SYNTHESIS, CHARACTERIZATION AND THERMAL PROPERTIES OF CHITIN-*G*-POLY(CAPROLACTONE) COPOLYMERS BY USING CHITIN GEL

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Abstract

Chitin is known to be natural polymer and it is non-toxic, biodegradable and biocompatible. The chitin-*g*-poly(caprolactone) (Chitin-*g*-PCL) copolymer was prepared by the ring-opening polymerization of caprolactone onto chitin gel in the presence of tin(II) 2-ethylhexanoate catalyst by bulk polymerization method under homogeneous system. The prepared copolymer were characterized by FT-IR, ^{13}C-NMR, thermogravimetric analysis (TGA), differential thermal analysis (DTA), scanning electron microscopy (SEM), solubility and X-ray diffraction (XRD).The degree of substitution of chitin-*g*-PCL copolymer was found to be 0.48. The TGA analysis showed that chitin-*g*-PCL was slightly less thermal stability than original chitin. It was due to the grafting of PCL degraded the crystalline structure of chitin. DTA analysis of chitin-*g*-PCL showed the two exothermic peaks between 300 and 400 °C. The first peak at 342 °C was due to chitin peak and the second peak was due to PCL. These results suggested that chitin and PCL chains were mixed well at a molecular level. The XRD pattern analysis of chitin-*g*-PCL showed a weak and broader peak, which demonstrated that the conjugation of PCL with chitin suppressed the crystallization of both chitin and PCL to some extent. The SEM studies showed that the chitin gel seems have a smooth surface morphology, but the chitin-*g*-PCL showed slightly rough morphology due to the grafting of PCL into chitin. The surface morphology studies also confirmed the grafting reaction.

Introduction

Chitin, a naturally abundant mucopolysaccharide, and the supporting material of crustaceans, insects, is well known to consist of 2-acetamido-2-deoxy-β-D-glucose through a β (1→ 4) linkage. Its immunogenicity is exceptionally low, in spite of the presence of nitrogen. It is a highly insoluble material resembling cellulose in its solubility and chemical reactivity. It may be regarded as cellulose with hydroxyl at position at C-2 replaced by an acetamido group. Chitin is a white, hard, inelastic, nitrogenous polysaccharide and the major source of surface pollution in coastal areas [1, 2]. As most of the present-day polymers are synthetic materials, their biocompatibility and biodegradability are much more limited than those of natural polymers such as cellulose, chitin, chitosan and their derivatives. However, these naturally abundant materials also exhibit a limitation in their reactivity and processability [3, 4]. In this respect, chitin is recommended as suitable functional materials, because these natural polymers have excellent properties such as biocompatibility, biodegradability, non-toxicity, adsorption properties [5-8]. However, the practical use of chitin has been confined to the unmodified forms due to its insolubility in the most of the organic solvents. For a breakthrough in utilization, graft copolymerization onto chitin will be a key point, which will introduce desired properties and enlarge the field of the potential applications of

chitin by choosing various types of side chains [8-11]. Of the possible chemical modifications of this rigid polysaccharide, grafting with synthetic polymers has been explored as an interesting alternative method to develop novel hybrid materials, such as chitin-g-polystyrene, chitin-g-poly(methyl methacrylate), chitin-g-poly(γ-methyl L-glutamate), chitin-g-poly(2 –alkyl-2-oxazoline) and chitin-g-[poly(2-methyl-2-oxazoline have been reported so far. But these polymers have limited biodegradability because of the presence of their no degradable branches.

Poly(caprolactone) (PCL) is an attractive material which combines several interesting properties such as biodegradability, biocompatibility, good mechanical properties [12]. It may have multiple biomedical and environmental applications, as prostheses, bandages or controlled release matrix for active principles (ex. Drugs, pesticides and surgery repair materials) [13-16]. Therefore, it is promising material to combine chitin with PCL to produce a new biosynthetic polymer hybrid appreciable for a variety of purposes. PCL is generally prepared from catalyzed ring-opening polymerization of ε-caprolactone. Tin(II) 2-ethylhexanoate is a representative catalyst for its high efficiency and low toxicity. Chitin-*g*-PCL has been reported by using the water soluble chitin [16]. In the present study, we applied the homogenous reaction system of *ε*-caprolactone for chitinous polymer synthesis. Here, we are reporting synthesis and characterization of chitin-*g*-PCL copolymer by using chitin-*ε*-caprolactone gel in the presence of tin(II) 2-ethylhexanoate catalyst under homogeneous system by bulk polymerization method.

Material and Methods

Materials

Chitin (M_W-206,000, degree of acetylation 72.4 %) was received from Katakura Chikkarin Ltd. *ε*-Caprolactone and tin (II) ethyl hexanote were received from Wako Pure Chemicals. All other materials used were of analytical grade.

Preparation of chitin-ε-PCL gel

10 g of chitin was mixed with 1 L of saturated $CaCl_2.2H_2O/CH_3OH$ solution (1% w/v chitin solution). After the pressure filtration 5 L of distilled water was added to the above solution. The chitin hydrogel suspension was dialyzed for 1 week and followed by centrifugation. The finally chitin hydrogel was formed. 1 g of chitin hydrogel was converted into chitin-methanol gel by solvent exchange with centrifugation. In the same way the chitin-methanol gel was converted to chitin-*ε*-caprolactone gel. The chitin-methanol-*ε*-caprolactone gel was evaporated at 40 ºC to remove the methanol fraction. Finally chitin-*ε*-caprolactone gel was formed.

Preparation of chitin-g-PCL copolymer

30 g of chitin-*ε*-PCL gel with 0.1 ml of tin (II) ethyl hexanote was added into a 100 ml flask. The reaction mixture was stirred continuously for 24 h at 140 ºC. After 24 h the reaction mixture was poured into 150 ml toluene. To precipitate the graft copolymer into 350 ml of methanol: toluene mixture (3:7). The precipitate was filtered and washed with methanol. The precipitate was dried in vacuum oven at 37 ºC for 24 h. The yield of the product was 70 %. The synthesis of chitin-*g*-PCL copolymer was shown in Figure 1.

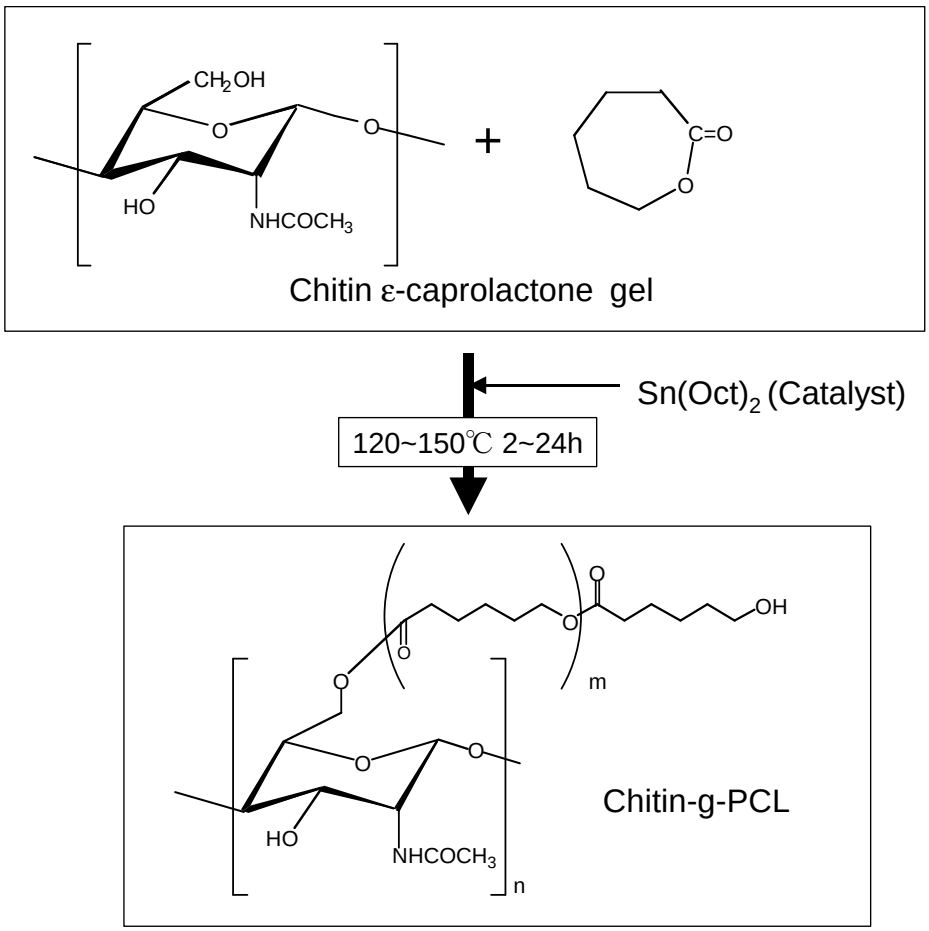


Figure 1 : Synthesis of chitin-*g*-PCL copolymer

Measurements

The IR spectra of the polymers were recorded in a Perkin Elmer FT-IR 2000 series spectrophotometer at room temperature with the KBr pellet method. The ^{13}C-NMR spectra of the polymers were recorded with a JEOL JMN-GSX-400 MHz spectrometer. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were performed with a SII TG-DTA 6200 thermal analyzer using 2 mg of the sample at a heating rate of 10 ºC/min in nitrogen. The surface morphology of samples was analyzed by scanning electron microscopy (SEM) by using JEOL JSM-6700 microscope. X-ray diffractograms were recorded according to a powder method with a Mac Science M_3X (model no. 1030) diffractometer using CuKα radiation. The solubility of the polymers was tested in various polar and non polar solvents by taking 10 mg of polymers in 2 mL of different solvents in a closed test tube and set aside for one day. The solubility of the polymers was noted after 24 h.

Results and Discussion

Synthesis of chitin-g-PCL

Chitin-*g*-PCL copolymers were prepared in different conditions according to Table 1. In this work we focused graft copolymerization under homogeneous system by bulk polymerization. From this system we got high yield. The grafted copolymer was easily separated by using toluene and methanol solvent mixture. The grafted copolymer was easily soluble in the solvent mixture. The grafted copolymer was partially soluble in most of the organic solvents and swelling in chloroform, toluene, acetone and ethyl acetate. The degree of substitution was around 0.48 and the degree of polymerization (*m*) was 3.2.

Run	Chitin (dry, (mmol))	ε-caprolactone (mmol)	$Sn(Oct)_2$ ml	ε-caprolactone/chitin	Temperature (ºC)	Time (h)
1	2.56	98.97	-	50.24	150	24
2	2.56	98.97	0.1	50.24	140	2
3	2.56	215.9	0.1	84.34	140	5
4	2.56	215.9	0.1	84.34	140	10
5	2.56	215.9	0.1	84.34	140	24
6	2.56	215.9	-	84.34	140	24

Table 1: Synthesis data of chitin-*g*-PCL

Characterizations

FT-IR Spectra

The FT-IR spectra of chitin, chitin-*g*-PCL and PCL were shown in Figure 2. A strong absorbance peak between 2800 and 3000 cm^{-1} were due to methylene groups. It confirms the successful introduction of the PCL chain into the chitin. The ester carbonyl stretching band was observed at 1727 cm^{-1}. The peak of amide carbonyl stretching band (amide I band) and an N-H bending band (amide II band) were appeared at 1667 and 1559 cm^{-1}, respectively.

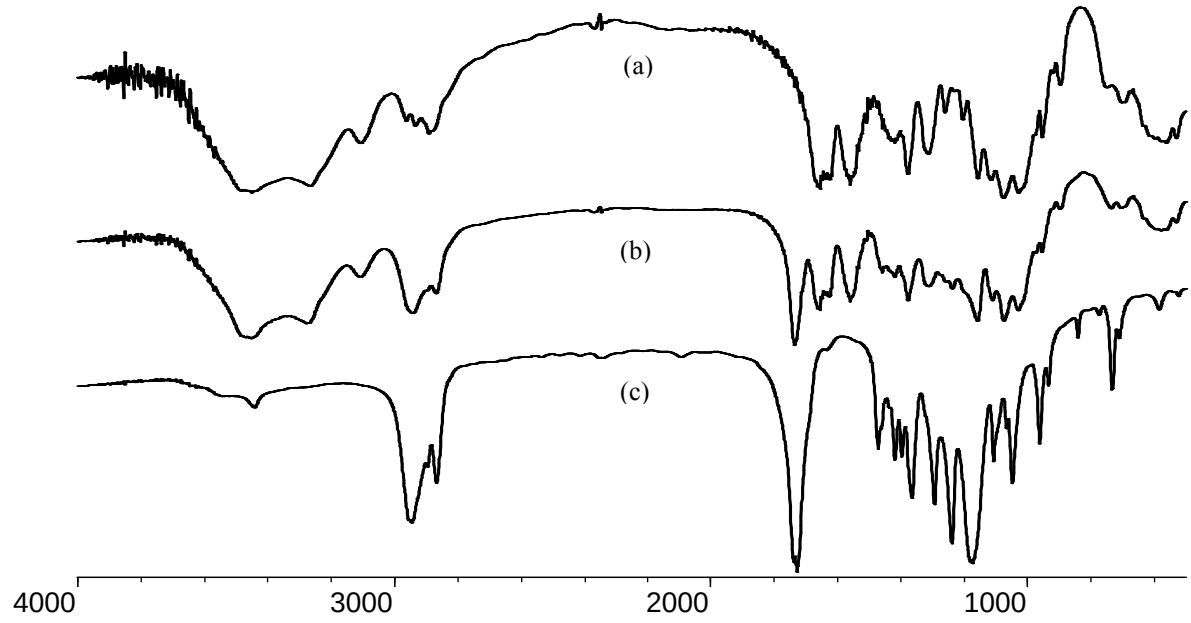


Figure 2 : FT-IR Spectra of (a) Chitin, (b) Chitin-*g*-PCL and (c) PCL

Solid state ^{13}C-NMR Spectra

The ^{13}C-NMR spectra of chitin, chitin-*g*-PCL and PCL were shown in Figure 3. The substitution of polyester chain was confirmed by the ^{13}C-NMR analysis. The ester formation connected directly to the pyranose ring occurs, the C-6 carbon attached to the ester oxygen should shifts by 2-3 ppm toward a lower magnetic field from the C-6 carbon bearing hydroxyl group. Actually, partially 6-*O*-actylated chitosan showed the ester-*O*-linked C-6 carbon signal at 65.48 ppm, while the C-6 carbon signal with a hydroxyl group of the chitosan derivative was detected at 62.79 ppm. As another example, it was reported that the C-6 carbon signal of ethyl *β*-D-glucopyranside appears at 62.0 ppm, whereas the ester-*O*-linked C-6 carbon of the glycoside derivative having poly(ε-PCL) segment the 6-position is observed in 64.3 ppm [17]. In our study, no peaks were detected around the 64-65 ppm region except for the above mentioned C-ε carbon signal at 64.12 ppm. The ester oxygen connected carbonyl carbon appears at around 61.10 ppm (C-6). The other methylene group in the PCL ring appears at 64.12 (C-ε), 36.15 (C-*α*) 29.12 (C-*δ*),

25.82 (C-β) and 26.11(C-γ) ppm. The methyl group in the chitin ring shows the signal 21.15 ppm. The solid state ^{13}C-NMR spectra confirm the structure chitin-*g*-PCL.

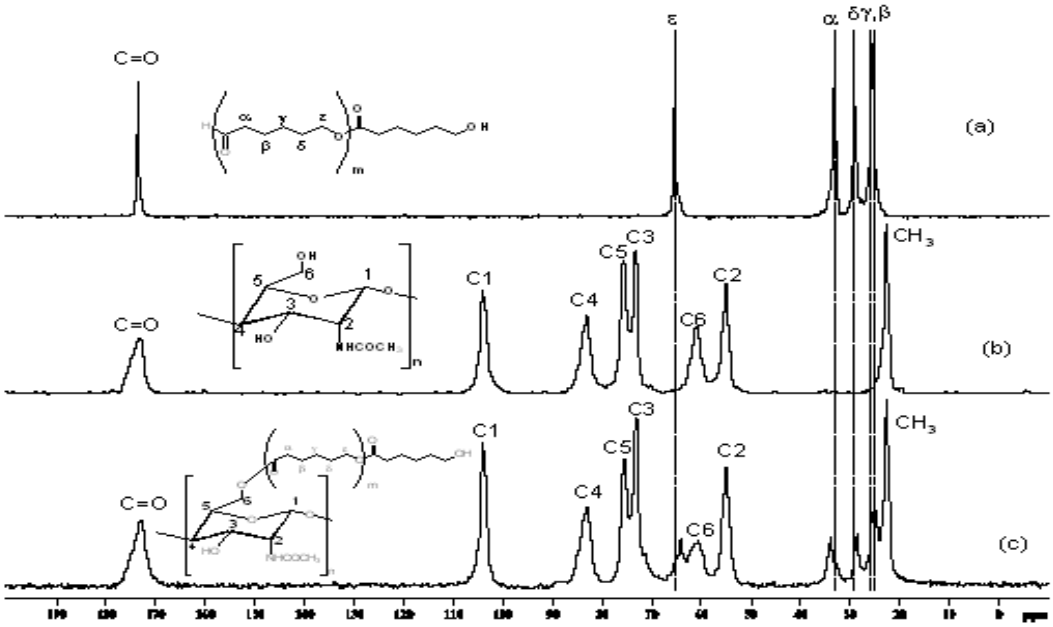


Figure 3 : ^{13}C-NMR spectra of (a) PCL, (b) Chitin and (c) Chitin-*g*-PCL

Thermal Properties

Figure 4 show the thermogram of chitin, chitin-*g*-PCL and PCL. In the thermogram of chitin (Fig. 4a) two decomposition steps could be observed, the first occurs in the range of 50–110 °C, and is attributed to water evaporation. The second occurs in the range of 300–400 °C and could be attributed to the degradation of the polysaccharide structure of the molecule, including the dehydration of polysaccharide rings and the polymerization and decomposition of the acetylated and deacetylated units of chitin. In the chitin-*g*-PCL thermogram (Fig. 4b), three decomposition steps were observed. The first peak was similar to be found in chitin, the peak was occurring in the range of 50–100 °C. The second peak was occurring in the range of 220-350 °C. The second decomposition step occurred at a lower temperature than observed for the chitin decomposition. After that the degradation of chitin-*g*-PCL occurs gradually. These results suggested that chitin-*g*-PCL was slightly less thermal stability than chitin. It was due to the grafting of PCL degraded the crystalline structure of chitin.

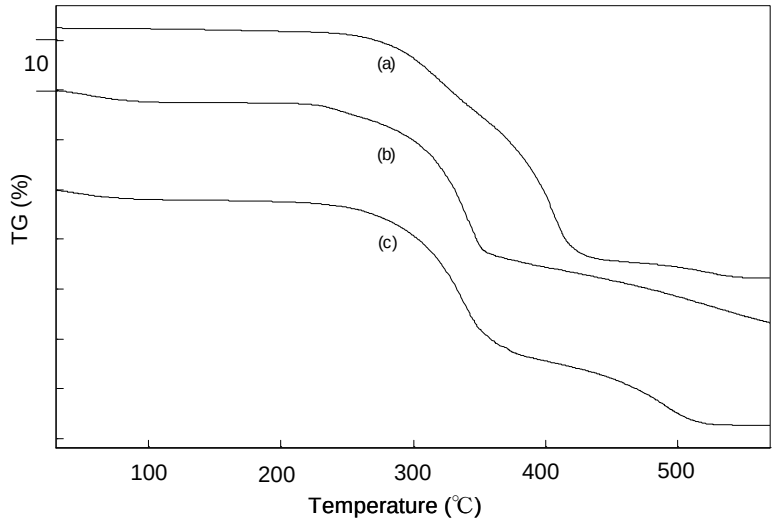


Figure 4 : TGA of (a) Chitin, (b) Chitin-*g*-PCL and (c) PCL

Figure 5 shows the differential thermal analysis of chitin, chitin-*g*-PCL and PCL. Differential thermal analysis of PCL was showed the endothermic peak was obtained at 59.2 ºC was due to the melting of the PCL crystalline phase. The chitin-*g*-PCL showed the two exothermic peaks between 300 and 400 °C. The first peak at 342 °C was due chitin peak and the second peak was due to PCL. The exothermic the peak was also obtained at 443°C in the PCL. Similarly, the exothermic peak was observed at 481°C in the chitin-*g*-PCL. The PCL peak was slightly shifted due to the grafting reaction. These results were suggested that chitin and PCL chains were mixed well at a molecular level.

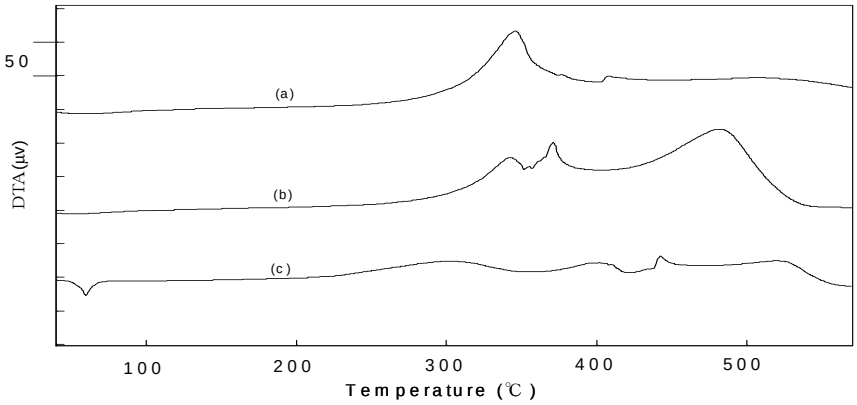


Figure 5 : DTA of (a) Chitin, (b) Chitin-*g*-PCL and (c) PCL

X-ray diffraction

The X-ray diffraction pattern of PCL, Chitin-*g*-PCL and chitin was shown in Fig. 6. It was known that the PCL homopolymer was easy to crystallize [3, 14, 15]. Compared with the original chitin, chitin-*g*-PCL showed a weak and broader peak at 2θ= 15-25º region, which demonstrated that the conjugation of PCL with chitin suppressed the crystallization of both chitin and PCL to some extent. It was confirmed that chitin and PCL chains were mixed well at a molecular level. As shown in Figure 9b and 9c exhibited a broad signal centered at 2θ= 19º, which is attributed to the GlcN sequences. Similarly, the intensity of broad signal centered at 2θ= 9º due to GlcNAc sequences. The chitin-*g*-PCL shows partially crystalline in nature. But the experiment carried out under heterogeneous system was showed amorphous in nature.

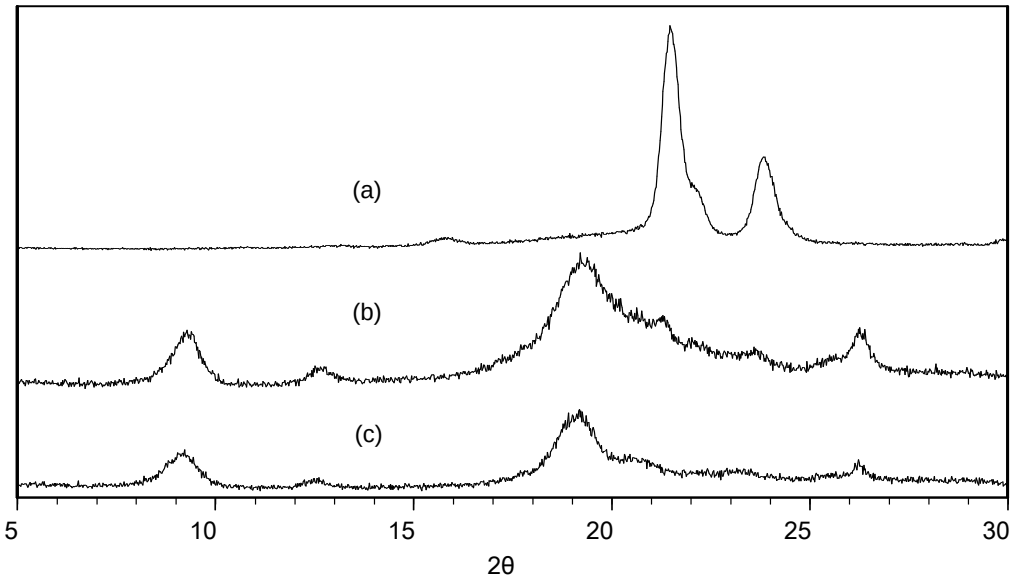
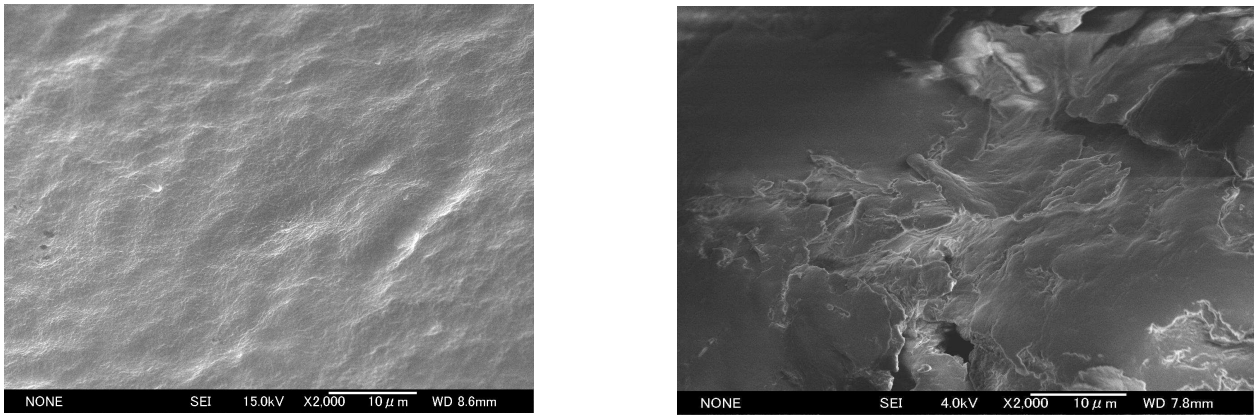


Figure 6 : XRD diffraction pattern of (a) PCL, (b) Chitin-*g*-PCL and (c) Chitin

SEM studies

The SEM pictures of chitin gel and chitin-*g*-PCL were shown in Fig. 7. The chitin gel seems have a smooth surface morphology. But the chitin-*g*-PCL showed slightly rough morphology. This may be due to the grafting of PCL into of chitin. It shows a relatively homogeneous aspect with a tightly packed structure. The surface morphology studies confirmed the grafting reaction of chitin.



(a) (b)

Figure 7 : SEM analysis of (a) Chitin gel and (b) Chitin-*g*-PCL

Solubility

The solubility data of chitin and chitin-*g*-PCL was shown in Table 2. The resulting chitin-*g*-PCL copolymer exhibited an improved affinity for organic solvents, compared with the original chitin.

Solvent	Chitin	Chitin-*g*-PCL
Water	Insoluble	Swelling
DMF	Insoluble	Swelling
DMSO	Insoluble	Swelling
CHCl3	Insoluble	Partially Soluble
Toluene	Insoluble	Swelling
1% CH3COOH	Insoluble	Highly Swelling

Table 2: Solubility data of chitin and chitin-*g*-PCL

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