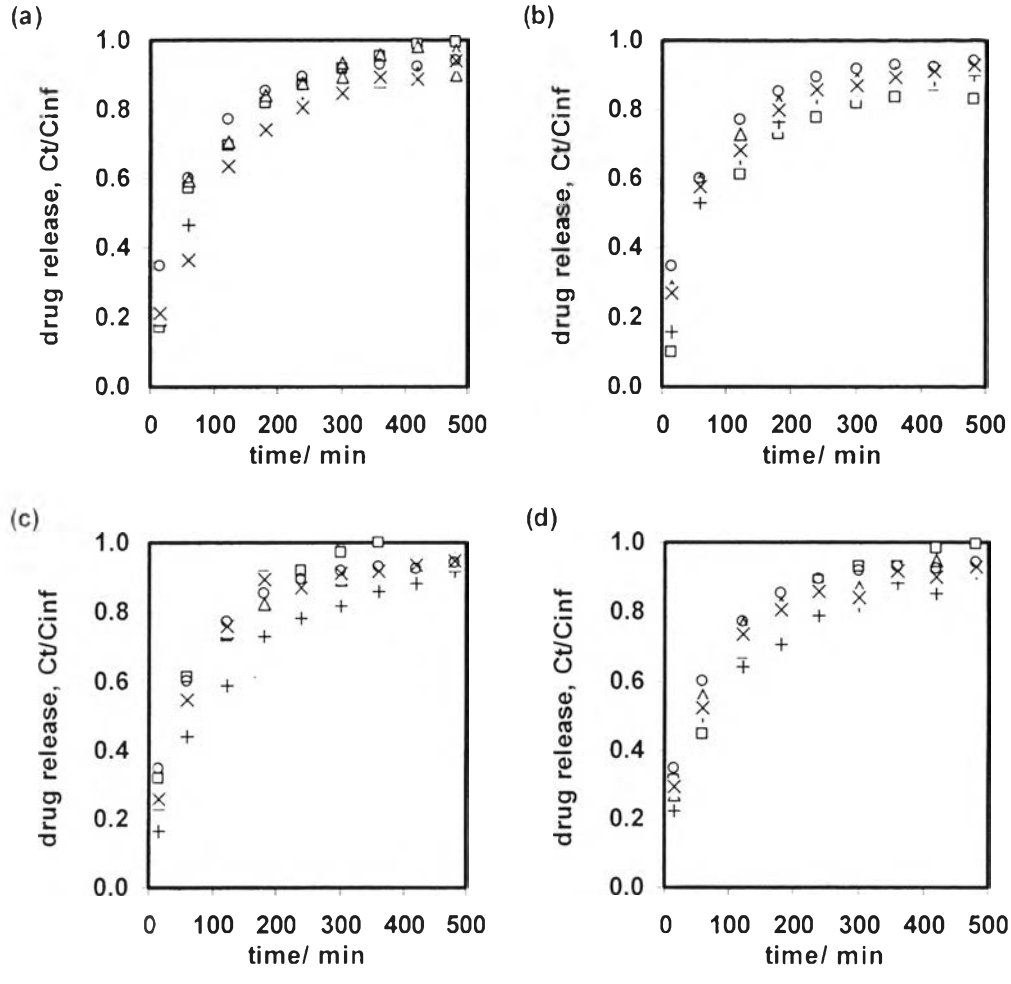

Figure 5.7 Relative drug release as a function of time of (a) unmodified MAG-OFF magnetic field, (b) unmodified MAG-ON magnetic field, (c) silane-modified MAG-OFF magnetic field, and (d) silane-modified MAG-ON magnetic field. No magnetic nanoparticle added (○), 0.5% added (□), 5.0% added (△), and 10.0% added (x). C_{inf} represents cumulative mass release at 24 h (N=3). The degrees of crosslinking of all samples were fixed at 50%.

The effect of external magnetic field is also studied by comparing between Figure 5.7 (a) with Figure 5.7 (b) and Figure 5.7 (a) with Figure 5.7 (b). The drug release profiles are slightly decreased after applying external magnetic field. Lui *et al.* explained drug release behavior of gelatin hydrogel and magnetite composite in ON-OFF magnetic field that the magnetic responsiveness of composite

hydrogel decreased under switching "ON" mode but increased upon switching "OFF" mode [6]. The responsive property can be attributed to the fact that the porosity or the pore size of the composite hydrogel change with the switching on and off mode. Lui suggested a close configuration of the composite hydrogel when the hydrogel was exposed to external magnetic field while an open configuration was suggested when the hydrogel was not in magnetic field and restore to original state.

5.5 Conclusions

Magnetic responsive chitosan hydrogel was successfully prepared by one-pot synthesis in aqueous system of PEG-crosslinked water-soluble chitosan. The drug (Vitamin B_{12}) was used as a model drug for controlled release demonstration. The release behaviors of drug loaded hydrogels were comparatively studied between with and without external magnetic field in aqueous solution. The external magnetic field and degree of crosslinking are found to affect the drug release profile while surface modification of magnetic nanoparticles by silane coupling agent and magnetic content show no significant change in drug release profile. Based on the responsiveness in magnetic field, the magnetic chitosan hydrogel can be potentially developed for application in smart drug release system.

5.6 Acknowledgements

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5.7 References

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