



CHAPTER IV

BIOMEDICAL CHITOSAN MATERIAL: WATER-BASED SYSTEM SUPERABSORBENT HYDROGEL AND AEROGEL

4.1 Abstract

A system of chitosan-hydroxybenzyl triazole aqueous solution mixing with dicarboxylated polyethylene glycol is an effective pathway to provide gelation at ambient temperature under the presence of carbodiimide water soluble conjugating reagent. The incorporation of clay in this hydrogel gives the chitosan-clay nanocomposite hydrogel. The swelling in water of nanocomposite hydrogel in water is high enough to give porous aerogel in nanometers after removing water molecule by freeze-drying. The mechanical properties of hydrogel and aerogel are improved when clay incorporated into system. The comparative studies on hydrogel properties of chitosan-unmodified clay (Cloisite Na⁺) and chitosan-modified clay (nanoclay) nanocomposite reveal that the chitosan- modified clay nanocomposites showed the decrease in swelling ratio as compared to chitosan-unmodified clay ones.

Keyword: Chitosan, Water-based system, Hydrogel, Aerogel

4.2 INTRODUCTION

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 [1]. 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. As the swelling of hydrogels in water is significant, the tensile strength needs to be improved. In order to solve this problem, various composites of hydrogels were proposed [2]. When water molecules in organic polymer hydrogels are removed under supercritical conditions without destroying the three-dimensional polymer networks, porous aerogels can be obtained [3]. Aerogels give a scaffold network with a highly porous structure. Scaffolds play an important role as supporters to repair and regenerate the tissues. The scaffold is produced from synthetic and natural polymers which are biodegradable and non-toxic materials. Natural polymer scaffolds provide several advantages over synthetic polymers because they facilitate cell attachment and maintain differentiation of cells [4].

Chitosan, the natural polysaccharide existing in the exoskeleton of crustaceans, is one of the most natural biodegradable polymers used as a tissue scaffold. Advantages of chitosan are non-toxicity, biocompatibility and biodegradability [5]. Moreover, chitosan can be fabricated into various forms, especially, porous structures for cell transplantation and tissue regeneration [6]. Many researchers reported the structural modification of chitosan at the molecular level to propose novel products, mostly for pharmaceutical and biomedical applications. As chitosan is lacking of water solubility, including organic solvent insolubility, based on its strong inter and intra-molecular hydrogen-bonded networks. Up to present, the use of an acidic solvent is the most practical way to materialize chitosan [7]. There, changing chitosan to organic salts, conjugating with hydrophilic groups or lowering the molecular weight to oligomer level are alternative strategies to dissolve chitosan in aqueous.

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. Currently, our group succeeded in conjugating reaction of chitosan in water. There, the mixture of water soluble chitosan and an equimolar of carboxylated polyethylene glycol at room temperature gives chitosan-PEG derivative.

Based on this reaction pathway, here, we propose a simple chitosan gelation by using water soluble conjugating agent and dicarboxylated polyethylene glycol to obtain chitosan hydrogel. As, mentioned above, chitosan hydrogels have poor mechanical strength and tensile strength; thus, it is necessary to improve chitosan hydrogels by using small amount of clay to enhance the performance of hydrogels and aerogels. Clay not only provides reinforcement to polymer matrices resulting in polymer nanocomposite structure but also is attractive to consider the hydrogel with clay nanocomposite based on thermal properties and mechanical properties. In the present work, we aim to develop chitosan-clay nanocomposite to obtain a unique hydrogel and aerogel.

4.3 EXPERIMENTAL

4.3.1 Materials

Chitosan (95% DD, M_v of 5.6×10^5) was supplied by Seafresh Chitosan (Lab) Company Limited, Thailand. Poly (ethylene glycol) (PEG) (M_n 1450 Dalton) was purchased from Sigma-Aldrich, Inc., USA. 1-Hydroxybenzotriazole monohydrate (HOBtH₂O) and 1-ethyl-3-(3-dimethylaminopropyl-carbodiimide) hydrochloride (EDC·HCl) were purchased from Wako Pure Chemical Industries Co. Ltd., Japan. Succinic anhydride was provided from Fluka Chemika, Switzerland. Pristine sodium montmorillonite (clay) with cation exchange capacity of 120 mequiv/100g (trade name: Cloisite Na⁺) was purchased from Southern Clay Product Inc. Montmorillonite clay surface modified with 15-35% octadecylamine (ODA) and 0.5-5 wt. % aminopropyltriethoxysilane (trade name: Nanoclay, Nanomer[®] I.31PS) was purchased from Sigma Aldrich Chemie GmbH, Germany (Figure 4.1).

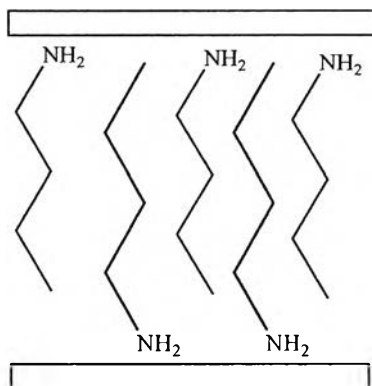


Figure 4.1 Structure of modified clay by ODA.

4.3.2 Instruments and equipment

Structural analysis

Fourier transform infrared (FTIR) spectra were obtained from an attenuated total reflection (ATR) accessory equipped on a Perkin Elmer Spectrum 1. The analysis was carried out with 32 scans at a resolution of 4 cm^{-1} over a frequency range of $4000\text{--}400\text{ cm}^{-1}$.

Surface analysis

The BET surface areas of aerogels were determined by using an Autosorp-1 gas sorption system (Quantachrome Corporation). The calcined samples were preheated in nitrogen for 3-4 h at $100\text{ }^{\circ}\text{C}$. Adsorption characteristics were measured under liquid nitrogen atmosphere.

Thermal analysis

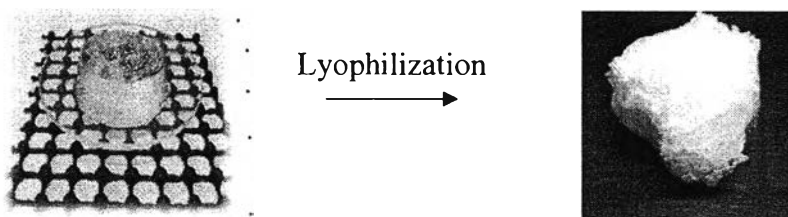
Thermogravimetric analyses were carried out using a Perkin Elmer Pyris Diamond with N_2 flow rate of 20 mL/min and a heating rate of $10\text{ }^{\circ}\text{C/min}$ starting from 30 to $600\text{ }^{\circ}\text{C}$.

Morphological studies

The effectiveness of clay exfoliation was studied by using wide angle X-ray diffraction (WAXD) by a RIGAKU RINT 2000 with $\text{CuK}\alpha$ as an X-ray source ($\lambda=0.154178\text{ nm}$). The scanning range was $2\text{--}15$ degree with a scanning rate of 2 degree/min .

4.3.3 Preparation of chitosan hydrogel

Chitosan-HOBt aqueous solution was prepared as reported previously [8]. Chitosan (0.1g, 0.61 mmol) was mixed with HOBt·H₂O (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 hours. 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). To the solution of chitosan-HOBt aqueous (10 mL), carboxy-terminated poly (ethylene glycol) 10% WSCl (0.235 g, 1.22 mmol, 10 mL) were added. The reaction was carried out at ambient temperature for 15 minutes to obtain chitosan hydrogel. The crude gel was dialyzed and lyophilized to obtain chitosan aerogel CD-1. In similar, chitosan aerogel CD-3, CD-5 and CD-7 were prepared by adding carboxy-terminated poly(ethylene glycol) 30%, 50% and 70% respectively.



Scheme 4.1 Chitosan in; (a) hydrogel, and (b) aerogel forms.

4.3.4 Chitosan/clay nanocomposite hydrogel and aerogel

Clay (cloisite Na⁺ or nanoclay) for 1-5 wt % and COOH-PEG-COOH (1.70 g, 1.53 mmol) was added into chitosan-HOBt 10 mL solution and stirred at room temperature for 24 h. WSC was added into clay suspension solution to obtain chitosan/clay nanocomposite hydrogel. The product was lyophilized to obtain chitosan/clay aerogel. The content ratios of chitosan and clay were varied to be 1:0.01, 1:0.02, 1:0.03, 1:0.03, 1:0.04 and 1:0.05 to obtain aerogels CU-1, CU-2, CU-3, CU-4 and CU-5. Similarly, in the cases of unmodified clay, the aerogels obtained are CM-1, CM-2, CM-3, CM-4 and CM-5 for the ratios of chitosan: modified clay for 1:0.01, 1:0.02, 1:0.03, 1:0.04, and 1:0.05, respectively (Scheme 4.2).

4.4 RESULTS AND DISCUSSION

4.4.1 Synthesis of PEG-crosslinked chitosan

Chitosan hydrogel is obtained by using dicarboxylated poly (ethylene glycol) as crosslinker. All reaction was carried out in water at room temperature to obtain chitosan hydrogel. The mole ratios were varied to clarify the optimal conditions.

From Figure 4.2, the appearances of hydrogels are depending on the mole ratio of crosslinker. CD-3 shows the good appearance while it retains shape.

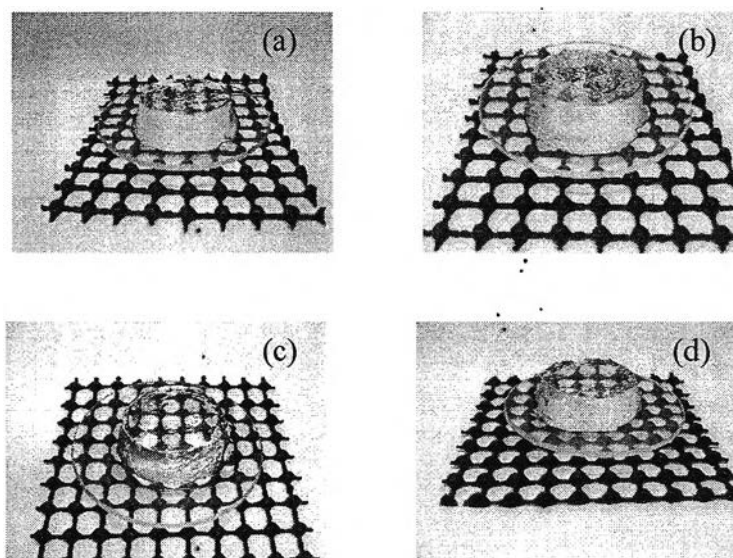


Figure 4.2 Appearances of chitosan hydrogel of ; (a) CD-1, (b) CD-3, (c) CD-5, and (d) CD-7 with vary mole ratio

Figure 4.3 shows how mole ratio of chitosan:dicarboxylated poly (ethylene glycol) affects the swelling ratio of hydrogels. Correspond with appearance, CD-3 shows the lowest degree of swelling as compared with other gels. This can be concluded that CD-3 was prepared under the optimal condition. In general, an increase in amount of crosslinker, will decrease the swelling due to the dense network structure [9]. However, CD-5 and CD-7 show highly increase in swelling ratio. This might be due to dicarboxylated poly (ethylene glycol) form dimeric network together with crosslink network with chitosan chains.

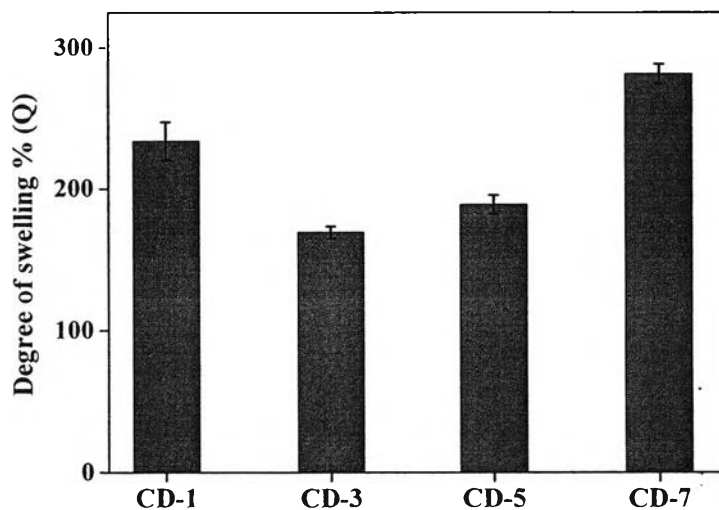


Figure 4.3 Degree of swelling of chitosan hydrogels.

Chitosan hydrogel was dialyzed and freeze-dried before analyzing by using ATR-FTIR. Chitosan shows a peak at 1646 cm^{-1} (amide I) (Figure 4.4(a)), whereas the chitosan gel (curve (b)) obtained shows the peaks at 1730 and 1650 cm^{-1} corresponding to C=O ester and amide I, (Figure 4.4(b)).

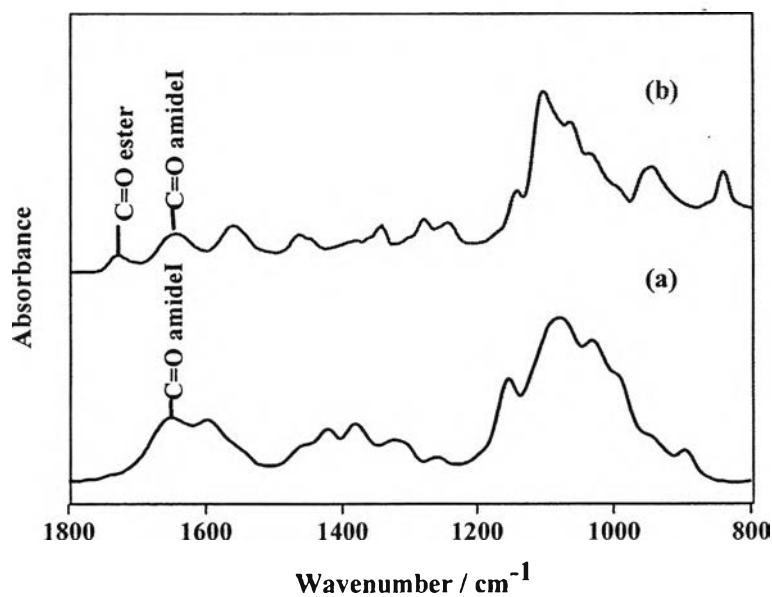


Figure 4.4 ATR-FTIR spectra of ; (a) chitosan, and (b) chitosan aerogel.

Figure 4.5 shows the most significant surface area of chitosan aerogel when dicarboxylic ethylene glycol is 10 wt%. This reflects the surface of each chitosan fiber when the crosslink was not completely formed. For CD-3 to CD-7, the surface area of aerogel increases with an increase of crosslinker content.

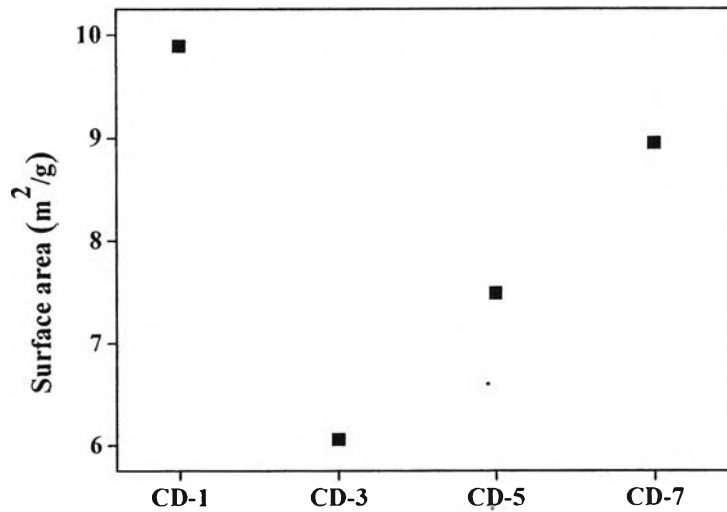


Figure 4.5 Surface area of chitosan aerogels.

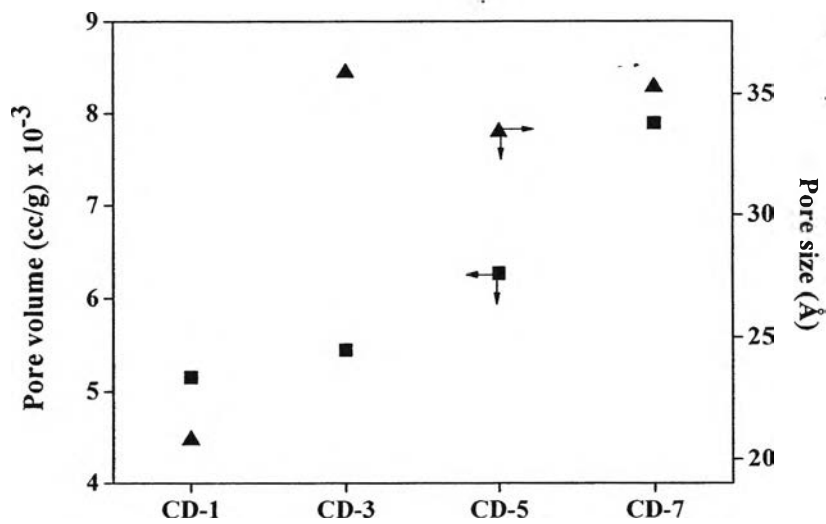


Figure 4.6 Pore volume (■) and pore size (▲) of chitosan aerogels.

Figure 4.6 shows the porosity of the aerogel. As compared to the surface area, the low pore size and pore volume of CD-1 reflects the incomplete crosslink network. For other aerogels, the pore sizes obtained are almost identical. The pore volume of CD-7 is the most significant. This reflects the small amount of chitosan (30 wt %) existed in the system as polymer matrices with physical and chemical crosslink of dicarboxylate polyethylene glycol.

Figure 4.7 shows the degradation temperature of chitosan and each aerogel. It should be noted that chitosan degrades at above 260 °C. At this temperature range (260-300 °C) all samples show the weight loss which reflects the degradation of chitosan. The weight loss at around 380 °C can be observed for all aerogels. This reflects the degradation of crosslink network. The slight weight loss at 200 °C might be due to the degradation of soft network since polyethylene glycol dicarboxylate crosslinker has a long chain which may provide the weak network.

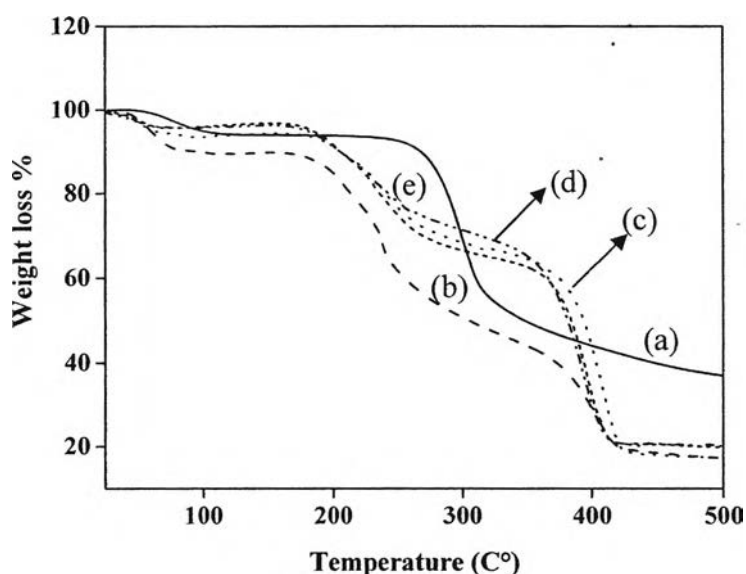


Figure 4.7 TGA thermograms of (a) chitosan flake, (b) CD-1, (c) CD-3, (d) CD-5 and (e) CD-7.

4.4.2. Synthesis of chitosan-PEG-clay aerogel nanocomposite.

Although the chitosan hydrogel (CD-3) has good appearance and gives the lowest degree of swelling, it was formed that the aerogel could be easily torn apart in water. To provide a stronger network, clay was used to incorporate with chitosan nanocomposite by adding 1-5 wt% of clay [10]. Here, two types of clay, i.e. nanoclay and organoclay were added into chitosan hydrogel before freeze-drying to obtain aerogel [11].

Chitosan is a semi-crystalline polymer which is insoluble in aqueous solution at pH more than 5.5. In order to prepare chitosan aqueous solution, dilute acid is required. This results in the protonation on amino groups of chitosan leading to fully dissolving chitosan in aqueous phase. For this reason, amino group of chitosan is in protonated form under complexation with HOBt. An electrostatic interaction is expected to take place between the positively charged $-\text{NH}_3^+$ groups and the negatively charged surfaces of clay [12]. Therefore, clay in chitosan nanocomposites functions as an effective multi-functional crosslinker [13].

Figure 4.8 shows the most significant swelling ratio of chitosan hydrogel as compared to other hydrogels. Based on the ionic interaction between chitosan and clay, the swelling ratio of hydrogel is significantly decreased. The comparative studies for swelling ratio between chitosan hydrogel with modified and unmodified clay are carried out. It is important to note that the decrease in swelling of chitosan hydrogel is significant for chitosan with modified clay system.

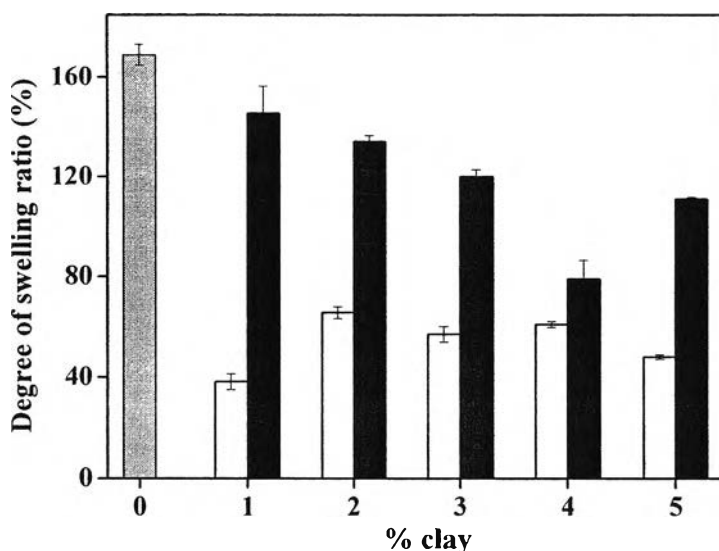


Figure 4.8 Swelling ratio for, (◻) chitosan gel without clay, (□) chitosan gel with modified clay, and (■) chitosan gel with unmodified clay.

This might be due to the fact that amino group of modified clay functionalized onto clay layers offers hydrogen bonds with OH groups on chitosan structure. This effective hydrogen bond between clay layers and chitosan provides the significant stability of this clay-chitosan hydrogel in aqueous atmosphere resulting in the decrease of swelling property.

Figure 4.9 shows the XRD patterns of clay and the aerogel obtained from freeze-dried hydrogel with clay 1-5 wt%. The unmodified clay shows a diffraction pattern with $7.17^{\circ}2\theta$ referring to d-spacing of 12.32 \AA . Considering the composites of chitosan and unmodified clay with 1-5 wt% loading content, the diffraction peak of clay is disappeared. This suggests the exfoliation pattern of clay layers in the chitosan matrices. In the case of modified clay, the diffraction peak reveals the decrease in 2θ ($4.220^{\circ}2\theta$, $d = 20.9212 \text{ \AA}$) implying the expansion of clay layers. This reflects the interaction of chitosan into clay galleries (Figure 4.9(b)). After incorporation of modified clay into chitosan matrices, 1% CS-clay aerogel shows no diffraction pattern referring to the complete exfoliation. However, when the organoclay content was 4-5 wt%, a peak at $4.6^{\circ}2\theta$ appears. This might be due to a specific flocculation clay layer with chitosan when clay amount in the aerogel exceeds the exfoliation.

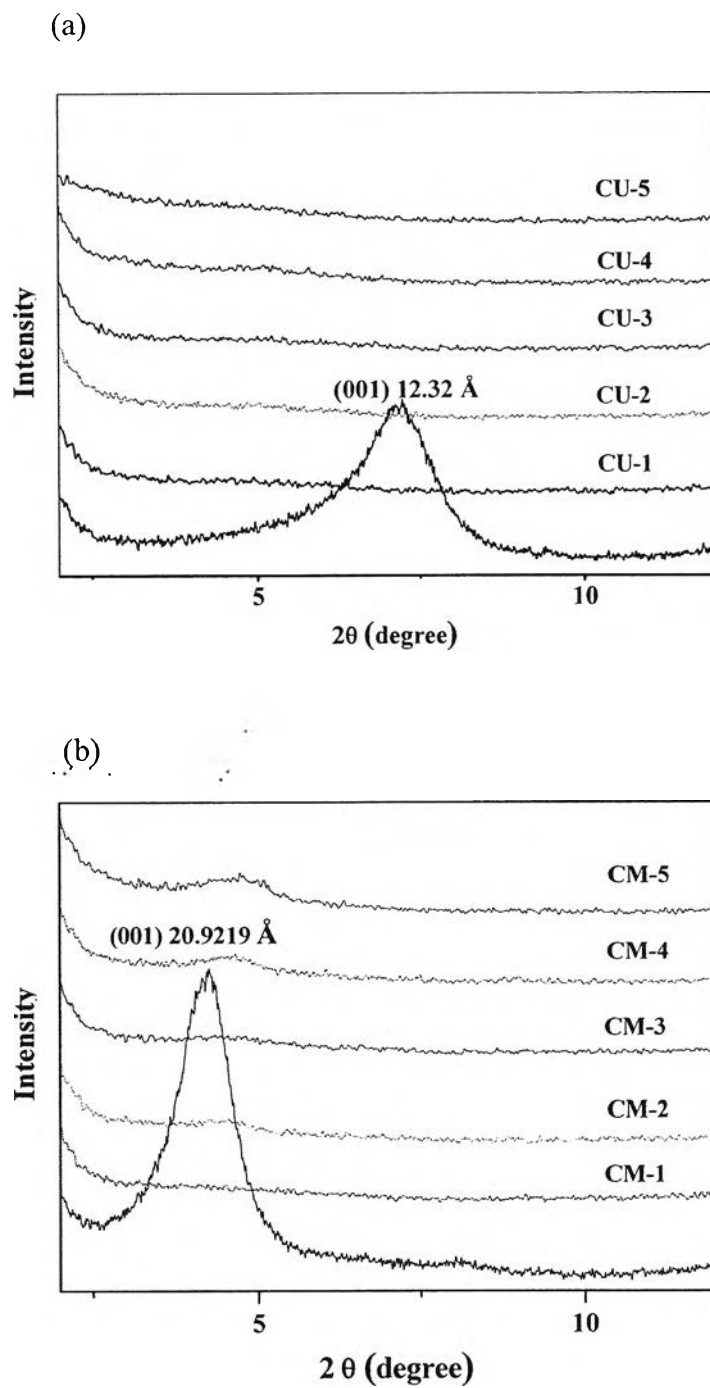


Figure 4.9 XRD patterns of ; (a) unmodified clay and its aerogel nanocomposites (CU series), and (b) modified clay and its aerogel nanocomposites.

The surface area, pore size and total pore volume of chitosan aerogel with unmodified and modified clay are summarized in Table 4.1. In general, chitosan nanocomposite aerogel BET surface areas range from 200-1000 m²/g [14]. Here the aerogel with either modified or unmodified clay for 1-5 wt% show similar results. This reflects the morphology was controlled by freezing and lyophilizing rate of chitosan hydrogel [15].

Table 4.1 Surface area, pore volume and pore size of chitosan clay nanocomposites

samples	Surface area (m²/g)	Pore volume (cc/g)	Pore size (Å)
Chitosan gel with unmodified clay 1-5 wt%	16-38	0.013-0.025	25-34
Chitosan gel with modified clay 1-5 wt%	12-53	0.009-0.046	29-35

The degradations of aerogels with clay are similar to those of aerogel in Figure 4.10. There are 3 regions related to (i) the weak aerogel matrices starting to degrade at around 220 °C, (ii) the chitosan mainchain degradation at around 300 °C, and (iii) the crosslink network degradation at above 350 °C.

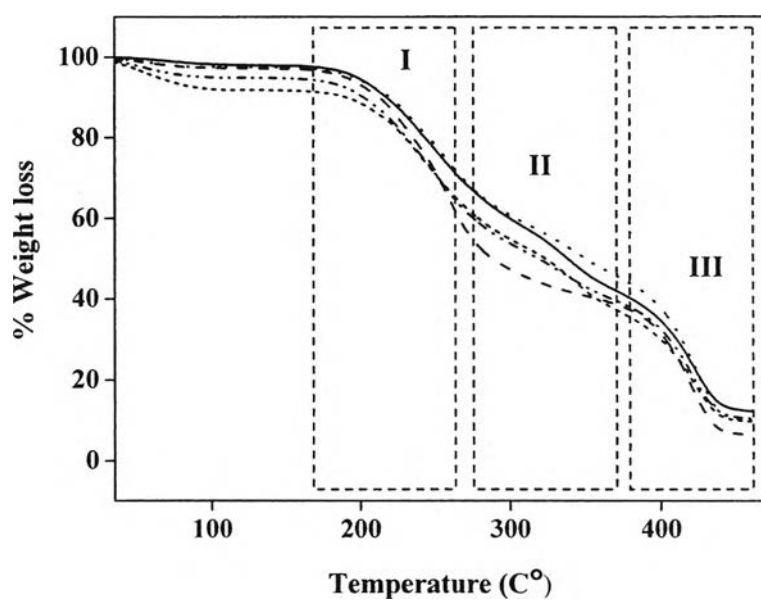


Figure 4.10 TGA thermograms of chitosan with unmodified clay; CU-1 (---), CU-2 (.....), CU-3 (.....), CU-4 (— · ·), and CU-5 (—).

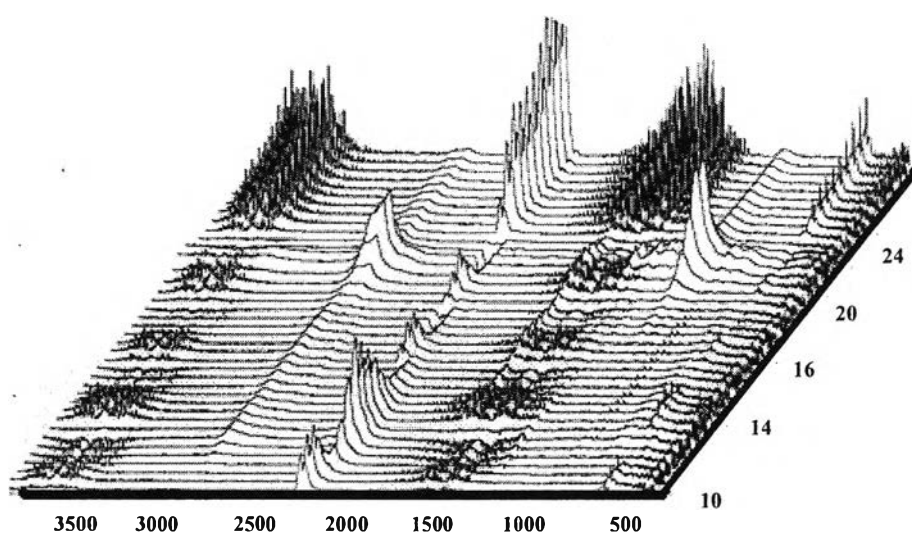


Figure 4.11 TGA-FTIR of chitosan with CU-1 as a function of time.

To confirm the structure of volatile materials, the TGA-FTIR was applied. Figure 4.11 shows that the first degradation is related to the functional group $\text{N}=\text{C}=\text{O}$ as identified from the peak at 2280 cm^{-1} . This reflects the degradation of

chitin-chitosan copolymer. The decomposition temperature at above 24 min (above 240 °C) gives the peak at 2868 cm^{-1} and 1120 cm^{-1} . This reflects the degradation of dicarboxylated poly (ethylene glycol).

4.5 CONCLUSIONS

The chitosan-clay nanocomposite was successfully prepared by using chitosan-hydroxybenzyl triazole aqueous solution mixing in water-based system mixing with dicarboxylated polyethylene glycol as the crosslinking agent. It was found that clay improved the swelling of hydrogel.

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