สำนักงานวิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER III

VINYL-CHITOSAN MACROMONOMER: AN APPROACH FOR CONTROLLED STRUCTURE CHITOSAN

Abstract

Chitosan macromonomers prepared by conjugating low molecular weight chitosan with acrylic acid monomer are discusssed. The optimal conditions to obtain vinyl-chitosan macromonomer and vinyl-chitosan polymer are clarified. The characterization of the compound in each step by FTIR, ^{1}H-NMR, WAXD are summarized. The swelling behavior of the polymer gels obtained are reported.

Keywords: Low molecular weight chitosan, Macromonomer, Acrylic acid monomer, Polymeric chain layer, Polymer gel, Swelling

Introduction

For decades, there has been interest in chitin-chitosan for practical applications because of its bioactivity[1], biocompatibility[2], biodegradability[3], chemical-[4], and physical-[5] properties. However, most applications have been achieved by physical modifications such as bead preparation, gel formation and membrane casting. Since the preparation of functional chitosan derivatives always faces the problems of high molecular weight and strong inter- and intramolecular hydrogen bonding, successful chemical modifications of chitosan products are rare. The chemical reactions of chitin-chitosan are limited because of its insolubility in common solvents. In most cases, the reactions have to be done in heterogeneous conditions such as tosylation[6] , phthaloylation[7] and N-acylation[8].

To overcome these problems, some chemical modifications have been proposed for the complete organic or aqueous soluble derivatives, such as carboxymethylchitin/ chitosan[9], phthaloylchitosan[10]. Although those reactions bring chitosan to a soluble polymer, the chemical structure of chitosan is already changed which limits the variety of possible derivatives in the following step.

Thus, much attention on the chemical modification of chitin/chitosan is about improving its solubility for homogeneous effective reactions. Depolymerization is an alternative way to improve the solubility and reactivity of chitosan. Up to now, it is known that the effective depolymerization of chitosan can be achieved by three methods, enzymatic degradation, γ-ray irradiation, and chemical treatment. Enzymatic hydrolysis is an attractive way because of the mild reaction conditions and high specific cleavage. For example, endochitinase performs the chain scission of chitin at the β-(1,4) glycosidic linkage of N-acetylglucosamine within the chain whereas exochitinase exhibits the cleavage at the end of the chain. However, this method requires many steps in the enzyme preparation process. Photoirradiation is an approach to that can be applied on a large scale with no purification step required. Yoksan *et al.*[11] determined that the optimum γ-ray irradiation conditions of 25 kGy made it possible to obtain 75% chain degradation without crosslinking or changes in structure. It is also found that the reduction of molecular weight reached a steady state at 10^5 level when the starting molecular

weight is above $7x10^5$ gm/mole. Chemical treatment is an effective method to obtain low molecular weights at the $\sim10^4$ gm/mole level although the chemical waste and purification are involved.

It is important to note that by reducing the molecular weight of chitosan, we can achieve reactions that are controlled at the molecular level. Yoksan *et al.*[11] proposed chitosan nanospheres by using low molecular weight chitosan conjugated with hydrophilic chains of polyethylene glycol. The present work proposes an original concept of chitosan reactions using a chitosan macromonomer. This can be possible when the introduction of monomer or polymerizable reactive species is conjugated onto the chitosan chain. By polymerizing chitosan macromonomers, a copolymers with specific designed chemical structure can be obtained.

Recently, layers of polymers have received much interest and have been reported as nanocomposite materials[12]. In nature, crab and shrimp shells are good examples of bio-nanocomposite polymers consisting of chitin-chitosan, protein, and inorganic metal species, especially calcium and magnesium. Thus, it is our challenge to develop chitin-chitosan layered structures with synthetic polymer chains that mimic the structure and function of crab and shrimp shells by using chitosan macromonomer concept.

The present work is based on the unique strategy of modifying the low molecular weight chitosan with a reactive vinyl group attached on either the amino or hydroxyl functional group of chitosan to obtain chitosan macromonomers. The polymerization of chitosan macromonomers with vinyl monomers can be expected to lead to a layered structure with a polymer chain of chitosan alternated with synthetic polymer.

Experimental Section

Materials. Chitin-chitosan with a degree of deacetylation (%DD) of 87 was locally supplied from the SEAFRESH Company, Bangkok, Thailand. Ethanol, and acetone were from BDH Laboratory Supplies, England. Acrylic acid and deuterated oxide were purchased from Fluka Chemika, Switzerland. Potassium persulphate was from Asia Pacific Specialty Chemicals Limited, Australia. Methanol was purchased from J.T Baker, USA. Deuterated acetic acid was from EURISO-TOP, France. All chemicals were used without further purification.

Instruments and Equipment. Qualitative and quantitative FT-IR spectra were obtained from a Bruker Equinox 55/S Spectrometer with 32 scans at a resolution of 4 cm^{-1}. A frequency range of 4000-400 cm^{-1} was observed using a deuterated triglycinesulfate detector (DTGS) with a specific detectivity, D^*, of $1x10^9$ $cm.Hz^{1/2}w^{-1}$. A DuPont Thermal Gravimetric Analyzer was used to study thermal properties of the samples under N_2 with a flow rate of 20 mL/min and heating rate of 20°C/min from 30°C to 600°C. ^{1}H-NMR was obtained from a 500 MHz JEOL JNM-A500 instrument at 70±1°C using deuterated acetic acid (CD_3COOD) and deuterated oxide (D_2O) with tetramethylsilane (TMS) as solvent. X-ray diffraction patterns were obtained from a Rigaku RINT 2000, using Cu K_α (λ = 0.154 nm) scanning for 2θ of 5-90° operating at 40 kV, 30 mA with Ni filter. Intrinsic viscosity [η] was measured with a calibrated viscometer Connon-Ubbelohde (No.2, A149) in 0.1 M sodium acetate/0.2 M acetic acid aqueous solution at 30±0.05°C. Molecular weight was calculated using the Mark-Houwink equation with $K=1.64\times10^{-30}\times DD^{14}$ and $a=(-1.02\times10^{-2}\times DD)+1.82$ as proposed by Wang *et al.*[13].

Vinyl-chitosan macromonomer. Low molecular weight chitosan, **1** (0.3 g), dissolved in deionized water (20 mL) having acrylic acid monomer (0.1 mol equivalent of chitin-chitosan unit). The reaction was carried out at 70°C for 2 h After the solution was concentrated, the crude product was obtained by reprecipitating in acetone to obtain **2** (Scheme I).

Polymerization of LMCS-acrylic acid macromonomer. Compound **2** (80 mg) and acrylic acid monomer (20, 30, 40, and 50 moles equivalent to chitin-

chitosan unit) were polymerized in water (15 mL) at 90°C for 3 h using potassium persulfate (0.3 g) as an initiator. The crude product was washed thoroughly by water followed by methanol for several times to obtain **3** (Scheme I).

Swelling Behavior. Compound **3**, chitosan, and poly(acrylic acid) were weighed in dry state. All three types of samples were immersed in water at room temperature for 5 h. The samples were thoroughly wiped by filter paper and weighed. The degree of swelling (SW) was calculated by $SW(wt.\%) = (W-W_0)\times 100/W_0$, where W and W_0 are the weights of swollen state and dry state, respectively.

Results and Discussion

Low molecular weight chitosan was prepared using 1.7 N hydrochloric acid at 100°C. It was confirmed that the structure of chitosan after acid hydrolysis was maintained with the molecular weight at 10^4 level as reported elsewhere[14].

LMCS-acrylic acid macromonomer. The macromonomer of low molecular weight chitosan was designed by fixing the amount of acrylic acid at 0.1 mole equivalent per chitosan unit. This might bring a possibility to achieve a chain of chitosan with a single unit of reactive vinyl group to satisfy the macromonomer condition. The reaction time was varied from 30 minutes to 8 hours. It was observed that when the reaction proceeded the solution became homogeneous. As the reaction proceeded, the conjugation between acid and amino group of chitosan provide amide bonds as shown in Scheme I. The reaction was traced by FTIR using crude product to observe the peak at 1655 cm^{-1}. The conjugation of acrylic acid onto chitosan may occur at the amino group or the primary hydroxyl group. The spectrum of the product obtained shows a new peak at 1434 cm^{-1} referring to acrylic acid monomer where the amide I and II also appeared at the same position with slightly increase of amide I band (Figure 1). This implies that the reaction occurs mainly at the amino group.

After the reaction, the product was washed thoroughly by methanol to remove unreacted acrylic acid monomer. ^{1}H-NMR supported the FTIR result as seen from the peaks at 6.2-6.6 ppm belonging to the protons of vinyl group (Figure 2), while other peaks of chitosan were clarified. The degree of conjugation evaluated by ^{1}H-NMR using the ratio of 6.2-6.6 ppm to C_2 chitosan peak was found to be 7.7%.

XRD was applied to study how the change in the molecular packing structure can be observed after macromonomer generated. The XRD patterns of low molecular weight chitosan and LMCS-acrylic macromonomer are shown in Figure 3. The pattern of conjugated low molecular weight chitosan was different from the starting material with a new peak at 15°2θ.

Polymerization of LMCS-acrylic acid macromonomer. The polymerization between macromonomer and acrylic acid monomer was achieved by

using potassium persulfate as a radical initiator. When the polymerization proceeded, the solution became a gel.

Figure 4 shows **3** with a new sharp peak at 1714 cm^{-1} due to the -COOH of acrylic acid and a characteristic peak at 895 cm^{-1} referred to glucopyranose ring. It is important to note that when the amount of acrylic acid increased, the peak at 1714 cm^{-1} increased as shown in Figure 4(a)-(d). This suggests the structure of copolymer is between chitosan and acrylic acid. It should be noted that the after the reaction proceeded, the solution became a gel.

The product was also studied by Wide angle X-ray diffraction (WAXD) to confirm the reaction. Figure 5 shows the XRD patterns of vinyl-chitosan macromonomer, and polymer with different amount of acrylic acid. It was found that the peaks at 10°2θ and 20°2θ were shifted and the peak obtained was broader. This implies a decrease in crystallinity. The polymerization between chitosan-acrylic acid macromonomer and acrylic acid might produce a long chain polymer as a layer in between chitosan chains. The chain chitosan and poly(acrylic acid) might be extended as the poly(acrylic acid) chain was polymerized. As a result, the intra-and intermolecular hydrogen bondings of chitosan were eliminated. The XRD patterns support this speculation.

Swelling Behavior. It is expected that the products show the good absorption due to the chain layer of chitosan and poly(acrylic acid). In this step, we focused on the swelling ability in water. The degree of swelling is expressed as the amount of water absorbed per gram of dry polymer. Figure 6 illustrated swelling behavior of chitosan, poly(acrylic acid) and LMCS-acrylic acid polymer in water at room temperature. Since poly(acrylic acid) is a water-soluble polymer, the product was dissolved completely before half an hour. At the molar ratio of macromonomer : acrylic acid at 1 : 40, the water uptake appears to be nearly 2000 times. Referring to the graft copolymerzation products[15] which are about 20-30 times, we discuss our system as follows. Our chitosan macromonomer and the vinyl monomer might be polymerized in chain layer structure. The high percentage of swelling implied the interaction of water molecules with both chitosan and acrylic acid chains in the layer by layer level. It should be noted that when the swelling time was above 4 h in the case of 1:50 (LMCS-acrylic acid macromonomer: acrylic acid), the product started to

dissolve during swelling measurement. This implied the chitosan chain in the present work and poly(acrylic acid) are water soluble polymer. The swelling behavior achieved with the water soluble property is also another useful point to apply in drug delivery system. It should also be noted that the gel produced in the present system does not require crosslinking agent as glutaraldehyde which is not practical in biosystem for most cases.

Conclusions

Vinyl-chitosan macromonomer can be prepared by conjugating our low molecular weight chitosan with acrylic acid monomer. The obtained macromonomer was confirmed the structure by FTIR, XRD, and ^{1}H-NMR. Chitosan chain layer structure was successfully prepared by the polymerization between LMCS-acrylic acid macromonomer and acrylic acid. This polymer shows very high water absorption.

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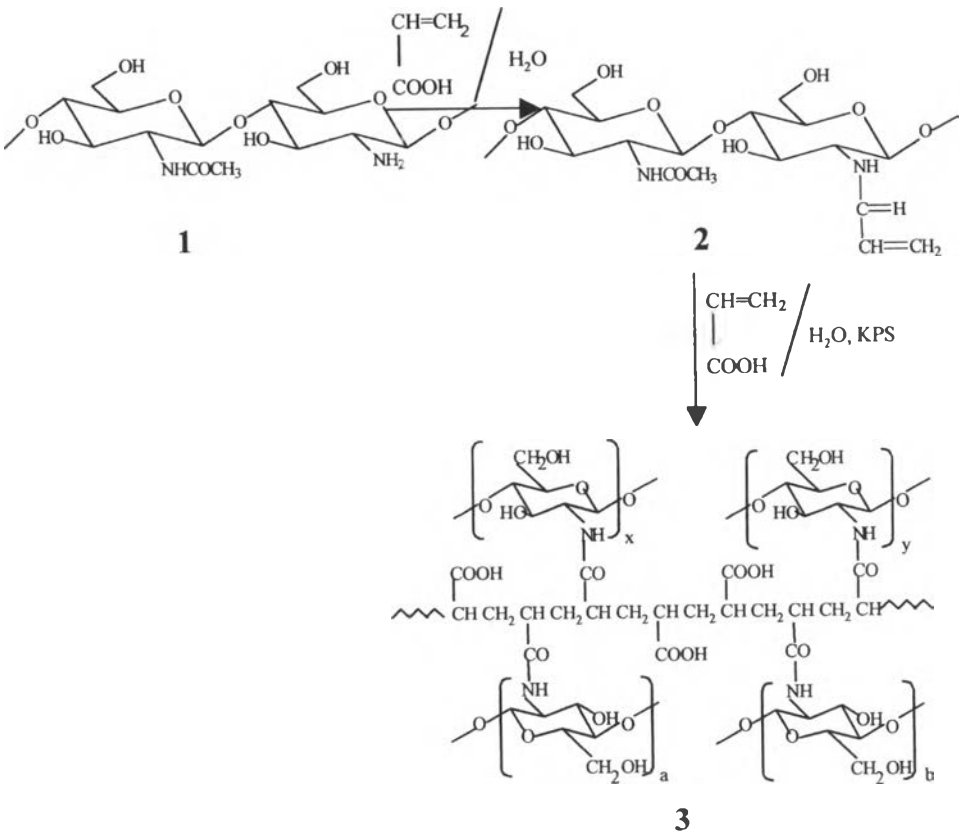


Scheme I. (Kosum et al.)

Figure Captions

Figure 1. FTIR spectra of: (a) low molecular weight chitosan, and (b) chitosan macromonomer.

Figure 2. ^{1}H-NMR of: (a) low molecular weight chitosan, and (b) chitosan macromonomer.

Figure 3. X-ray diffractograms of: (a) low molecular weight chitosan, and (b) chitosan macromonomer.

Figure 4. FTIR spectra of LMCS-acrylic polymer at the molar ratios of LMCS macromonomer: acrylic acid for: (a) 1:20, (b) 1:30, (c) 1:40, and (d) 1:50.

Figure 5. X-ray diffractograms of: (a) LMCS macromonomer, and LMCS-acrylic polymer at the molar ratios of LMCS macromonomer: acrylic acid for: (b) 1:20, (c) 1:30, (d) 1:40, and (e) 1:50.

Figure 6. Swelling percentage of: (a) chitosan, and LMCS-acrylic polymer at the molar ratio of LMCS macromonomer: acrylic acid for: (b) 1:20, (c) 1:30, (d) 1:40, and (e) 1:50.

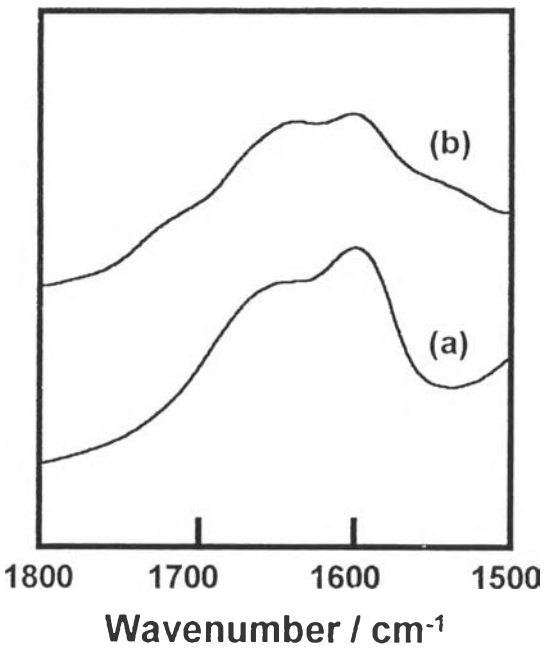
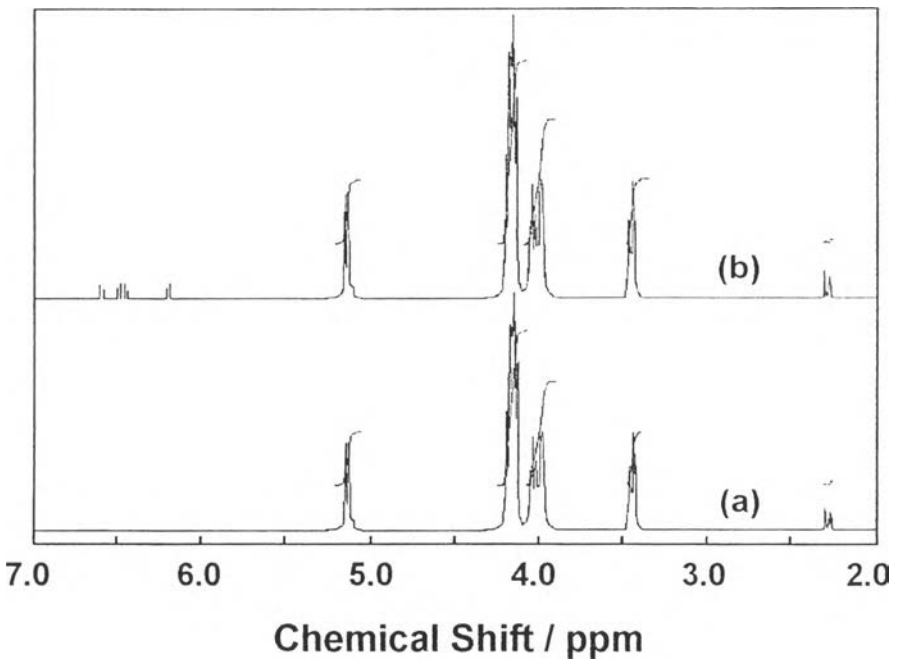


Figure 1 (Kosum et al.)



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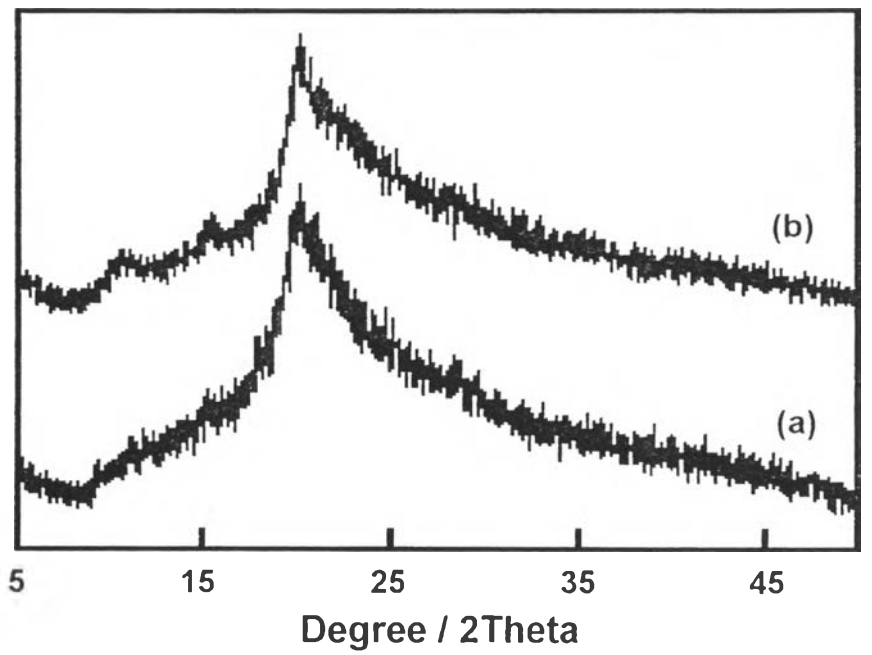

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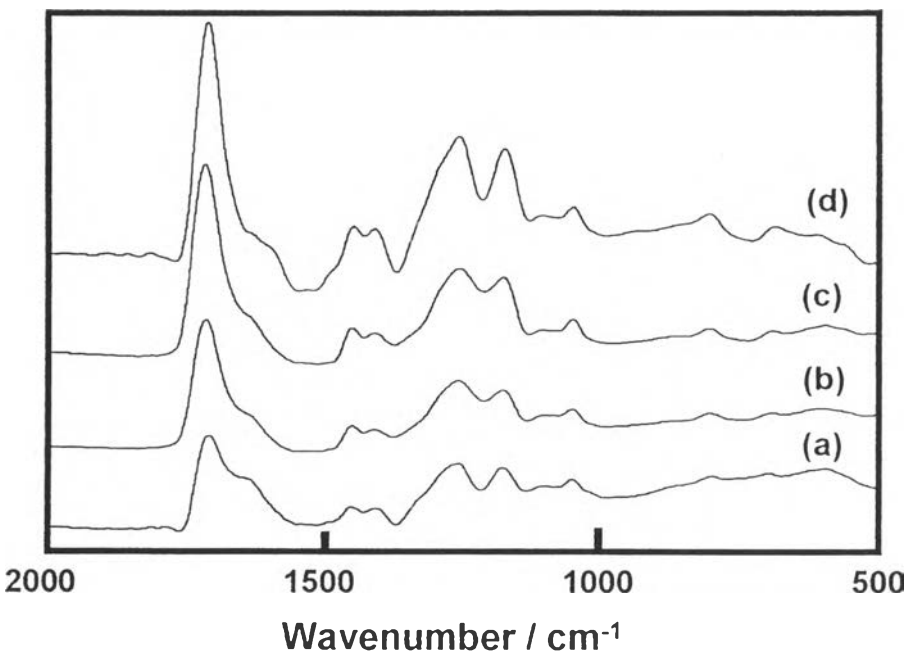
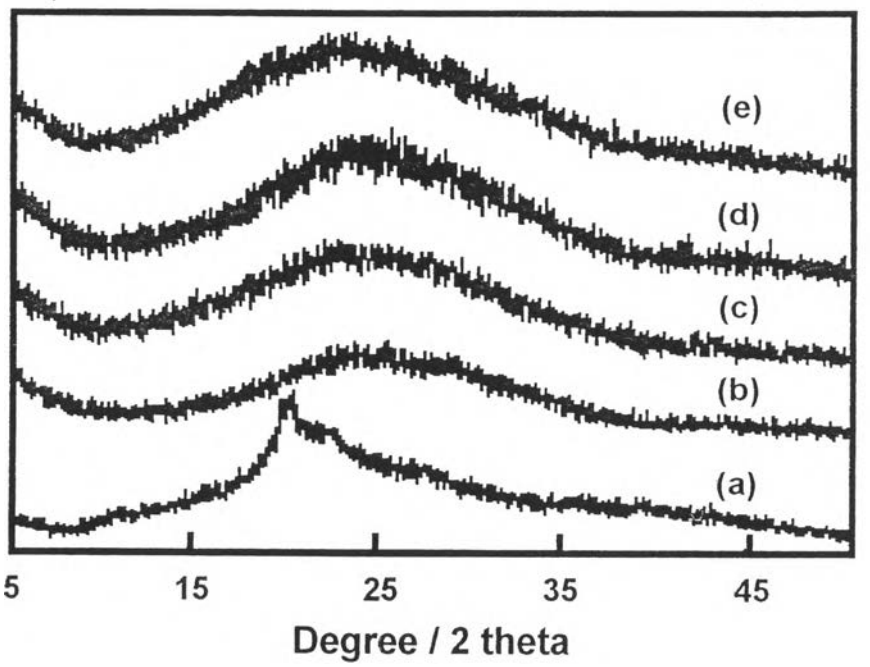


Figure 4 (Kosum et al.)



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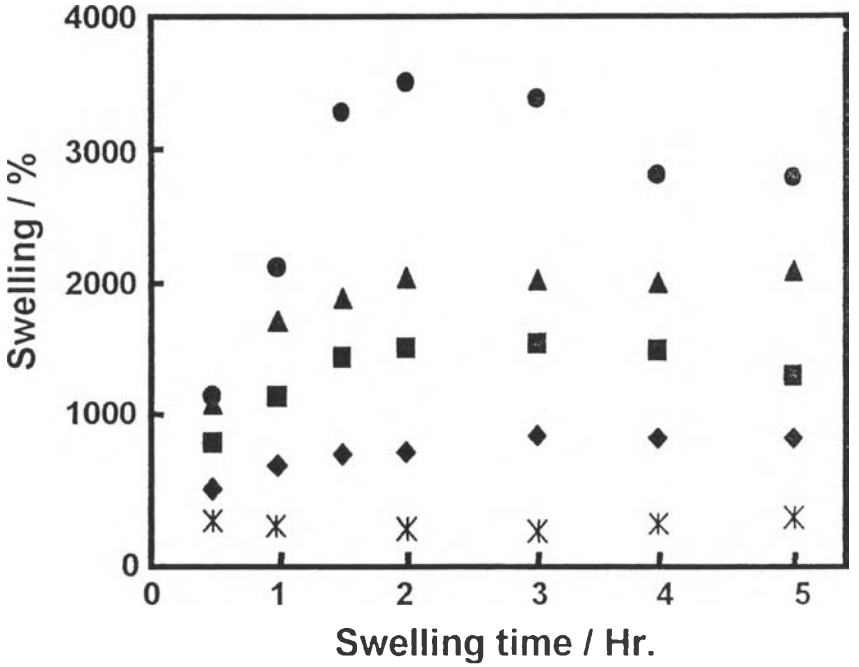

Figure 6 (Kosum et al)