CHAPTER IV

CONTROLLED HYDROPHOBIC/HYDROPHILICITY OF CHITOSAN FOR SPHERES WITHOUT SPECIFIC PROCESSING TECHNIQUE

Abstract

Chitosan is functionalized with phthalic anhydride at amino group and poly(ethylene glycol) methyl ether at hydroxyl group via homogeneous reaction. The product regenerated forms particles in either aqueous solution or organic solvents. The particles are spheres with the size of few micrometers as observed from SEM. The present work demonstrates that by simply adjusting the hydrophilicity and hydrophobicity, we can directly obtain spheres without specific processing technique.

Introduction

Chitin-chitosan is a material suitable for drug delivery system (DDS) related to its biocompatibility,[1,2] biodegradability,[3,4] and bioactivity,[5,6] which items of beads,[7,8] membranes,[9,10] and gels[11] are well known. Although chitosan conjugations with drug are pathways to achieve prodrugs,[12,13] the problems about drug active sites have to be considered in the reaction. It is known that sphere is an alternative DDS material to stabilize drugs.[14] Up to now, simple physical modifications such as, suspension crosslinking,[15] spray-drying coagulation,[16] and emulsification/solvent evaporation,[17] have been reported for various spherical particles in the range of 10~700 μm. However, the particles achieved by means of the processing technique face the problems related to the particle size, the drastic regeneration condition, including the efficient amount of drug incorporated. Recently, Akashi et al. proposed the conjugation of the hydrophilic chains onto polystyrene to obtain core-corona nanospheres, which are well dispersed in an aqueous solution and show the immobilization properties for biomolecules, peptide drugs, and antibodies.[18] For the past few years, we have focused on the controlled structure of chitosan by adjusting the polarity on the chain.[20] Herein, we propose a potential pathway to achieve chitosan spheres in both polar and non-polar media without specific processing technique. This brings us chitosan spheres, which is a useful model for incorporation with either hydrophilic or hydrophobic drug molecules.

Materials and Methods

Poly(Ethylene Glycol) Methyl Ether terminated with Carboxyl Group (mPEG-COOH, 2a-2c)

Poly(ethylene glycol) methyl ether (mPEG, M_n 2000, 3.00 g, 1.5×10^{-3} moles) was reacted with succinic anhydride (0.15 g, 1 mole equivalent to mPEG) in 1 mL DMF at 60°C overnight in the presence of catalytic amount of pyridine. The mixture was reprecipitated in diethyl ether, and dried in vacuo to obtain mPEG-COOH, **2b**. As comparing with mPEG (Figure 1(a)), compound **2** shows the peak at 1735 cm^{-1} referring to carbonyl groups, while the peaks at 2875 cm^{-1} and 1105 cm^{-1} belong to methylene groups and ether bonds (C-O-C), respectively (Figure

1(b)). ^{1}H NMR confirmed the new peak at 2.5 ppm belonging to methylene protons of succinic anhydride. Compounds **2a**, and **2c** were similarly prepared by using different molecular weights of mPEG, i.e., 550, and 5000, respectively.

Phthaloylchitosan-mPEG (3)

Chitosan (M_v = 1.7 × 10^5, and degree of deacetylation (%DD) = 90, provided by the Seafresh Chitosan (Lab) Thailand) was modified to be phthaloylchitosan, **1**, as reported by Nishimura et al.[19] The phthalimido group was substituted for 80% to the chitosan chain as determined from EA.[20] Compound **2b** (3.25 g, 0.40 moles equivalent to **1**) was stirred with 1 (1.00 g, 3.86 x 10^{-3} moles) in 20 mL DMF solution containing 1-hydroxy-1H-benzotriazole, monohydrate (HOBt, 0.71 g, 3 moles equivalent to **2b**) at room temperature until clear solution. 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide, hydrochloride (WSC, 0.89 g, 3 moles equivalent to **2b**) was reacted for overnight. The mixture was dialyzed in water and thoroughly washed with methanol to obtain white particles, **3e**. For comparative studies, a series of the compounds **3a**-**3d** were also prepared by varying the amount of **2** for 0.02, 0.05, 0.10, and 0.20 moles equivalent to **1**. Comparing with chitosan (Figure 1(c)) and phthaloylchitosan (Figure 1(d)), the successful reaction for **3** was identified. For example, **3e** is confirmed from the peaks at 2875, 1360, and 1345 cm^{-1} belonging to methylene and methyl groups, respectively (Figure 1(e)). The peaks at 1714 and 1776 cm^{-1} possess to C=O in anhydride whereas 1714 cm^{-1} also belongs to C=O in ester. ^{1}H NMR exhibited the peaks at 2.5 and 3.4 ppm belonging to methylene protons of succinic anhydride and mPEG, whereas the peaks at 3.1, 2.9-4.7 and 7.9 refer to methyl, hexosamine, and aromatic protons, respectively.

Results and Discussion

Colloidal Formation

It is important to point out that the reaction producing **3** proceeded in a homogeneous system, where the effective mPEG conjugation to the chain of chitosan can be expected. The mPEG was modified to be mPEG-COOH and conjugated with chitosan by WSC via ester linkage. Although the mPEG-COOH crude product

might contain unreacted mPEG, in this case, the reaction of mPEG could be accomplished with chitosan through WSC via ether linkage. Thus, polyethylene glycol chains were functionalized onto the chitosan chain. When white particles of **3** were added to water (Figure 2) or organic solvents (Figure 3), either polar or non-polar ones, such as methanol, ethanol, iso-propanol, chloroform, toluene, and hexane, followed by sonication for few minutes, the solutions became white turbid. We speculated that the colloidal phenomenon was induced via the interaction between **3** and the solvent molecules. One of the evidences to support our speculation is that even **1** shows the increasing in turbidity after sonication. This might be due to the tendency to form micelle-like structure owing to the hydrophobicity of phthalimido groups on chitosan chain. It is necessary to consider that plain chitosan and most derivatives cannot be dissolved in water and common organic solvents but soon precipitated out. Thus, we hardly see the colloidal solutions. When the hydrophilicity and hydrophobicity on the chain balance at a certain level, the turbidity is significant implying the formation of a micelle-like structure. The order of the turbidity is **3e** > **3d** ≥ **3c**; moreover, all sonicated solutions of **3** maintained the turbidity for more than a week at room temperature. This also supports our speculation about the interaction and the high stability between water molecules and mPEG chains on chitosan.

Sphere Appearance

Compounds **1** and **3** were taken from the solution and dried to observe the appearance by scanning electron microscope (SEM). Figure 4(a) clarifies that chitosan starting material is irregular flake, while **1** is partially round in shape (Figure 4(b)). The appearance of **1** implies the initial step of sphere formation as a result of phthalimido groups in chitosan chain. This effect is much more significant for a series of **3** which well-defined spheres in shape and size are observed (Figure 4(c) and 4(d)). For example, spheres of **3e** are on average the size of 1500 nm (Figure 4(d)).

Core-Corona Concept and Particle Size Evaluation

It should be noted that after separating spheres from the solution, SEM photographs indicate individual spheres and some aggregation (Figure 4(c) and (d)). Serizawa et al.[21] reported the evaluation of polystyrene nanospheres under the concept of core-corona structure where mPEG refers to the corona part and polystyrene to the core. In that case, when mPEG chain is longer, the hydrophilic interaction of corona is significant and leads to the dense core; as a result, the appearance of particle size is small. In our case, we consider chitosan moiety as a core and expect to see the effect of mPEG chain length and its content. Particle size analyzer is applied to evaluate the overall of the core-corona size. However, we have to accept that by this technique either the individual spheres or aggregates will be identified as particles, thus, we intentionally define the size borderline at 3 μm for small ones (Figure 5). Figure 5(a) indicates that the higher the molecular weight of mPEG is, the smaller the particle size will be. This implies the mPEG chain contracts chitosan core. The result that mPEG 5000 showing an amount of small particles as high as 60% (when mPEG 5000 content is 0.40 moles equivalent to **1**) also supports the mPEG effect (Figure 5(a)). We, then, focused on mPEG 5000 but varied its content. Figure 5(b) gives two important results. First, it clearly identifies that **1** rarely show small particles, which might be related to the lack of mPEG. Second, the high content of mPEG enhances the preferable condition to form small particles. Taking this into our consideration, we concluded that the phenomena about the suspension, sphere formation, well dispersion in medias, and stability are controlled by mPEG side chain on chitosan.

Acknowledgement

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References

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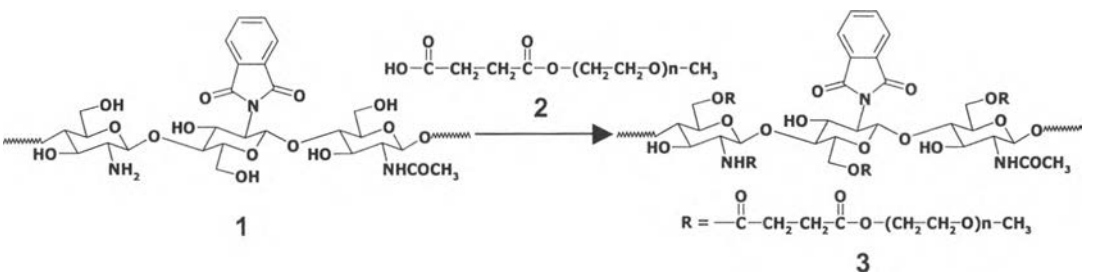
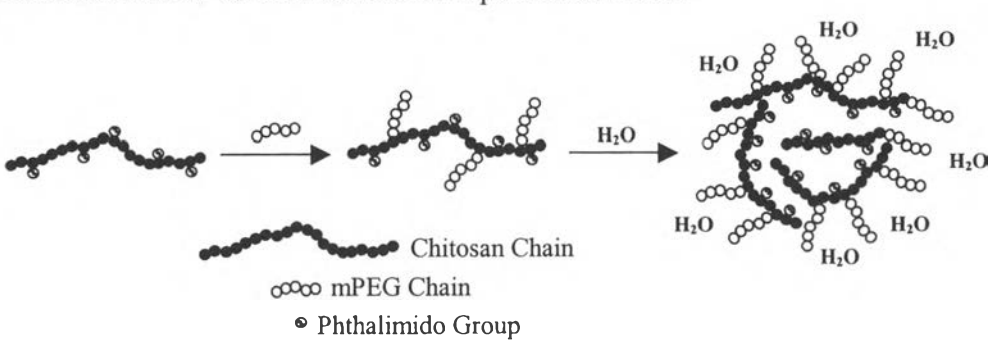
Notes

Elemental analysis of 1: Anal. Calcd. for 80% substitution $(C_{14}H_{13}O_6N)_{0.8}$ $(C_6H_{11}O_4N)_{0.1}$ $(C_8H_{13}O_5N)_{0.1}$: (%) C, 56.17; H, 4.75; and N, 5.20; Found: (%) C, 56.18; H, 4.45; and N, 4.35.

(a) Reaction of N-phthaloylchitosan grafted mPEG



(b) Schematic draw of the reaction and sphere formation



Scheme 1 (Yoksan et al.)

Figure Captions

Figure 1 Fourier transform infrared (FTIR) spectra of (a) mPEG, (b) **2b** (M_n of mPEG = 2000), (c) chitosan, (d) **1**, and (e) **3e** (mPEG content = 0.40 moles equivalent to **1**).

Figure 2 Turbidity and stability of **1**, **3c**, **3d**, and **3e** in water (a) before, and (b) after sonication and left for a week.

Figure 3 Turbidity and stability of **3e** in water, methanol, ethanol, isopropanol, chloroform, toluene, and hexane (from left to right) (a) before, and (b) after sonication.

Figure 4 Scanning electron microscopy (SEM) photographs at 25 kV of (a) chitosan (magnification of 15,000), (b) **1** (magnification of 20,000), (c) **3e**, magnification of 10,000), and (d) **3e** (magnification of 35,000).

Figure 5 Various particle sizes under the effects of (a) molecular weight of **2** (mPEG content = 0.40 moles equivalent to **1**) and (b) content of **2c** (open bar: for particle size smaller than 3 μm; stippled bar: for particle size larger than 3 μm).

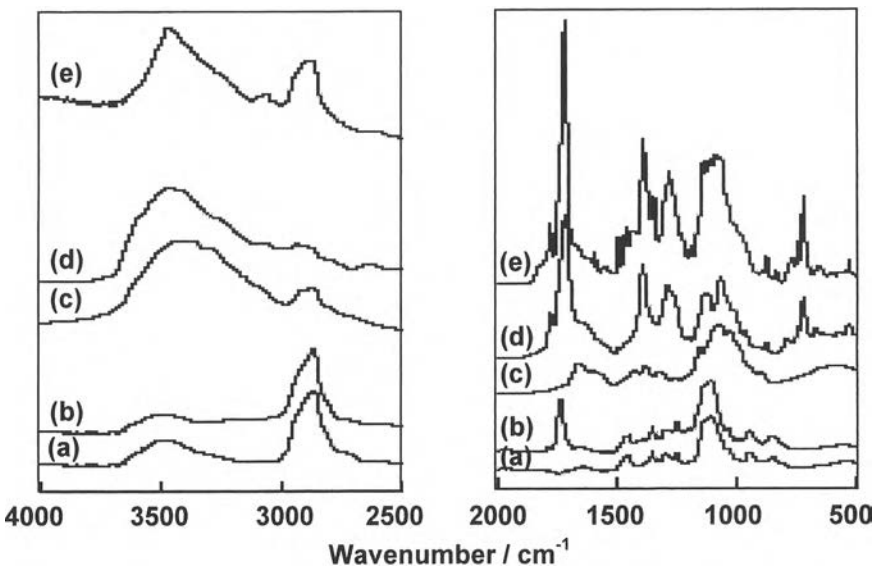


Figure 1 (Yoksan et al.)

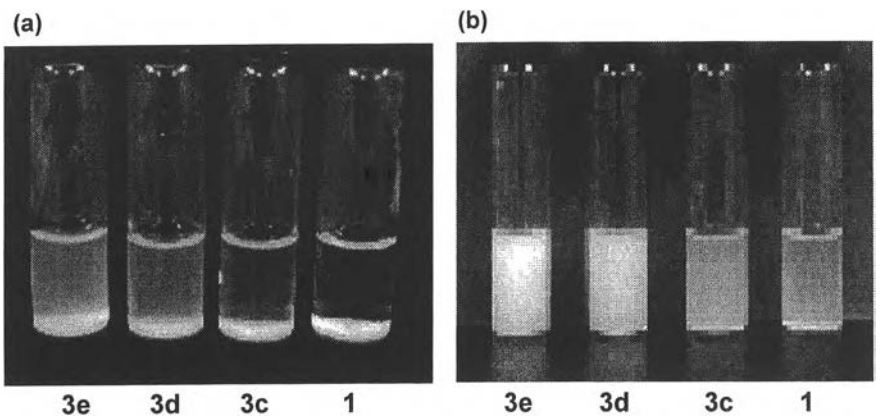


Figure 2 (Yoksan et al.)

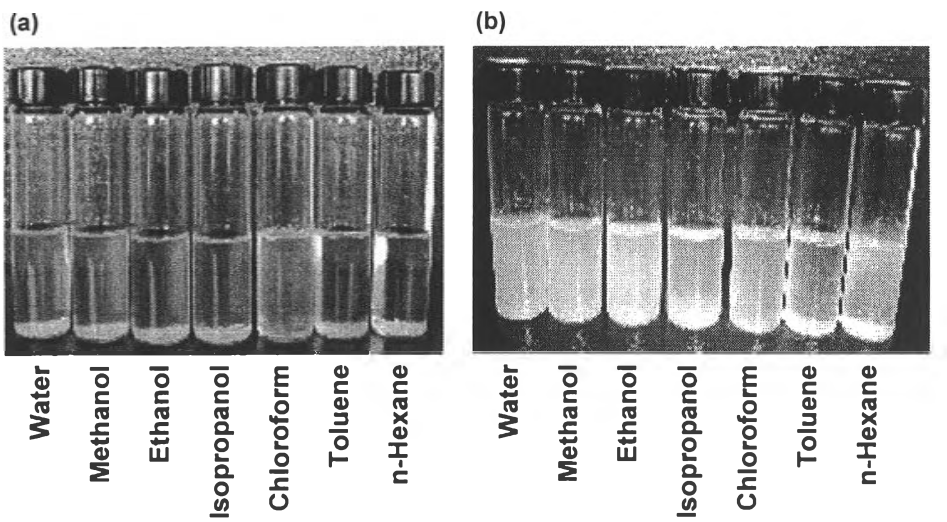


Figure 3 (Yoksan et al.)

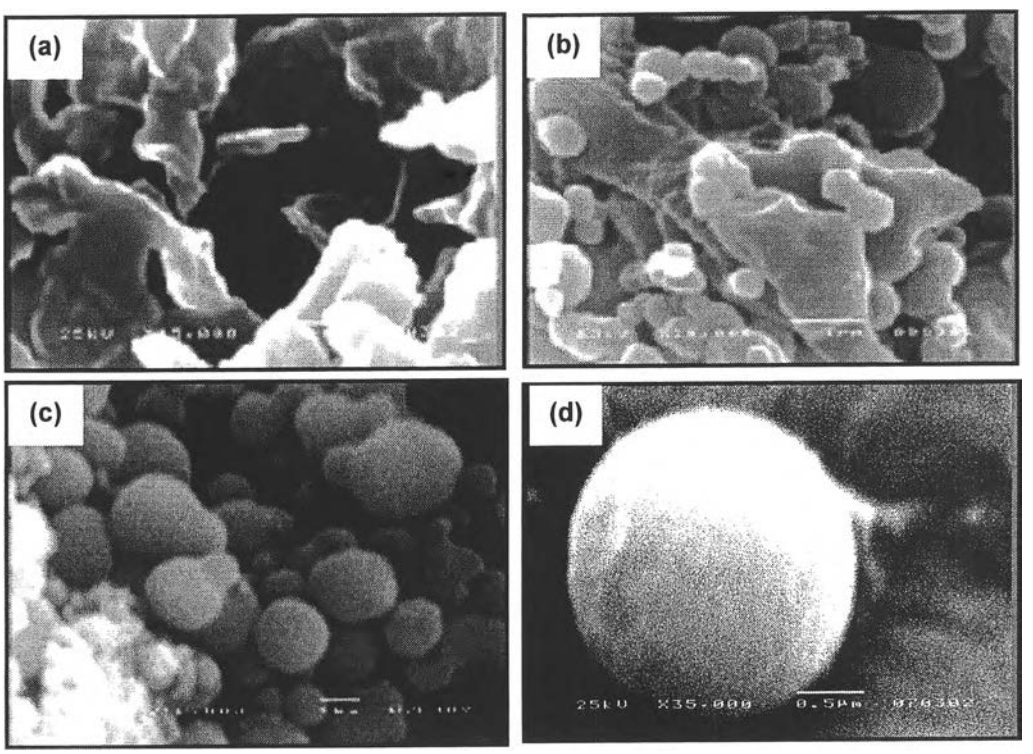


Figure 4 (Yoksan et al.)

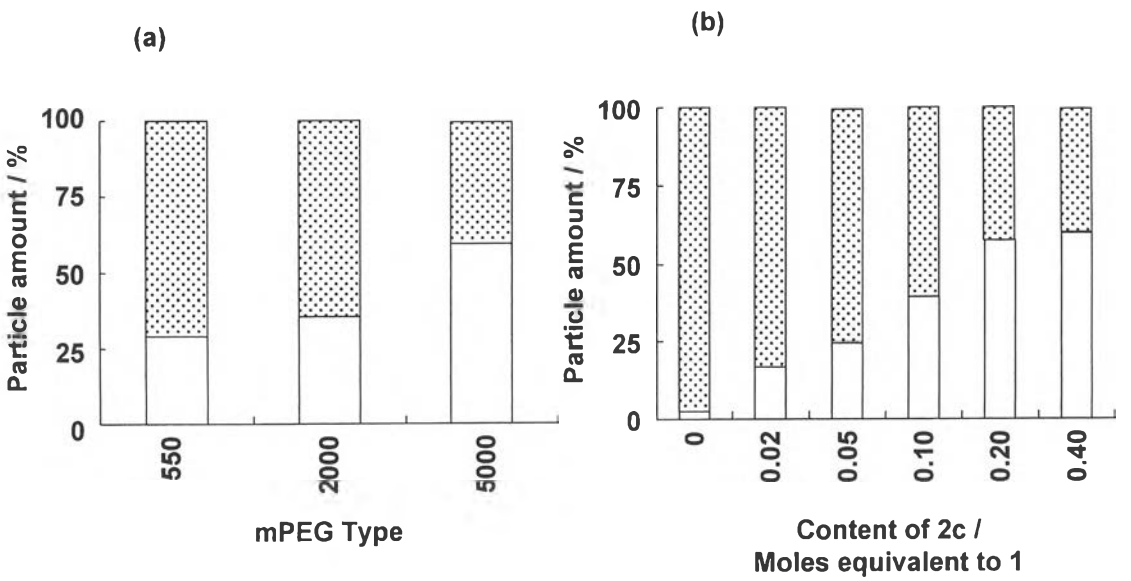


Figure 5 (Yoksan et al.)

Supplementary Materials: Figure Captions

Figure I. ^{1}H NMR of mPEG.

Figure II. ^{1}H NMR of **2**.

Figure III. ^{1}H NMR of **3**.

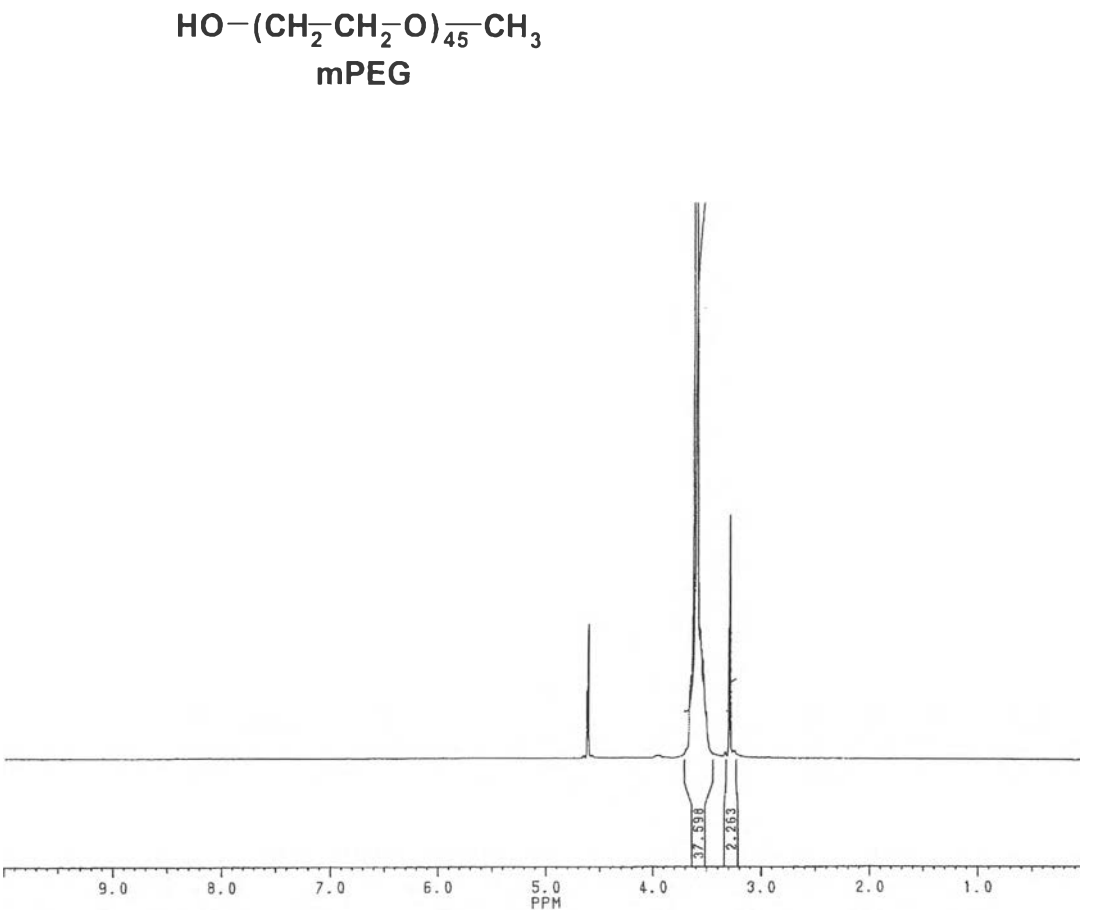
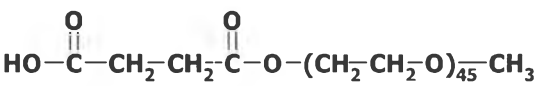


Figure I (Yoksan et al.)



2

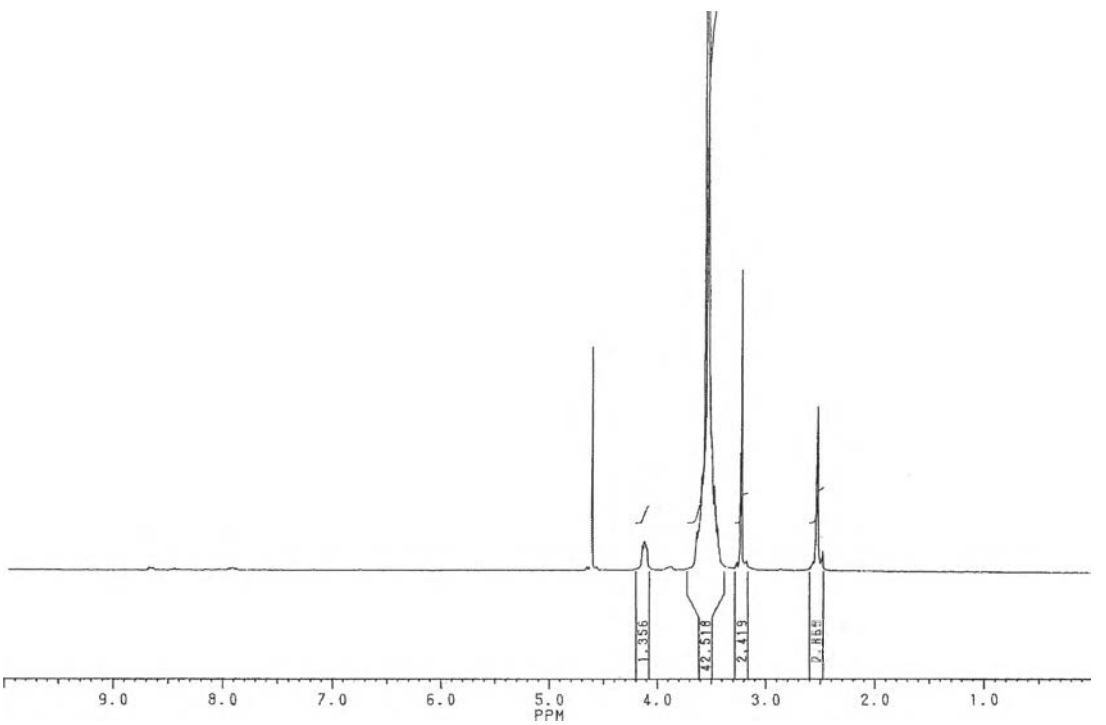
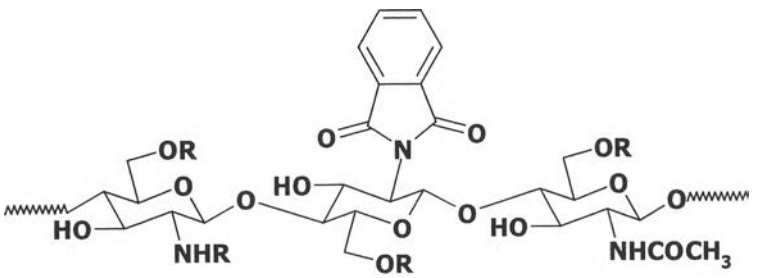


Figure II (Yoksan et al.)



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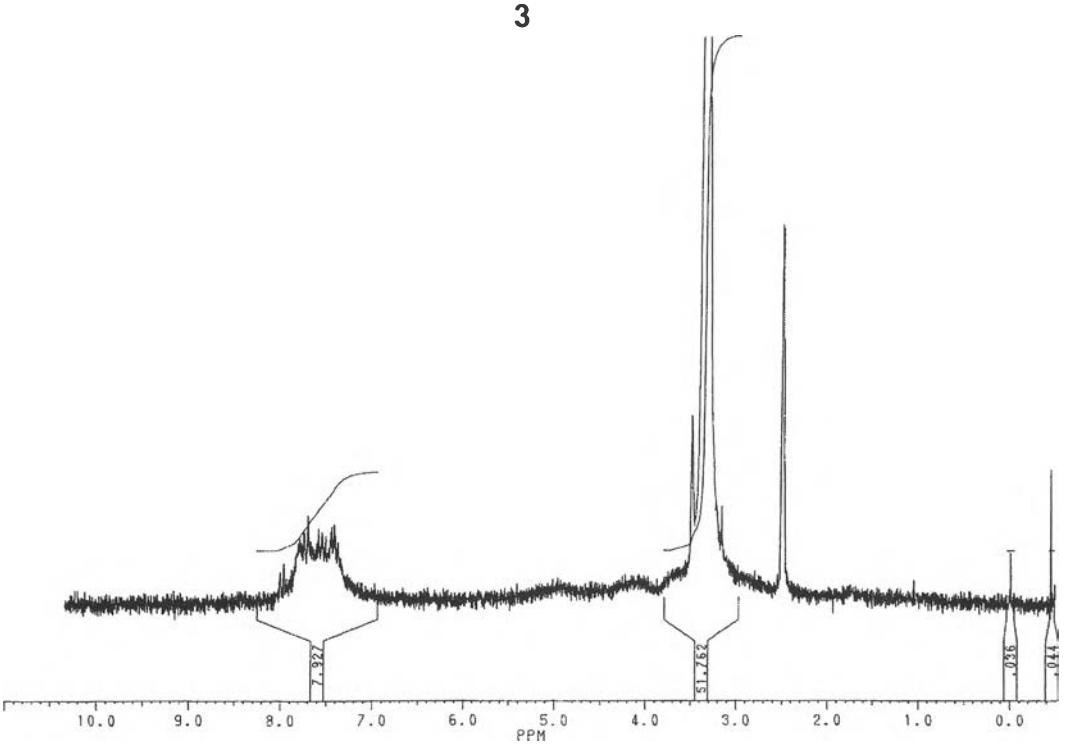

Figure III (Yoksan et al.)