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ABSTRACT

Graft polymerization of styrene on chitosan derivative was carried out in order to obtain a new amphiphilic hybrid material of synthetic and natural polymers using *N,N*-dimethylformamide (DMF) soluble 6-*O*-tritylchitosan and azo compound having carboxylic acid groups. 6-*O*-Tritylchitosan was coupled with 4,4'-azobis(4-cyanovaleric acid) (ACVA). Graft polymerization of styrene on the 6- *O*-tritylchitosan was carried out in DMF using the pendant ACVA moiety as an initiator. After deprotection of trityl groups of the polymerization products, graft copolymer (PSt-*g*-chito) was obtained. Contact angle measurement and transmission electron microscope observation for the films of the PSt-*g*-chito were carried out. The obtained PSt-*g*-chito showed a micro phase separated morphology.

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INTRODUCTION

Polysaccharides are hydrophilic natural polymers having many hydroxyl groups and generally have relatively good biocompatibility. Therefore, many approaches have been carried out to apply polysaccharides for biomedical or biodegradable materials. Chitosan is a polysaccharide having primary amino groups obtained by *N*-deacetylation of a natural polymer chitin and noteworthy as non-toxic, biocompatible and non-immunogenetic polysaccharides [1, 2]. Therefore, a wide variety of applications of chitosan for biomedical materials, such as controlled drug release devices [3-5], drug carriers [6, 7], wound-healing dressings [8], gene carriers [9, 10] and others [11] have been reported over the last decades. Although highly reactive primary amino groups of the chitosan are convenient for chemical modification, one of the major problems is its low solubility in water and common organic solvents for chemical modification of chitosan to apply as functional materials. 6-*O*-Trityl protection of chitosan through *N*-phthaloylchitosan [12, 13] is an effective method to achieve homogeneous chemical modification of chitosan at the amino groups in good yield.

Recently, block or graft copolymers having both hydrophobic and hydrophilic segments have been reported to form various types of microstructures and have been applied as biomaterials. In particular, amphiphilic block copolymer of styrene and 2-hydroxyethylmethacrylate (HEMA) shows a micro phase separated structure, which leads to good blood compatibility [14, 15]. Saccharides and polysaccharides have many hydroxyl groups and have been used as hydrophilic and bioactive segments in some hybrid-type biomaterials. Many kinds of glycopolymers having saccharide moieties on their side chain [16-19] and block or graft copolymer of polysaccharides and synthetic polymers [20-22] have been synthesized and studied as biofunctional materials.

In this paper, we report a convenient method for graft polymerization of a vinyl monomer on chitosan to obtain a new amphiphilic hybrid material of synthetic and natural polymers. We employed styrene as a common and hydrophobic vinyl monomer and carried out graft polymerization of styrene on chitosan using *N,N*-dimethylformamide (DMF) soluble 6-*O*-tritylchitosan and carboxylic acid derivative of azo compound, 4,4'-azobis(4-cyanovaleric acid) (ACVA), as a radical initiator. After deprotection of trityl group, we studied the contact angle and the morphology of the obtained graft copolymer, PSt-*g*-chito, to evaluate its hydrophilicity and microstructure.

EXPERIMENTAL

Materials

Chitosan (M_w = 1.5 x 10^5, degree of *N*-deacetylation >99%) was obtained from Kimitsu Ind. Co., Japan. ACVA was purchased from Wako Pure Chem. Ind., Japan, and purified by recrystallization from water. Styrene was purified by distillation. DMF was distilled under reduced pressure from calcium hydride (CaH_2). The other organic solvents were purified by the usual distillation method. The other materials were of commercial grade, and used without further purification.

Measurements

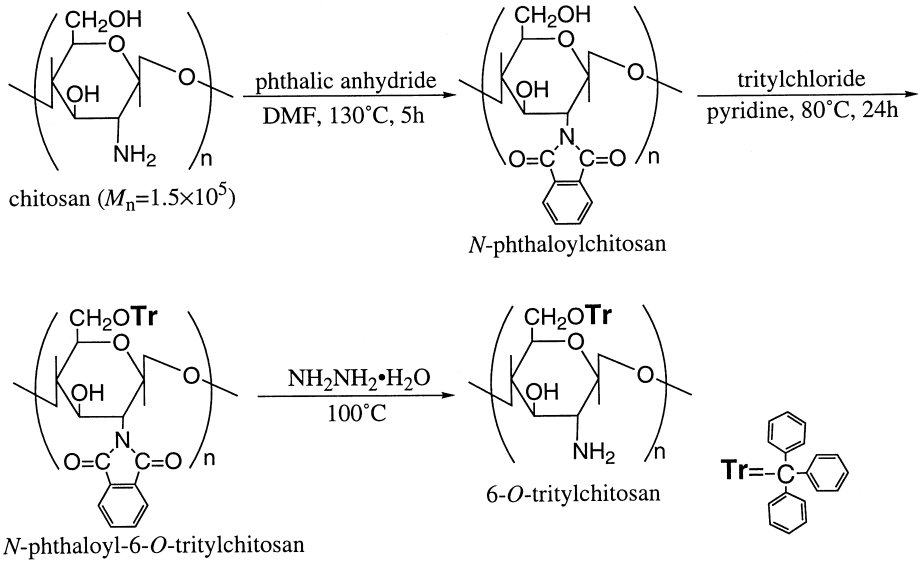
^{1}H-NMR spectra were recorded on a JEOL GSX-400 using tetra-methylsilane (TMS) as an internal reference. Infrared (IR) spectra were measured with a Perkin-Elmer 1600 Series FT-IR. The apparent molecular weight was estimated by gel-permeation chromatography (GPC) (column: Toso TSK Gel ($G4000H_{XL}$ + $G2500H_{XL}$), detector: UV (254nm) and refractive index (RI), eluent: tetrahydrofuran (THF), standard: polystyrene). Glass transition temperature (T_g) was determined by a differential scanning calorimeter (DSC) (Rigaku TAS-200). The specimen was heated from about 0 to 200˚C with a heating rate of 10˚C/min. Contact angle with water was measured by the droplet method using cast films from DMF after annealing in boiled water. TEM images were obtained on a JEOL JEM-1210 electron microscope.

Synthesis

The synthesis of 6-*O*-tritylchitosan were carried out by a similar procedure in references [12, 13] according to Scheme 1.

N-Phthaloylchitosan

Chitosan (23.7g, 147 mmol sugar unit) was reacted with 64.5 g (441 mmol) of phthalic anhydride in 100 mL of DMF at 130°C for 5 hours under a nitrogen atmosphere. The reaction mixture was poured into a large amount of ice-cold water to precipitate *N*-phthaloylchitosan. The precipitate was collected by filtration and rinsed with hot ethanol 4 times and ethyl ether to give a yellow ocher solid. Yield: 36.2 g (84.6%).



Scheme 1. Synthetic route of 6-*O*-tritylchitosan

IR (KBr): 3465 (O-H), 2943 (C-H), 1770, 1711 (imide C=O), 1468 (aromatic C=C), 1391 (C-N), 1112 (C-O-C), 1089 (C-OH), 721 670 cm^{-1} (aromatic C-H, C=C).

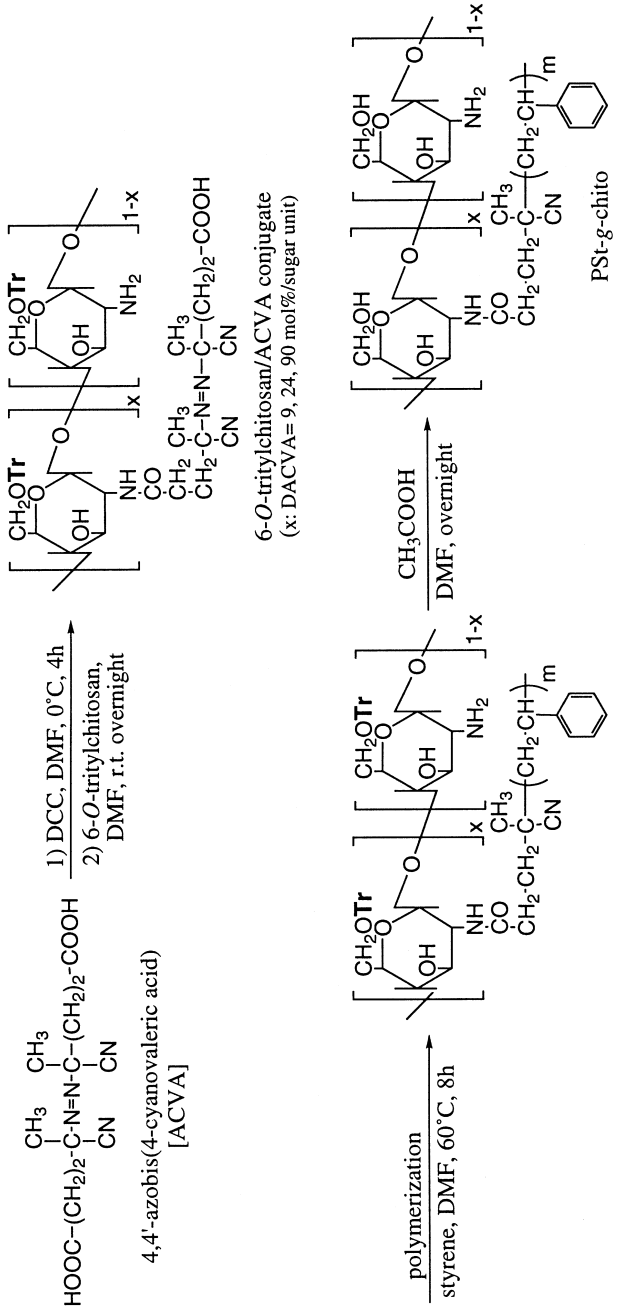
N-phthaloyl-6-O-tritylchitosan

The obtained *N*-phthaloyl-chitosan (16 g, 55.0 mmol sugar unit) was reacted with 100 g (360 mmol) of trityl chloride in 80 mL of pyridine at 80°C for 24 hours under a nitrogen atmosphere. The reaction mixture was poured into a large amount of ice-cold water to precipitate *N*-phthaloyl-6-*O*-tritylchitosan. The precipitate was collected by filtration and rinsed with ethanol 4 times and ethyl ether to give a brown solid. Yield: 22.8 g (77.8%).

IR (KBr): 3477 (O-H), 3057 (aromatic C-H), 2927 (C-H), 1777, 1716 (imide C=O), 1490, 1447 (aromatic C=C), 1387 (C-N), 1112 (C-O-C), 1066 (C-OH), 746, 720, 704, 632 cm^{-1} (aromatic C-H, C=C).

6-O-Tritylchitosan

The obtained *N*-phthaloyl-6-*O*-tritylchitosan (22.7g, 42.6 mmol) was allowed to react with a large amount of hydrazine monohydrate (200 mL) in water (400 mL) at 100°C for 24 hours under a nitrogen atmosphere. The reaction mixture was poured into large amount of ethanol to precipitate 6-*O*-trityl-



Scheme 2. Synthetic route of PSt-*g*-chito

chitosan. The precipitate was collected by filtration and rinsed with ethanol for 4 times and ethyl ether to give brown solid. The degree of tritylation was estimated to be about 95% from the elemental analysis data. Yield: 13.8 g (80.4%).

IR (KBr): 3443, 3372 (O-H, N-H), 3057 (aromatic C-H), 2875 (C-H), 1595 (N-H), 1490, 1447 (aromatic C=C), 1155 (C-O-C), 1073 (C-OH), 765, 703, 633 cm^{-1} (aromatic C-H, C=C).

Elemental Analysis: Calcd for $C_{25}H_{25}O_4N \cdot H_2O$: C, 71.24; H, 6.46, N, 3.32%. Found, C, 71.04; H, 6.51, N, 3.35.

The synthesis of PSt-*g*-chito were carried out according to Scheme 2.

6-O-Tritylchitosan/ACVA Conjugate

A certain amount of ACVA (60, 360 or 872 mg, 0.21, 1.28 or 3.10 mmol) was reacted with 0.5 equivalent of dicyclohexylcarbodiimide (DCC) at 0°C for 4 hours in DMF (2 mL). The reaction mixture containing dicyclohexylurea was added dropwise to 6-*O*-tritylchitosan (800 mg, 2.12 mmol sugar unit) in DMF (18 mL) and stirred overnight at room temperature. After the removal of very small amount of insoluble products (crosslinked products) by filtration, the obtained mixture was subjected to gel-filtration chromatography (column: Sephadex LH-60, f3,5 x 25 cm, eluent: DMF) to remove free ACVA and dicyclohexylurea. The high molecular weight fraction was isolated and evaporated. The introduction of ACVA group was estimated by ^{1}H-NMR in DMSO-*d6* to be 9.0, 24, 90 mol%/sugar unit, respectively. The following spectral data was obtained for 6-*O*-tritylchitosan/ACVA conjugate having 90 mol% of DACVA value.

^{1}H-NMR (DMSO-*d*6): d = 1.65, 1.69 (s, CH_3), 2.7, 2.9 (t, CH_2), 7.2 ppm (phenyl).

IR (KBr): 3391 (O-H, N-H), 3056 (aromatic C-H), 2900 (C-H), 2290 (C∫N), 1662 (amide C=O), 1490, 1448 (aromatic C=C), 1155 (C-O-C), 1061 (C-OH), 748, 701, 631 cm^{-1} (aromatic C-H, C=C).

Graft Polymerization of Styrene

The obtained 6-O-tritylchitosan/ACVA conjugate was dissolved in DMF (16 mL). The solution and styrene were placed in a glass tube. The tube was sealed under reduced pressure. The sealed tube was heated in an oil bath at 60˚C for 8 hours. The reaction mixture was poured into a large amount of methanol to precipitate polymerization products containing homopolystyrene. The precip-

itate was washed with methanol 4 times. The homopolystyrene were removed by Soxhlet extraction with benzene for about 24 hours.

IR (KBr): 3400 (O-H), 3024 (aromatic C-H), 2921 (C-H), 1665 (amide C=O), 1600, 1450, 1492 (aromatic C=C), 1156 (C-O-C), 1068 (C-O), 757, 697 632 cm^{-1} (aromatic C-H, C=C).

The obtained graft copolymer having trityl groups was poured into a mixture of aqueous acetic acid solution (50%) and DMF (1/1, v/v) for removal of the trityl group. The suspension was stirred overnight at room temperature. After evaporation, the suspension was poured into a large amount of ethanol to precipitate PSt-*g*-chito. The precipitated obtained was washed with ethanol 4 times. This reaction was repeated again to confirm complete deprotection. No release of hydrolysis product of trityl group was detected at the second reaction. The weight contents of polystyrene and chitosan in PSt-*g*-chito were estimated from elemental analysis.

Estimation of Molecular Weight of Graft Chain

PSt-*g*-chito (10 mg) was added to 50 mL of conc. HCl aq. solution. The solution was stirred at 80°C overnight. After the complete hydrolysis of chitosan units, homopolystyrene was extracted 3 times with chloroform. The organic layer was washed 3 times with sat. NaCl aq. solution. The apparent molecular weight of the polystyrene obtained was estimated by GPC.

Observation by TEM

Samples for TEM observation were prepared by the following procedure. The cast films from DMF of the PSt-*g*-chito were stained with osmic acid vapor and embedded in an epoxy resin as embedding medium. The embedded films were cut in ultrathin sections of 50 nm thickness. TEM images were obtained on a JEOL JEM-1210 electron microscope.

RESULTS AND DISCUSSION

Preparation of PSt-*g*-chito

Preparation of 6-*O*-tritylchitosan was performed to obtain organic-solvent-soluble chitosan derivative having reactive amino groups. The synthesis of 6-*O*-tritylchitosan were carried out according to Scheme 1. Characterization of the products in each step was carried out by IR absorption spectra. The synthe-

sis of *N*-phthaloylchitosan was confirmed by the appearance of IR absorption of typical imide C=O bands at 1770 and 1711 cm^{-1}, and aromatic (C=C, C-H) bands at 1468, 721 and 670 cm^{-1}. Characterization of N-phthaloyl-6-*O*-tritylchitosan was carried out by the appearance of stronger C-H stretching absorption of aromatic ring of trityl group at 3057 cm^{-1} and new aromatic (C=C, C-H) bands assigned to mono-substituted phenyl group at 746, 704 cm^{-1}, respectively in the IR spectra. The tritylation reaction was reported to proceeded selectively at 6-*O* position because of steric hindrance of the bulky protective group and the higher reactivity of the primary hydroxyl groups [12]. The deprotection of *N*-phthaloyl group to give 6-*O*-tritylchitosan was confirmed by complete disappearance of absorption of imide C=O bands around 1700 cm^{-1}. The obtained 6-*O*-tritylchitosan was soluble in DMF and DMSO.

The synthesis of PSt-*g*-chito was carried out according to Scheme 2. The coupling reaction of ACVA and amino group of 6-*O*-tritylchitosan was carried out by using DCC as a condensation reagent. The introduction of ACVA unit was confirmed by the appearance of absorption of C=O and C∫N bonds at 1665 and 2290 cm^{-1}, respectively in the IR spectra, and signals from methyl and methylene protons at d = 1.65, 1.69 and 2.7, 2.9 ppm in ^{1}H-NMR spectra. The degree of substitution of ACVA group per sugar unit (DACVA) for 6-*O*-tritylchitosan/ACVA conjugate was estimated by ^{1}H-NMR spectroscopy based on the area ratio of signals from methyl protons of ACVA groups at d = 1.65 and 1.69 ppm and protons of trityl group at 7.2 ppm to be 9.0, 24 or 90 mol%/sugar unit when the 0.1, 0.6 or 1.5 equivalent of ACVA to amino group of 6-*O*-tritylchitosan was used, respectively. The graft polymerization was carried out using the 6-*O*-tritylchitosan/ACVA conjugates as macromolecular initiators in DMF at 60°C for 8 hours. The conversions of styrene were checked by analysis of unreacted styrene in the supernatant and washing, and were over 90% for all of the experiments. Homopolystyrene was removed by Soxhlet extraction. The graft polymerization of styrene on 6-*O*-tri-tylchitosan was confirmed by the enlargement of absorption of C-H bands at 2900-3050 and around 750 cm^{-1}, and aromatic C=C bands at 1490-1600 cm^{-1} in the IR spectra (Figure 1) compared with other absorption bands assigned to the chitosan unit. Before and after deprotection trityl group with acetic acid, the obtained graft copolymers could be effectively swollen to give a low viscous fluid with organic solvents (DMF, THF, chloroform and ethyl acetate) which dissolve polystyrene, however, it was not completely soluble. This is probably due to the partial crosslinked structure of the graft copolymer as a result of the radical recombinational termination reaction. Therefore, the molecular weights of the graft copolymers were not determined.

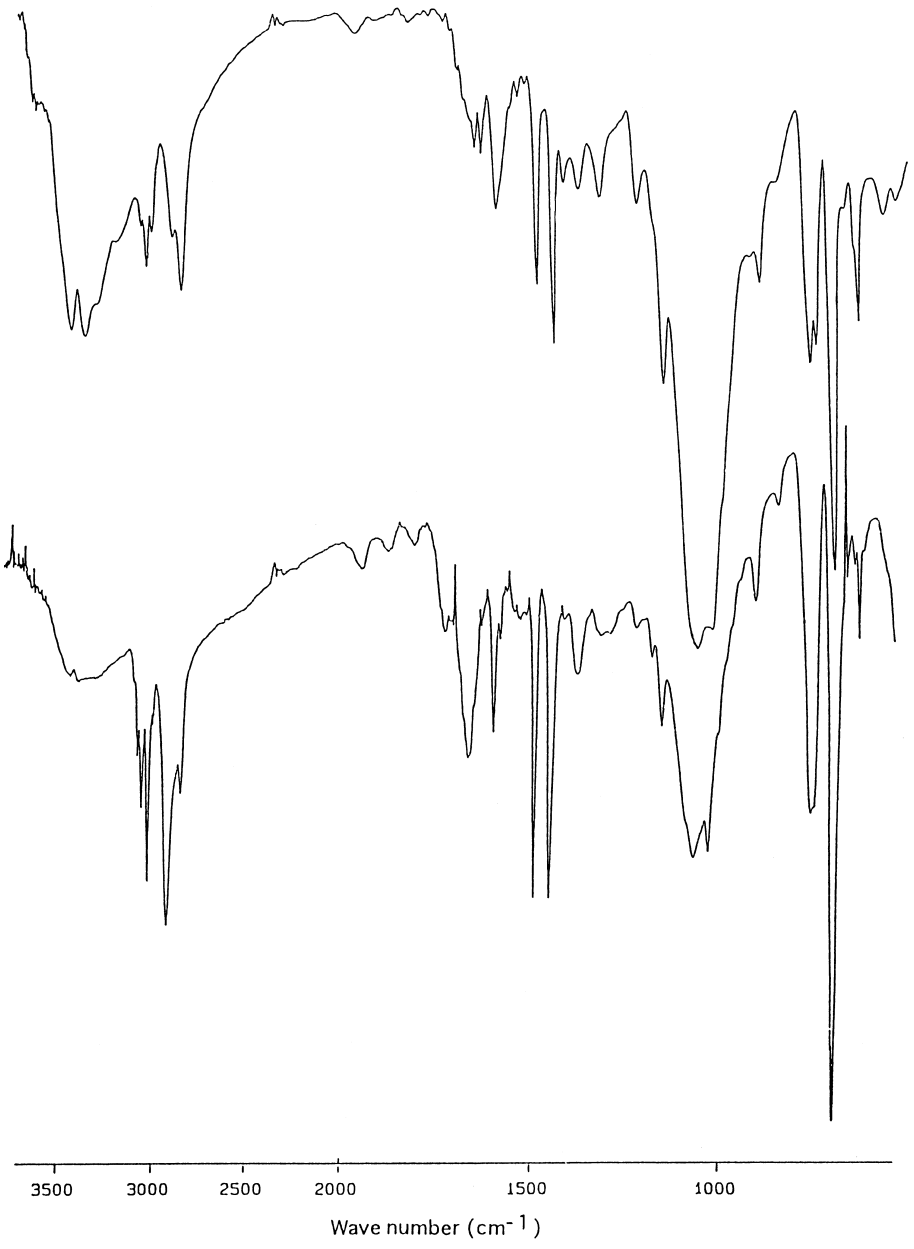


Figure 1. IR spacer of 6-*O*-tritylchitosan (upper) and polymerization product with styrene and 6-*O*-tritylchitosan/ACVA conjugate (DACVA = 9 mol%/sugar unit, mole ratio styrene in feed to ACVA = 2000) (bottom).

Table 1 summarizes the reaction conditions, the results of graft polymerization, and characteristics of the PSt-*g*-chito obtained after deprotection with acetic acid treatment. M_n of PSt means number average of molecular weight of grafted polystyrene segment for each PSt-*g*-chito determined by GPC after acidic hydrolysis of chitosan unit. Chitosan content means weight content of chitosan unit in PSt-*g*-chito. Because formation of homopolystyrene was unavoidable in these experiments and some amount of graft copolymer might be removed together with homopolystyrene in Soxhlet extraction procedure, the yields of the experiments were not so high. The yield tended to be higher when the ACVA value was high and styrene in feed was large. T_g values of the PSt-*g*-chitos were almost constant in the range of 105-111°C. These values were not so different from that of isotactic polystyrene. Figure 2 shows the relationship between M_n of grafted polystyrene and mole ratio of styrene in feed to ACVA (M/I). M_n of polystyrene and M/I showed relatively good linearity for all of the experiments. These results mean that the length of graft chain can be controlled by varying M/I value. As shown in Table 1 (runs 1-3), the chitosan content decreased with an increase of styrene in feed when 6-*O*-tritylchitosan having 9% of DACVA value was used. On the other hand, when the styrene in feed was constant (runs 3, 4 and 6), chitosan content decreased with an increase of DACVA value of 6-*O*-tritylchitosan. These results mean that the weight content of chitosan in PSt-*g*-chito can also be controlled by varying styrene in feed and DACVA value of 6-*O*-tritylchitosan.

Contact Angle

Contact angles with water of the films of PSt-*g*-chito after annealing in boiled water were measured to investigate the hydrophilicity of the films. Figure 3 shows the relationship of the contact angle of the film of PSt-*g*-chito and weight content of chitosan in the PSt-*g*-chito. The contact angles of polystyrene and chitosan were 89°C and 75°C, respectively. The contact angles of PSt-*g*-chito films were almost constant and similar with that of polystyrene when the chitosan content in PSt-*g*-chito was under 16 wt%. However, when the chitosan content in PSt-*g*-chito was over 20 wt%, the contact angle decreased with an increase in the chitosan content and became close to that of chitosan. These results suggest that a small amount of chitosan (less than 16%) did not affect the hydrophilicity of the graft copolymer film. However, when the chitosan content was high (over 20%), some of the chitosan segments were located on the surface of the film and changed its hydrophilicity.

TABLE 1. Synthesis of PSt-*g*-chito

Run	Graft polymerization[a]				PSt-*g*-chito		
	DACVA[b] mol%	Styrene in feed g (mmol)	M/I [c]	Yield [d] mg (wt%)	Mn of PSt × 10^{-4} [e]	Tg [f] °C	Chitosan content [g] wt%
1	9	1.29 (12.4)	340	130 (8.7)	2.5	106	26.9
2	9	2.58 (24.8)	680	130 (4.6)	3.8	111	24.4
3	9	7.74 (74.4)	2000	150 (1.8)	9.3	113	15.3
4	24	7.74 (74.4)	810	1170 (14.7)	5.1	107	3.9
5	90	1.29 (12.4)	48	160 (10.7)	0.6	106	20.8
6	90	7.74 (74.4)	290	2450 (30.8)	3.6	105	2.2

a) Polymerization was carried out using 200mg of 6-O-tritylchitosan/ACVA conjugate in DMF at 60°C for 8 h. Homopolystyrene was removed by Soxhlet extraction.

b) Degree of substitution of ACVA group per sugar unit for 6-O-tritylchitosan/ACVA conjugate estimated from ^{1}H-NMR.

c) Mole ratio of stylene in feed to ACVA unit.

d) Before deprotection.

e) Mn of grafted polystyrene chain measured by GPC after acidic hydrosysis of chitosan unit.

f) Measured by DSC.

g) Weight content of chitosan in PSt-g-chito estimated from elemental analysis data.

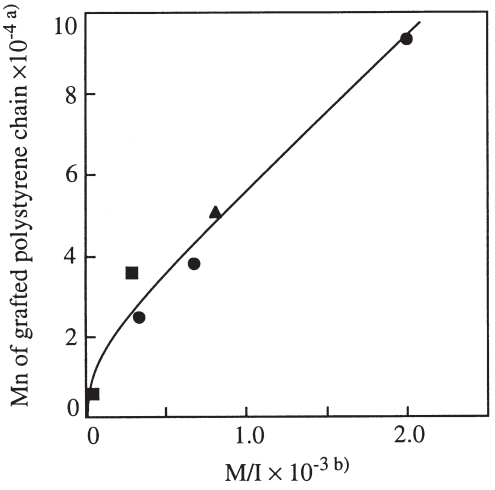


Figure 2. Plots of M_n of grafted polystyrene chain vs. M/I ratio.
(●) PSt-*g*-chito prepared from 6-*O*-tritylchitosan/ACVA conjugate (DACVA = 9 mol%/sugar unit); (Δ) PSt-*g*-chito prepared from 6-*O*-tritylchitosan/ACVA conjugate (DACVA = 24 mol%/sugar unit; (■) PSt-*g*-chito prepared from 6-*O*-tritylchitosan/ACVA conjugate (DACVA = 90 mol%/sugar unit).
a) Number-average molecular weight of grafted polystyrene chain estimated by GPC after acidic hydrolysis of chitosan.
b) Mole ratio of styrene in feed to ACVA unit.

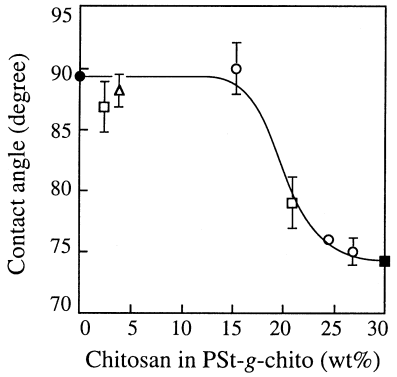


Figure 3. Relationship between contact angle and weight content of chitosan in PSt-*g*-chito. (O) PSt-g-chito prepared from 6-*O*-tritylchitosan/ACVA conjugate (DACVA = 9 mol%/sugar unit); (Δ) PSt-*g*-chito prepared from 6-*O*-tritylchitosan/ACVA conjugate (DACVA = 24 mol%/sugar unit; (❑) PSt-*g*-chito prepared from 6-*O*-tritylchitosan/ACVA conjugate (DACVA = 90 mol%/sugar unit); (●) polystyrene (M_n = 4.5 x 104) ; (■) chitosan (M_n = 1.5 x 105).

TEM Observation of the PSt-*g*-chito

Figure 4 shows TEM micrographs of the graft copolymers (the content of chitosan in graft copolymer = 26.9 or 15.3 wt%). The dark domains is considered to be chitosan rich phases stained with osmic acid. The graft copolymers showed micro phase separated morphology. The micro phase separated structure for the PSt-*g*-chito having 26.9 wt% of chitosan content was very fine. The domain size was around 5nm. On the other hand, the micro phase separated structure for the PSt-*g*-chito having 15.3 wt% of chitosan content was relatively rough compared with the other sample. Relatively large islands of polystyrene phases having 10-30nm of diameters were observed. These results suggest that the micro phase separated morphology of the PSt-*g*-chito may be controlled by control of the chitosan content in graft copolymer.

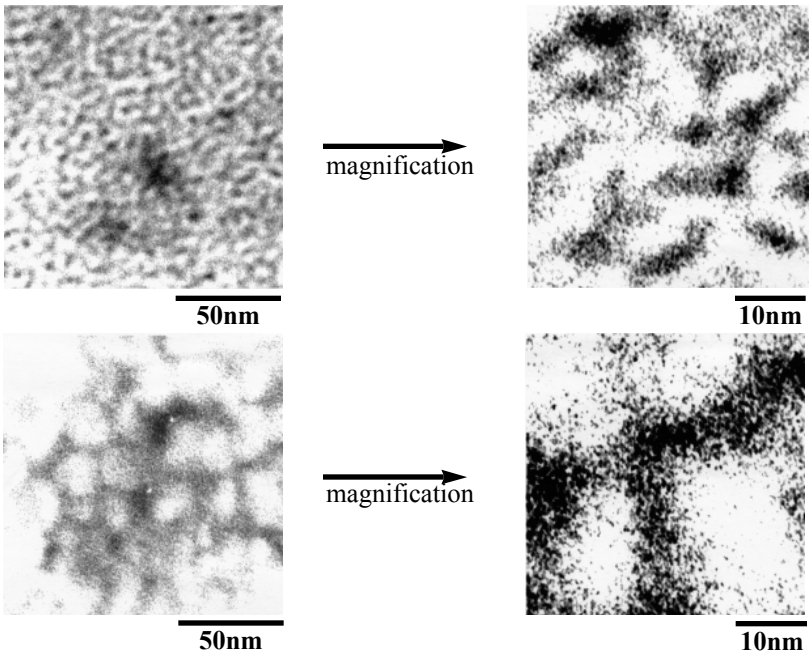


Figure 4. TEM view of PSt-*g*-chito stained with 4% OsO_4 solution. Upper: PSt-*g*-chito (chitosan content = 26.9 wt%) prepared from 6-*O*-tritylchitosan/ACVA conjugate (DACVA = 9 mol%/sugar unit); bottom: PSt-*g*-chito (chitosan content = 15.3 wt%) prepared from 6-*O*-tritylchitosan/ACVA conjugate (DACVA = 9 mol%/sugar unit).

CONCLUSION

PSt-*g*-chito could be obtained by graft polymerization of styrene on a 6-*O*-tritylchitosan immobilizing ACVA unit as a macromolecular radical initiator. The length of the grafted polystyrene chain and weight content of chitosan in graft copolymer could be controlled by varying the mole ratio of styrene in feed, to ACVA (M/I), amount of styrene in feed, and DACVA value. The surface hydrophilicity of the graft copolymer film tended to increase with increasing the weight content of chitosan in PSt-*g*-chito. The PSt-*g*-chito showed micro phase separated morphology. The micro phase separated pattern was changed by the weight content of chitosan in the graft copolymer.

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REFERENCES

[1] S. Hirano, H. Seino, Y. Akiyama, and I. Nonaka, *Progress in Biomedical Polymers*, C. G. Gebelein and R. L. Dunn, Eds., Plenum Press, New York, 1990, p. 283.

[2] S. Hirano, H. Seino, Y. Akiyama, and I. Nonaka, *Polym. Mat. Sci. Eng., 59*, 897 (1988).

[3] Y. Ohya, T. Takei, and T. Ouchi, *J. Bioact. Compat. Polym., 7*, 242 (1992).

[4] Y. Ohya, T. Takei, and T. Ouchi, *J. Microencap., 10*, 1 (1993).

[5] Y. Ohya, M. Shiratani, H. Kobayashi, and T. Ouchi, *J. Mac. Sci.-Pure & Appl. Chem., A31*, 629 (1994).

[6] T. Ouchi, T. Banba, M. Fujimoto, and S. Hamamoto, *Makromol. Chem., 190*, 1817 (1989).

[7] T. Ouchi, T. Banba, T. Matsumoto, S. Suzuki, and M. Suzuki, *J. Bioact.Compat. Polym., 4*, 362 (1989).

[8] R. A. Olsen, D. Schwartzmiller, W. W. Weppner, and R. Winandy, *Chitin and Chitosan. Proc. 4th Intern. Conf. Chitin and Chitosan*, G. Skjak-Braek, T. Anthonsen, and P. Sandord, Eds., Elsevier Applied Sci., London, 1989, p. 813.
[9] J. Murata, Y. Ohya, and T. Ouchi, *Carbohydr. Polym., 29*, 69 (1996).
[10] J. Murata, Y. Ohya, and T. Ouchi, *Ibid, 32*, 105 (1997).
[11] *Chitin Derivatives in Life Science, Proc. Intern. Symp. Chitin Derivatives in Life Science*, S. Tokura and I. Azuma, Eds., 1992.
[12] S. Nishimura, O. Kohgo, K. Kurita, C. Vittavatvong, and H. Kuzuhara, *Chem. Lett.*, *243* (1990).
[13] S. Nishimura, O. Kohgo, K. Kurita, and H. Kuzuhara, *Macromolecules, 24*, 4745 (1991).
[14] T. Okano, S. Nishiyama, I. Shinohara, T. Akaike, Y. Sakurai, K. Kataoka, and T. Tsuruta, *J. Biomed. Mater. Res., 15*, 393 (1981).
[15] T. Okano, M. Urano, N. Sugiyama, S. Shimada, I. Shinohara, K. Kataoka, and Y. Sakurai, *J. Biomed. Mater. Res., 20*, 1035 (1986).
[16] R. Schnaar, *Anal. Biochem., 143*, 1 (1984).
[17] S. Nishimura, M. Matsuoka, and K. Kurita, *Macromolecules, 23*, 4182 (1990).
[18] K. Kobayashi, H. Sumitomo, and Y. Ina, *Polymer J., 17*, 567 (1985).
[19] V. Chytry and H. Driguez, *Makromol. Chem. Rapid Commun., 13*, 499 (1992).
[20] G. Ziegast and B. Pfannemüller, *Makromol. Chem., 185*, 1855 (1984).
[21] K. Loos and R. Stadler, *Macromolecules, 30*, 7641 (1997).
[22] T. Ouchi, H. Nishizawa, and Y. Ohya, *Polymer, 39*, 5171 (1998).

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