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Structural aspects of poly(methyl methacrylate)-grafted β-chitin copolymers initiated by ceric salt

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Ceric salt-initiated graft copolymers of methyl methacrylate (MMA) onto β-chitin in water are investigated. The properties of the graft copolymers depended on the grafting yields. A reproducible swelling of grafted β-chitin was observed in common organic solvents, and the degrees of swelling were less than for native β-chitin. The change in crystal structure of β-chitin after grafting was studied by X-ray diffraction and compared with that for α-chitin. At lower grafting yields, graft polymerization occurred both on the normal plane (010) of the crystal and in the amorphous parts of β-chitin. When the grafting yield was higher than 260%, the diffraction of the plane (010) disappeared, which suggested conversion of the crystalline portion of β-chitin to an amorphous state by grafting. However, the grafting did not obviously affect the crystalline region of α-chitin even when the grafting yield was increased up to 620%. Evidence is presented for decrystallization of β-chitin by grafting, which in contrast with α-chitin, induced a discontinuous linear increase in hygroscopicity.

INTRODUCTION

Chitin is a linear β-(1 → 4) homoglycan composed of 2-acetamido-2-deoxy-D-glucopyranosyl (GlcNAc) residues. On the basis of crystal structures, three polymorphic forms have been recognized, known as α, β and γ-chitins (Rudall, 1963). The crystal structure of chitin has been studied mainly by X-ray diffraction, infrared spectroscopy and by CP–MAS ^{13}C-NMR (Takai *et al.*, 1988). X-ray diffraction showed that the three forms of chitin differ in the packing and polarities of the adjacent chains in a series of sheets. In β-chitin, sheets are all arranged in a parallel manner, and only intrasheet hydrogen bonds are formed (Gardner & Blackwell, 1975). In α-chitin, the sheets are antiparallel, and strong intrasheet as well as intersheet hydrogen bonds are present (Minke & Blackwell, 1978). IR spectra suggested differences between α and β-chitins both in the hydrogen bonding network and in the amide I vibration mode (Darmon & Rudall, 1950; Pearson *et al.*, 1960). Some applications of natural chitin and its modified derivatives have attracted much attention for possible use as a biomaterial (for example, Tokura *et al.*, 1992). Since chitin has poor solubility due to its high crystallinity, chemical modification has been employed as an important technique for converting such physical and chemical properties as solubility and hygroscopicity.

Fine structure and morphology of grafted cellulose copolymers initiated by ceric ion have been extensively investigated (Bhattacharyya & Maldas, 1984). X-ray analyses of poly(methyl methacrylate) (PMMA)-grafted cellulose derivatives showed a change in crystallinity of cellulose; grafting occurred in the lower-ordered region of cellulose (Shirakashi *et al.*, 1963). PMMA is known as a colorless thermoplastic; generally because of its lack of complete stereoregularity and its bulky methyl side groups, it is amorphous and represents non-crystalline diffusion in X-ray diffraction. Large decreases in crystallinity, however, have been observed at very high grafting levels for cellophane–acrylamide graft copolymer (Hori *et al.*, 1968). In previous work (Ren *et al.*, 1993), we found that the ceric salt is an effective initiator for graft polymerization of MMA onto α-chitin. At high grafting yield, grafted α-chitin derivatives swelled in common organic solvents and exhibited gel-like properties. Under certain conditions, thin film was prepared from the gel material.

In the present work, the graft polymerization of MMA onto β-chitin is investigated, and the physical properties, the fine structures and morphology of

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β-chitin derivatives are compared with those of PMMA-grafted α-chitin derivatives. We find that, by contrast with α-chitin, grafting changes the crystal structure of the original β-chitin significantly. Changes in crystal structures of both chitins after introduction of PMMA side-chains account for increases in hygroscopicity that depend on grafting yields of the copolymers.

EXPERIMENTAL

Materials

Lyophilized β-chitin from the pens of *Loligo* squid was kindly supplied by Nippon Suisan Co. Ltd, and powdered before use. Cerium (IV) ammonium nitrate, 99% formic acid and dichloroacetic acid (Wako Pure Chemical Industries Ltd) were used without further purification. MMA, also obtained from Wako, was purified by distillation under reduced pressure.

Preparation of β-chitin film

β-Chitin (1·0 g) was dissolved in a formic acid–dichloroacetic acid mixture through a process of freezing β-chitin in formic acid (20 ml) for one day at −20°C and then adding the mixture of formic acid–dichloroacetic acid (1:3 v/v) at room temperature (Tokura *et al.*, 1979). β-Chitin film was prepared from the 2% chitin solution by casting on a glass plate, solidifying in acetone, and then thoroughly washing with 0·5% methanolic potassium hydroxide, distilled water and methanol, successively. The clear and flexible chitin film was obtained after drying at room temperature by pressing it between filter papers.

Graft polymerization

Ceric salt-initiated graft copolymerization of MMA onto β-chitin (powder or film) was carried out in water under nitrogen atmosphere according to a method described elsewhere (Ren *et al.*, 1993). Grafted copolymer was isolated after Soxhlet extraction of non-grafted homopolymer with acetone, and grafting yield (the weight of grafted PMMA / the weight of chitin used) was calculated from the weights of both products. Grafted α-chitin derivatives were prepared by the same method.

Hygroscopicity

Dried samples were kept in a desiccator with a relative humidity (RH) of 76% (over saturated calcium chloride solution at 20°C). Moisture sorption and moisture content were calculated by the following equations:

$$\text{Moisture sorption (\%)} = \frac{\text{weight of absorbed moisture}}{\text{weight of dry sample}} \times 100$$

$$\text{Moisture content (\%)} = \frac{\text{weight of absorbed moisture}}{\text{weight of chitin in dry sample}} \times 100$$

Weight-average molecular weight

Grafted PMMA side chains were isolated from grafted β-chitin copolymer following acid hydrolysis. Hydrolysis of 0·5 g of graft copolymer was carried out in 50 ml of 20 N H_2SO_4 at 60°C for 30 min. The solution was cooled, diluted with distilled water to a total volume of 300 ml, allowed to remain overnight at 20°C, and the acid rinsed out. Crude product from the hydrolysate was dissolved in acetone and purified by reprecipitation for molecular weight measurement. Intrinsic viscosities ($[\eta]$) of grafted PMMA chains and homopolymer (PMMA) separated from the copolymer were measured in acetone with a Ubbelohde type viscometer at 25°C. Weight-average molecular weight ($\overline{M}_w$) was calculated by the following equation (Chinai *et al.*, 1955):

$$[\eta] = 9{\cdot}6 \times 10^{-3} \times \overline{M}_w^{0{\cdot}69}$$

The apparent number of PMMA grafts (number of grafting), which is defined as the moles of PMMA grafts per 100 g of chitin, was calculated by the equation describing as below:

$$\text{Number of grafting} = \frac{\text{grafting yield (\%)} \times 100\,(\text{g})}{\text{molecular weight of PMMA grafts } (M_w)} \;/100\,\text{g of chitin}$$

Swelling behavior

The swelling of grafted β-chitin in solvent was determined with a method similar to that reported for α-chitin. For the first swelling procedure of the polymer, powdered sample (0·3 g) was immersed in 30 ml of solvent such as *N*, *N*-dimethylformamide (DMF) at 20°C for one week. The wet polymer was collected by centrifugation at 3000 rpm for 10 min, followed by weighing the swollen material after eliminating free solvent out with a filter paper. The degree of swelling was calculated from the ratio of the weight of solvent involved in the swollen material to the weight of dry polymer. After removal of the solvent from the swollen specimen by extensive rinsing with methanol, the sample was dried at 50° C *in vacuo*. Then, the second swelling procedure for the polymer was carried out in the solvent again by applying the same method as for the first one.

Measurements

Infrared spectra were measured in KBr pellets with a Nicolet 5DXB Fourier-transform infrared spectrometer at 4 cm^{-1} resolution. X-ray powder or film patterns were measured by the reflection method with nickel-filtered CuKα radiation in a Rigaku RAD–IISR X-ray diffractometer operated at the ω–2θ scanning mode between 5° and 55°. Surface images were obtained by scanning electron microscopy with a Hitachi SEM model S-510 operating at 15 kV after spattering with Au–Pd.

RESULTS AND DISCUSSION

Graft polymerization of MMA

On the basis of graft polymerization of MMA onto α-chitin, the molar ratio of MMA to GlcNAc residues was found to be the most important factor in the preparation of derivatives with extensive grafting (Ren *et al.*, 1993). These derivatives showed several changes in chemical and physical properties. The same conditions have been found useful for grafting to β-chitin. Figure 1 shows the effect of the molar ratio of MMA to GlcNAc residues of β-chitin on grafting yield and a comparison with grafting to α-chitin. The grafting yield increases steadily with an increase in the molar ratio. At the lower molar ratios, grafting yields of β-chitin derivatives agree very closely with the results for the graft polymerization of MMA onto α-chitin. When the molar ratio of MMA to GlcNAc in β-chitin is higher than about 60, the grafting yield is much larger than the value of grafting yield on α-chitin up to 2300%. At higher molar ratios, the grafting yield of β-chitin derivatives increases remarkably to even more than 4500%.

Molecular weight and the apparent number of grafts

The molar ratio of MMA to GlcNAc residues of β-chitin also affects the molecular weight of graft copolymer and the number of graft chains. Figure 2 represents the influence of grafting yield on the weight-average molecular weights of grafted PMMA side chains, homopolymer (PMMA) and the number of grafting (moles PMMA grafts / 100 g of chitin). Molecular weights of grafted PMMA chains increase with increasing grafting yield and reach the maximum value of $2{\cdot}5 \times 10^6$. When the grafting yield is higher than 400%, the weight-average molecular weights of grafted PMMA chains decrease even though there is an increase of grafting yield. It is also seen in Fig. 2 that the molecular weight of grafted PMMA chains is greater than that of the homopolymer (PMMA); the disparity in molecular weights between grafted PMMA and homopolymer is about 1×10^6. By contrast, the number of grafting increases steadily with increasing grafting yield. The grafting yield increases with the molar ratio of MMA to GlcNAc already seen in Fig. 1; i.e., for β-chitin, the branching profile of the copolymer is regulated by the molar ratio of MMA to GlcNAc.

Solubility of PMMA-grafted β-chitin derivatives

Solubility was changed considerably by graft polymerization. Grafted derivatives were readily soluble in strong acid, such as formic acid, and swelled markedly

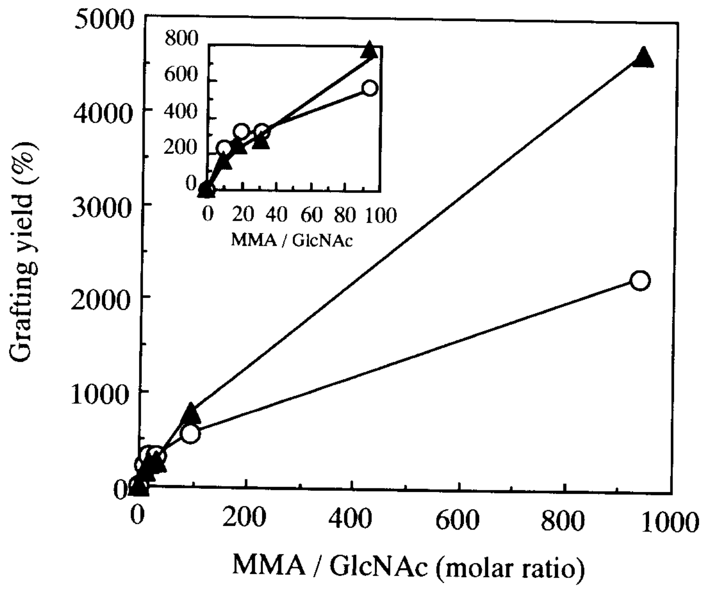

Fig. 1. Influence of molar ratio of MMA to GlcNAc residues of chitin powder on grafting yield. ○, Grafting onto α-chitin; ▲, grafting onto β-chitin. H_2O, 220 ml; [MMA], $1{\cdot}99 \times 10^{-1}$ M; [I], $4{\cdot}25 \times 10^{-3}$ M; [HNO_3], $3{\cdot}83 \times 10^{-2}$ M; *T*, 30°C; *t*, 12·5 h. Inset: magnified figure at low molar ratio.

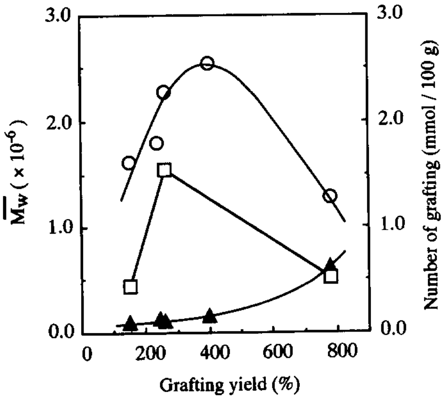

Fig. 2. Influence of grafting yield of β-chitin copolymer on the weight-average molecular weights of grafted PMMA chains (○) and homopolymer PMMA (□), and the number of grafting, i.e. mmol PMMA grafts / 100 g chitin (▲).

in common organic solvents. Figure 3 illustrates the effect of the grafting yield as a factor of swelling the modified β-chitin derivatives in DMF at 20°C for one week. Unmodified β-chitin shows a degree of swelling of about 20 in DMF, which is higher than for grafted β-chitin derivatives, and is probably due to the loose crystal structure of β-chitin. The swelling behavior of copolymers seems to depend on the grafting yield. A minimum degree of swelling of about 6 is observed in the copolymer with grafting yield of 244%. The swollen grafted sample is opaque yellow in color, which is different from the β-chitin itself. After the extraction of DMF from the swollen specimen by methanol and drying at 50°C *in vacuo*, swelling was reproducible for the grafted copolymers, but not for β-chitin, which decreased to *c.* 9. On the basis of the fact that the β-chitin was slightly shrunk by DMF treatment comparing with that of native β-chitin and did not clearly suggest any structural change by IR and X-ray diffraction , this decrease in swelling might be attributed to the process of reassembly of β-chitin chains during drying. In addition to DMF, the grafted copolymers also swelled in other organic solvents such as benzene, toluene, *N*, *N*-dimethylacetamide.

Hygroscopicity of graft copolymers

In Fig. 4, it is seen that β-chitin absorbs moisture more rapidly in the early stage, and reaches the equilibrium value (about 26%) of moisture sorption after 24 h. After grafting, much less moisture sorption occurs compared with β-chitin; all grafted copolymers absorb moisture 5–14% after 50 h. Generally, the moisture sorption of copolymer decreases with an increase in the grafting yield due to the grafting of hydrophobic PMMA. Similar decreases of moisture sorptions are also displayed in the α-chitin after grafting (not shown). In the PMMA-grafted β-chitin copolymers, however, unusual behavior in moisture sorption is observed with 244–260% of grafting yield. Chitin content in graft copolymer must affect the moisture absorption behavior of copolymer, since the chitin chains contribute hydrophilic hydroxyl and acetamide groups.

Moisture content, which is defined as the weight of absorbed moisture to the weight of chitin in dry sample, was used to estimate the hygroscopicity of the graft copolymers. Figure 5 shows the influence of grafting yield on moisture content in both grafted α- and β-chitin derivatives. In the case of grafted β-chitin, moisture content increased linearly with increasing grafting yield up to 244%, when a sharp rise occurred. When the grafting yield was higher than 260%, this trend is leveled off, and apparently increased again steadily with the grafting yield. For the α-chitin, the moisture content simply showed a linear increase with the grafting yield. Both moisture contents of α- and β-chitin after grafting increase linearly with an increase in grafting yield. The differences in their moisture contents at lower grafting may be attributed to their original crystal structures. The discontinuous increase in moisture content of

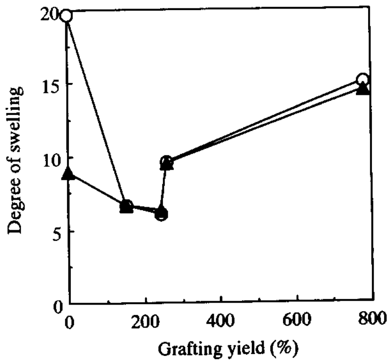

Fig. 3. Influence of grafting yield on the degree of swelling in DMF at 20°C for one week. ○, The first swelling procedure; ▲, the second swelling procedure after reprecipitation with methanol and drying at 50°C *in vacuo*.

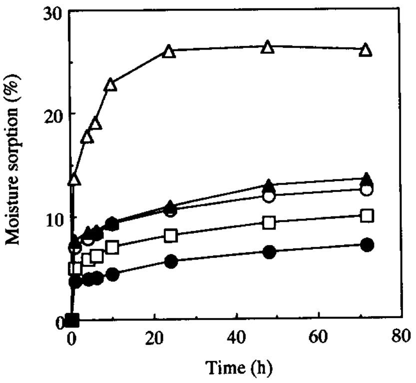

Fig. 4. Time course of moisture sorption by grafted β-chitin derivatives compared with β-chitin. β-Chitin (△); grafted copolymers with grafting yields of 152% (○), 244% (□), 260% (▲) and 780% (●).

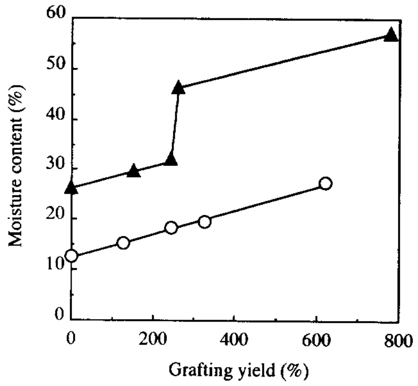

Fig. 5. Influence of grafting yield on moisture content of copolymer. ○, α-Chitin and its derivatives; ▲, β-chitin and its derivatives. Relative humidity, 76%; *T*, 20°C; *t*, 72 h.

β-chitin derivatives at higher grafting yield suggests that grafting changed the crystalline structure of β-chitin to an amorphous state, which will be discussed below. The linear increase in moisture content of α-chitin derivatives may be attributed to the grafting occuring predominantly in non-crystalline areas due to the predominant strong crystal structure of α-chitin.

IR examination of copolymer structure

Structure changes of β-chitin induced by grafting were analyzed by FT–IR as given in Fig. 6. A broad absorption band centered at 3440 cm^{-1}, the amide I band at 1651 cm^{-1} and the amide II band at 1560 cm^{-1} are similar to those observed in β-chitin. The absorption at 1311 cm^{-1}, due to the amide III group of β-chitin, decreases with increase in grafting yield. The strong absorption band seen in the grafted β-chitin derivatives at 1730 cm^{-1} is due to the carbonyl groups of grafted PMMA side chains.

X-ray diffraction study of structure in graft copolymers

Since α- and β-chitins show different crystal structures, the ordered arrangement in the grafted derivatives was examined in order to understand why their properties differed. The crystal structure of grafted β-chitin powder was studied by X-ray diffraction in the angular range of $2\theta = 5$–$55°$ at a scanning speed of 5°/min, as shown in Fig. 7. Two main diffraction peaks for β-chitin were observed at $2\theta = 8{\cdot}50°$ of (010), 19·68° of (020) and (1$\bar{1}$0) reflection planes. Diffraction intensities from these reflection planes were depressed remarkably by grafting, and several small broad diffractions, probably due to the grafted PMMA side chains appeared in the amorphous regions of β-chitin. The diffraction peak for the (010) reflection plane of copolymer was shifted from $2\theta = 8{\cdot}50°$ to 9·30° and the lattice spacing (d) was changed from 10·39 Å to 9·50 Å with increasing grafting yield. However, the diffraction peak for (020) and (1$\bar{1}$0) planes was maintained at $2\theta = 19{\cdot}68°$ when the grafting yield was lower than 244% (Table 1). The ratios of these two kinds of diffraction peaks were decreased with an increase in grafting yield from 0·48 (β-chitin) to 0·18. These results indicated that the graft polymerization of MMA onto β-chitin took place both on the normal (010) plane of crystal and in the amorphous regions of β-chitin at lower grafting yield, and that the crystalline structure of β-chitin was partly converted to an amorphous state by grafting. This conclusion was supported by later studies on grafted β-chitin films. When the grafting yield was higher than 260%, reflection from the

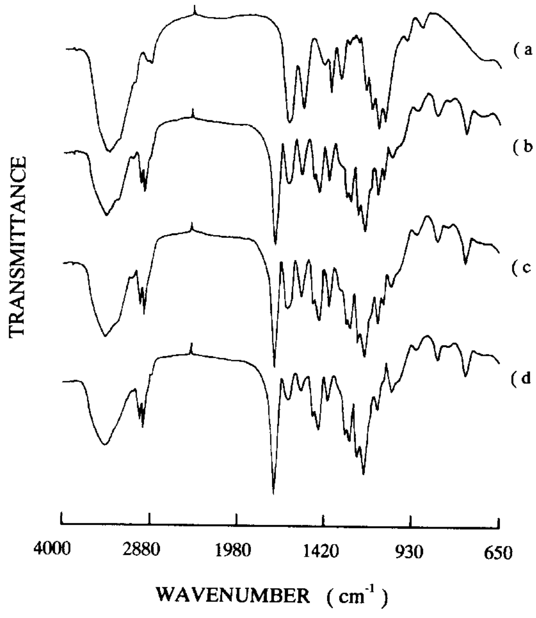

Fig. 6. FT–IR spectra of (a) β-chitin and its grafted derivatives with grafting yields of (b) 152%, (c) 260% and (d) 780%.

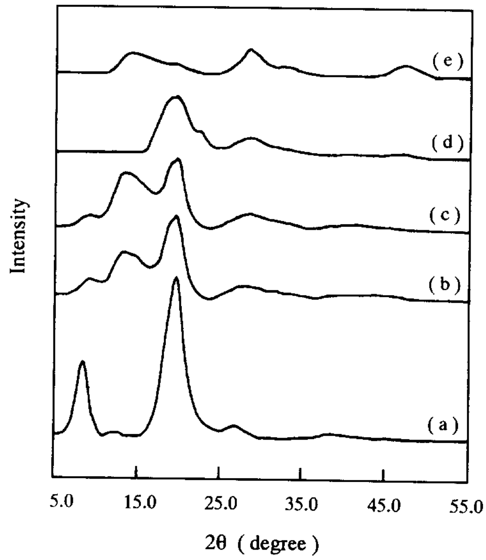

Fig. 7. X-ray diffraction patterns of (a) β-chitin and its grafted derivatives with grafting yields of (b) 152%, (c) 244%, (d) 260% and (e) 780%.

Table 1. X-ray diffraction data for β-chitin and grafted β-chitin derivatives with different grafting yields

Grafting yield (%)	$2\theta_1$(°)	d_1(Å)	$2\theta_2$(°)	d_2(Å)	I_1/I_2
0	8·50	10·39	19·68	4·51	0·48
152	8·94	9·88	19·68	4·51	0·23
244	9·30	9·50	19·68	4·51	0·18
260	0	0	19·62	4·52	0
780	0	0	19·00	4·67	0

2θ, diffraction angle; d, lattice spacing; I, intensity of diffraction: I_1, (010) plane; I_2, (020) and $(1\bar{1}0)$ planes.

(010) plane disappeared, and the diffraction patterns for (020) and $(1\bar{1}0)$ planes became quite broad and changed to $2\theta = 19{\cdot}00°$. That is to say, the crystalline structure of β-chitin collapsed completely at higher grafting, which might correspond to the discontinuous change in moisture content of its graft copolymers.

In the X-ray diffraction pattern of β-chitin film (Fig. 8), the conversion of the crystal structure by grafting was also observed. The ratio (1·33) of diffraction intensities of the (010) plane and the (020) and $(1\bar{1}0)$ planes for β-chitin decreased to 0·83 with the grafting yield of 123% for the grafted β-chitin film. The grafted chitin film became somewhat fragile but could be cut with a knife without cracking or shattering.

The contrasting decrease in crystallinity of grafted α-chitin derivatives is shown in Fig. 9. The major diffraction peak for α-chitin is at $2\theta = 9{\cdot}26°$ of the (020) plane and at $2\theta = 19{\cdot}18°$ of the (110) and (040) planes; the relative intensity ratio of these two peaks is about 0·4. After grafting, the positions of these two peaks, the corresponding relative intensity ratios and the lattice spacings are not changed significantly even when the grafting yield is enhanced to 620% (Table 2). Crystallinity of copolymer is decreased with an increase in grafting yield by introducing amorphous PMMA side chains. It seems that PMMA chain branches formed generally only in the non-crystalline region of α-chitin.

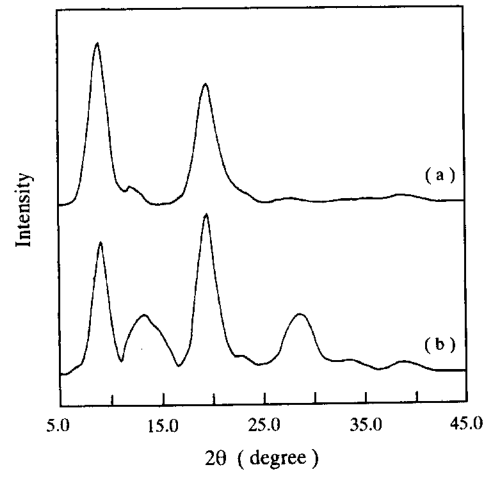

Fig. 8. X-ray diffraction patterns of (a) β-chitin film and (b) grafted PMMA β-chitin film with a grafting yield of 123%.

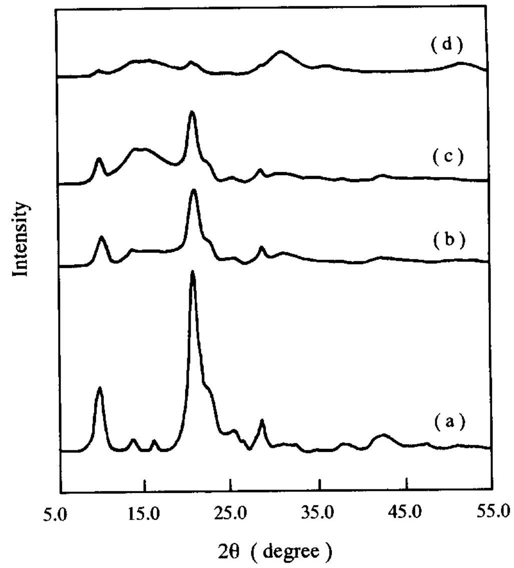

Fig. 9. X-ray diffractograms of (a) α-chitin and its derivatives with grafting yields of (b) 127%, (c) 244% and (d) 620%.

Table 2. X-ray diffraction data for α-chitin and grafted α-chitin derivatives with different grafting yields

Grafting yield (%)	$2\theta_1$(°)	d_1(Å)	$2\theta_2$(°)	d_2(Å)	I_1/I_2
0	9·26	9·54	19·18	4·62	0·37
127	9·30	9·50	19·18	4·62	0·30
244	9·24	9·56	19·12	4·64	0·38
326	9·32	9·48	19·06	4·65	0·36
620	9·32	9·48	19·00	4·67	0·47

2θ, diffraction angle; d, lattice spacing; I, intensity of diffraction: I_1, (020) plane; I_2, (110) and (040) planes.

SEM study on surface morphology of copolymer

Typical surface images observed by SEM are depicted in Fig. 10. β-Chitin (Fig. 10(a)) shows dispersed ribbons whose widths are less than 25 μm. At lower grafting yield, (Fig. 10(b)), these ribbons of β-chitin are shrunken, and the voids between ribbons are filled by grafting of MMA. When the grafting yield is higher than 260%, the ribbon shapes of β-chitin have been found to disappear into a dense assembly. As discussed with respect to in Fig. 7 and Table 1, poorly grafted copolymers are only partially decrystallized; the profile of surface structure differs from the highly grafted samples in which crystallinity decreases to a perfect amorphous state.

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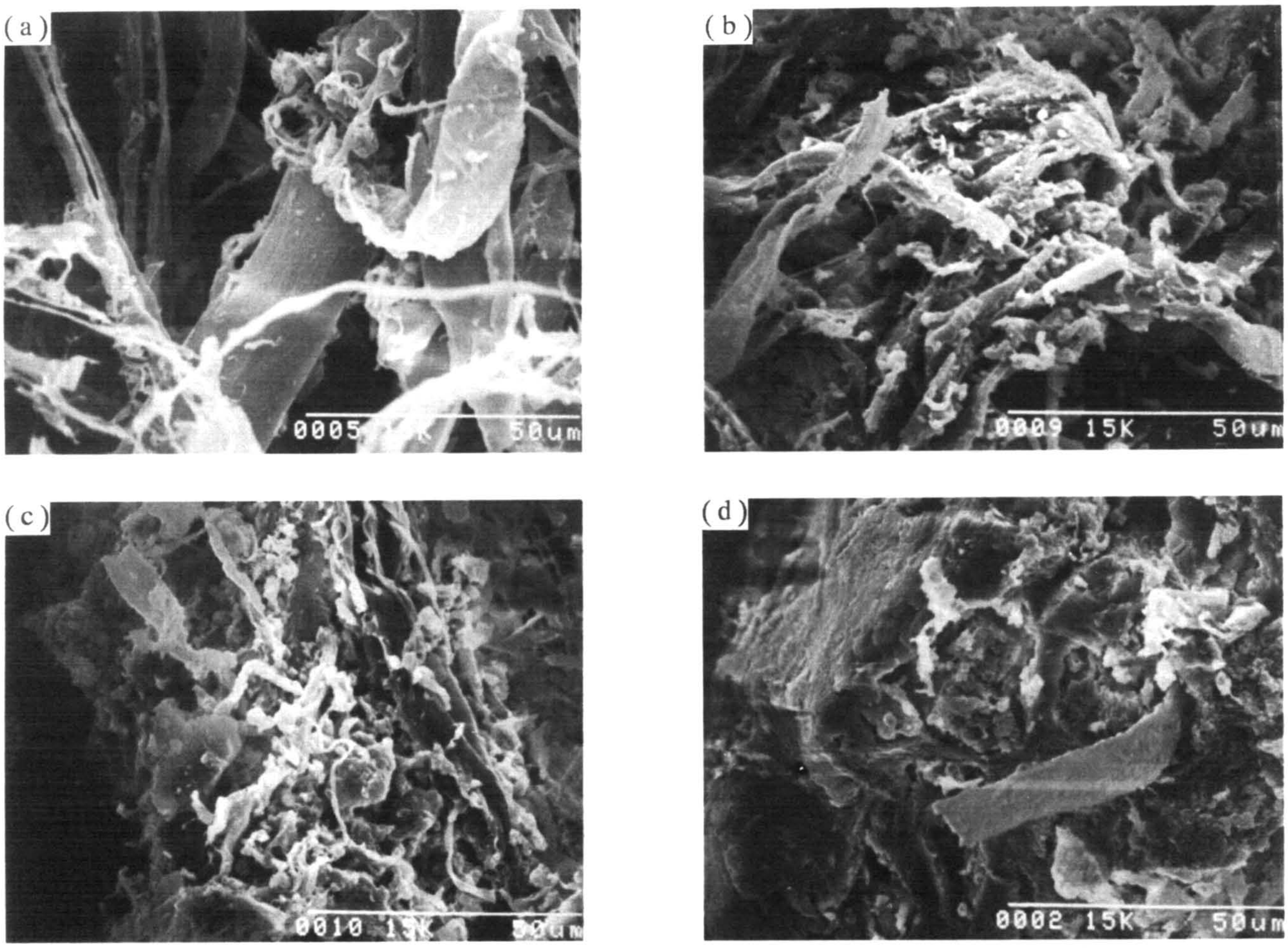

Fig. 10. SEM images of (a) β-chitin and grafted β-chitin derivatives with grafting yields of (b) 244%, (c) 260% and (d) 780%.

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