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Carbohydrate Polymers 63 (2006) 476–481

Carbohydrate Polymers

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Synthesis and properties of pullulan acetate. Thermal properties, biodegradability, and a semi-clear gel formation in organic solvents

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Received 2 March 2005; received in revised form 30 September 2005; accepted 5 October 2005
Available online 18 November 2005

Abstract

Pullulan acetate (AcPL) with various degree of substitution (DS: 1.0–3.0) was synthesized by the reaction of pullulan with acetyl chloride in the presence of pyridine. The product was characterized by gel permeation chromatography (GPC), infra-red (IR) and ^{1}H NMR spectroscopy. The weight average molecular weights of the products did not decrease less than 190,000 (GPC) in the acetylation reaction. Thermogravimetric analysis (TGA) revealed that AcPL has a higher decomposition temperature (306–363 °C) than unmodified pullulan (295 °C). Differential scanning calorimetry analysis (DSC) revealed that all the AcPLs exhibit a clear T_g, which decreased with increasing DS value in the range of DS 1.0–2.4. The AcPL with DS 2.4 showed the lowest T_g (153 °C), and the AcPL with DS 3.0 had a slightly higher T_g (163 °C). Tensile modulus of AcPL films was comparable to that of a popular cellulose acetate film. The biodegration rate of AcPL decreased with increasing degree of acetylation. The AcPL with DS 3.0 was found to form a semi-clear gel in organic solvents such as dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), and 1,4-dioxane.

Keywords: Pullulan; Acetylation; Pullulan acetate; Biodegradability; Organogel

1. Introduction

Thermoplastic polymer materials derived from renewable polysaccharides such as cellulose, starch, and pullulan offer interesting alternatives for synthetic polymers from the standpoints of conserving our petrochemical resources and providing biodegradation properties to the end product (Kaplan, 1998). Addition of hydrophobicity and thermoplasticity to such polysaccharides was the interesting subjects not only for environmentally friendly materials but also for biocompatible materials in pharmaceutical industries (Akiyoshi & Sunamoto, 1996; Shingel, 2004; Simon, Muller, Koch & Muller, 1998). Pullulan is a water-soluble, extracellular neutral polysaccharide with a linear flexible chain of 1,6-linked maltotriose units. Unlike starch and cellulose, pullulan is easily soluble in water and dimethyl sulfoxide (DMSO), even though its molecular weight is higher. Not many attempts have been made to modify pullulan chemically for imparting plasticity and hydrophobicity. Sunamoto and coworkers have reported the synthesis and properties of palmitoyl (Akiyoshi, Yamaguchi, & Sunamoto, 1991) and cholesteryl (Akiyoshi, et al., 1991; Akiyoshi, Deguchi, Moriguchi, Yamaguchi, & Sunamoto, 1993) modified pullulan derivatives, and Mocanu, Carpov, Chapelle, Merle, and Muller (1995) have reported the synthesis of adenine, thymine, and pyrene modified pullulan derivatives. Chloroalkylation (Mocanu, Vizitiu, Mihai, & Carpov, 1999), nitroalkylation (Heeres, Spoelma, van Doren, Gotlieb, Bleeker, & Kellogg, 2000), alkyl etherification (Shibata, Nozawa, Teramoto, & Yosomiya, 2002), and modification with isocyanates (Shibata, Asahina, Teramoto, & Yosomiya, 2001) and mesyl chloride (Mocanu, Constantin, & Carpov, 1996) have also been reported. Regarding pullulan acetate, Hijiya & Shiosaka (1975) have reported the preparation of pullulan acetate for adhesives, and Donabedian, Gross, and McCarthy (1992) have reported the plasticization of pullulan with acetic anhydride. On the other hand, Jung, Jeong, and Kim (2003) have investigated the morphology and self-association behavior of pullulan acetate for the application to controlled drug release. However, details of molecular weight, biodegradability, and mechanical properties were not reported in these reports.

In the present paper, AcPL was prepared by the reaction of pullulan with acetyl chloride in the presence of pyridine without reducing molecular weight, and thermal, mechanical

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doi:10.1016/j.carbpol.2005.10.008

and biodegradable properties were described. Furthermore we found that the AcPL with DS 3.0 formed a semi-clear organogel in polar organic solvents such as DMSO, *N*,*N*-dimethylformamide (DMF), and 1,4-dioxane. This is the first report about the AcPL gel in organic solvents.

2. Experimental

2.1. Materials

Pullulan, PF-20 grade kindly supplied by Hayashibara Biochemical Laboratories, Inc. ($M_n = 98{,}000$ g/mol, $M_w = 191{,}000$ g/mol, measured by GPC method using DMSO as eluent and injection solvent and using pullulan standards) was dried under vacuum at 70 °C for 24 h before use. The reagent grade *N*,*N*-dimethylacetamide (DMAc) was dried over molecular seives 5 Å for three days. Cellulose acetate (acetyl content 39.8%, DS 2.4, $M_n = $ ca 30,000) was purchased from Aldrich. Other reagents, acetyl chloride, pyridine, sodium hydroxide, and ethanol (Kanto Chemical Co. Ltd., Japan) were used without further purification.

2.2. Acetylation of pullulan

To a fine suspension of pullulan 15.0 g (hydroxyl group 0.278 mol) in DMAc 300 ml was added pyridine 16.5–44.1 g (0.209–0.557 mol), then acetyl chloride 10.9–29.1 g (0.139–0.371 mol, corresponding theoretical DS 1.5–3.0) dropwise. The mixture was stirred at 60 °C for 10 h, followed by neutralization with aqueous sodium hydroxide. After the concentration in vacuo, the reaction mixture was poured into excess ethanol. The formed precipitate was filtered and washed with ethanol several times, and dried at 60 °C for 12 h in vacuo to give AcPL 18.2–23.7 g as a colorless or pale brown solid.

2.3. Characterization

IR spectra of AcPL were recorded on an FT-IR 8100 spectrometer (Shimadzu Co. Ltd., Japan) by KBr method. GPC analysis was performed at 80 °C using a Shimadzu LC10A series equipped with two linear PLgel 5 μm Mixed-D columns (Polymer Laboratories Ltd), using DMSO as eluent and injection solvent. The elution rate was 0.5 mL/min and the detector was the refractive index type. Pullulan standards were used to generate a calibration curve for the molecular weight determination. ^{1}H NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 MHz, using tetramethylsilane (TMS) as an internal standard. The degree of substitution (DS) of AcPL for the lowest acetyl chloride feed ratio (corresponding theoretical $\mathrm{DS} = 1.5$) was calculated from the integration value (A) of acetyl protons at 1.8–2.2 ppm and that (B) of OH protons and H-1 protons of pullulan moiety observed at more than 4.7 ppm. Then DS values are expressed by the following equations: $\mathrm{DS} = 4A/(3B + A)$, derived from the equation: $\mathrm{A}/3\mathrm{x} = \mathrm{B}/\{1 + (3 - x)\}$ (corresponding to the area for one hydrogen in a glucose unit), where x equals DS. When the acetyl chloride feed ratio increased (corresponding theoretical $\mathrm{DS} \geqq 2.5$), DS was calculated from the integration value (A) described above and that (C) of OH protons and H-1 to H-6 protons of pullulan moiety observed at more than 3.5 ppm. Then DS values are expressed by the following equations: $\mathrm{DS} = 10A/(3C + A)$, derived from the equation: $\mathrm{A}/3\mathrm{x} = \mathrm{C}/\{7 + (3 - \mathrm{x})\}$ (corresponding to the area for one hydrogen in a glucose unit), where x equals DS. The difference in DS calculation is due to the shift of C–H protons to lower magnetic field by acetylation.

Differential scanning calorimetry (DSC) measurements were performed using a Perkin–Elmer Pyris 1 DSC instrument with a heating rate of 20 °C/min under a nitrogen atmosphere. The heating process from 20 to 240 °C was repeated, and the glass transition temperature (T_g) was determined from the DSC chart at the second heating process. The experimental error on determination of T_g by DSC was low and within ±2 °C. Thermogravimetric analysis (TGA) was performed on a Perkin–Elmer TGA-7 instrument with a heating rate of 20 °C/min under a nitrogen atmosphere. The decomposition temperature (T_d) was determined as the onset temperature of decline in the TGA chart. The experimental error on determination of T_d by TGA was within ±5 °C. Measurement of mechanical properties, i.e. tensile strength, modulus, and elongation, was performed on a Shimadzu mechanical tester model AGS-500C in the air-conditioned room (temperature, 23 ± 2 °C; relative humidity, $65 \pm 5\%$). A modified pullulan film with 0.6 ± 0.2 mm thickness was prepared by casting from DMF solution on a PTFE-coated tray and drying at 50 °C for 24 h under ambient pressure, followed by further drying at 40 °C for 48 h in vacuo. The film was cut into strips of 10 mm × 70 mm. The crosshead space was 50 mm, and the crosshead speed was 10 mm/min. Six measurements were conducted and the result was obtained as an average of four samples with mean values.

Biodegradability was determined by measuring a biochemical oxygen demand (BOD) under aerobic conditions. To the phosphate buffer (pH 7.4, 200 mL) containing 0.25 mM $CaCl_2$, 0.09 mM $MgSO_4$, 0.09 mM NH_4Cl, and 0.9 μM $FeCl_3$ in a glass bottle was added 4.35 mL of activated sludge containing 30 mg insoluble part, which was obtained from sewerage facilities of Chiba Institute of Technology. 20 mg of polymer powder were added, and the dispersion was steadily stirred with a magnetic stir bar. BOD was measured at 25 °C by using Taitec BOD tester 200F. Carbon dioxide was absorbed into 50% sodium hydroxide aqueous concentrate in a cup equipped within the glass bottle. The volume of the consumed oxygen was directly measured with a scaled cylinder.

Swelling experiments were carried out by soaking a piece of dried AcPL (DS 3.0) of known weight (W_d) in excess organic solvents at 25 °C. Swollen gel was taken out, wiped briefly, and weighed to give W_s at scheduled time intervals. The swelling degree (S_w) was expressed by $S_w = (W_s/W_d - 1)$. Photographs of swollen gels were taken by an Olympus Camedia C-40 digital camera.

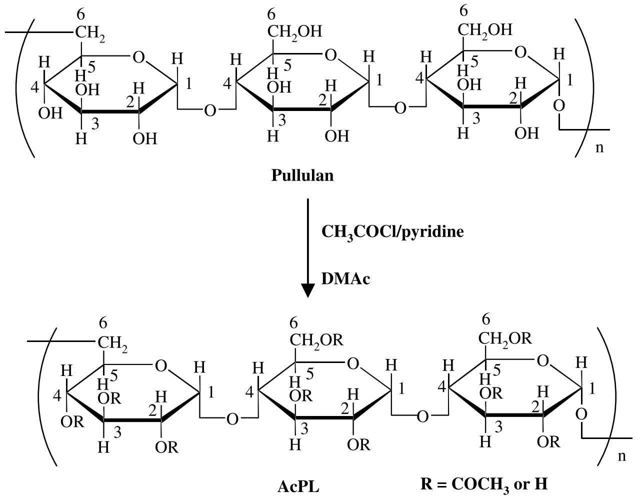

Fig. 1. Synthetic scheme of AcPL.

3. Results and discussion

3.1. Synthesis and characterization of pullulan acetate

Pullulan acetate (AcPL) was synthesized by the reaction of pullulan with acetyl chloride in the presence of pyridine at 60 °C in DMAc (Fig. 1) instead of the frequently reported method using acetic anhydride/pyridine (Donabedian, et al., 1992; Hijiya & Shiosaka, 1975; Jung, et al., 2003). The use of acetyl chloride afforded the product with a higher molecular weight, compared to the acetic anhydride method in our prior study (unpublished data). The synthetic data of AcPL are summarized in Table 1. The degree of substitution (DS) was varied by altering the feed ratio of acetyl chloride to one glucose unit of pullulan. In the IR spectra of AcPL, the absorption peak at ca 3300 cm^{-1} related to stretching vibration of hydroxyl group became weak, and the peak at ca 1750 cm^{-1} characteristic of ester group increased with increasing feed ratio of acetyl chloride (Fig. 2), indicating that DS of the product increases. The absorption peak around 3300 cm^{-1} did not disappear even for AcPL DS 3.0 (Exp. 4), implying that trace amounts of hydroxyl group are remaining or that moisture could not be completely removed by the conventional drying method.

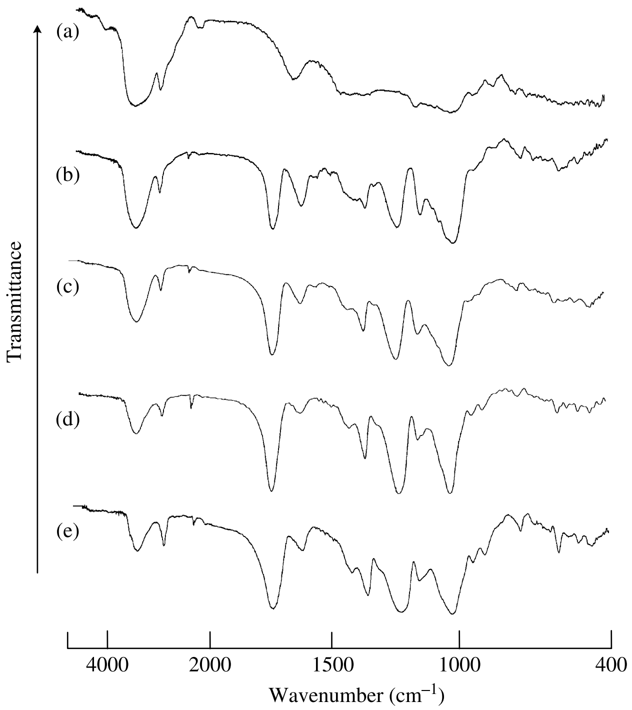

Fig. 2. IR spectra of (a) pullulan, (b) AcPL(Exp. 1, DS 1.0), (c) AcPL(Exp. 2, DS 1.7), (d) AcPL(Exp. 3, DS 2.4) and (e) AcPL(Exp. 4, DS 3.0).

Fig. 3 shows a typical ^{1}H NMR spectrum of AcPL (Exp. 3) in DMSO-d_6. Hydroxyl proton signals, observed at 4.5–5.6 ppm in the ^{1}H NMR spectrum of pullulan, decreased, and methyl proton signals at 1.8–2.2 ppm, which are assigned to the acetyl groups, appeared in the ^{1}H NMR spectrum of the AcPL. Some methine proton signals of pullulan shifted to lower magnetic field by acetylation. These results indicate that acetylation certainly proceeded. The precise assignment of each proton observed at 3 ppm and above could not be made because of the broadening and complexity of the signals. The degree of substitution (DS) for acetyl groups measured by ^{1}H NMR peak integrals is summarized in Table 1. The observed DS increased

Table 1
Synthetic data of AcPL

Exp. No.	Acetyl chloride[a]	Pyridine[a]	Theoretical DS	Observed DS[b]	M_n	M_w
1	1.50	2.25	1.50	1.0	95,500	194,000
2	2.50	3.75	2.50	1.7	107,000	225,000
3	3.50	5.25	3.00	2.4	118,000	301,000
4	4.00	6.00	3.00	3.0	–	–

[a] Molar ratio of feed reagent to a glucopyranosyl group of pullulan.
[b] ^{1}H NMR method.

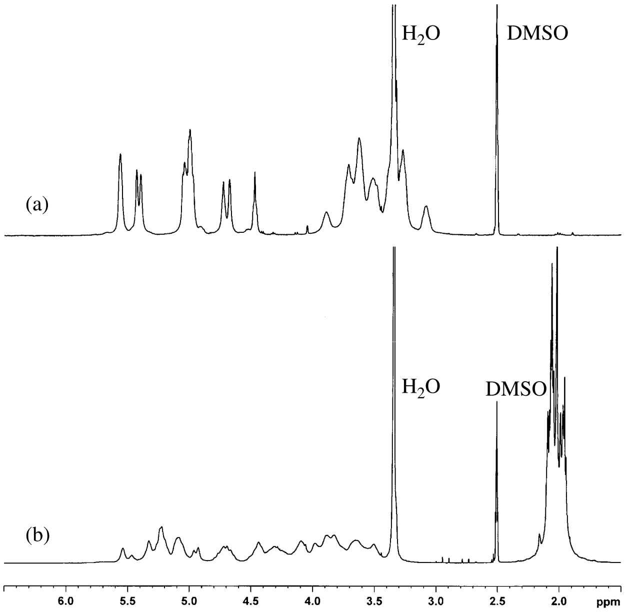

Fig. 3. ^{1}H NMR spectra of (a) pullulan and (b) AcPL (Exp. 3, DS 2.4) in DMSO-d_6.

with increasing feed ratio of acetyl chloride. Although the AcPL with the highest DS (Exp. 4) was insoluble in DMSO-d_6, the NMR spectrum could be obtained from the resulting gel.

The AcPLs (Exp. 1, 2 and 3) showed a little lower number-average molecular weight (M_n) than the theoretical M_n (123, 000, 141,000, and 159,000) calculated from their observed DS (1.0, 1.7, and 2.4) and the M_n (98,000) of pullulan. This result may be attributed to some depolymerization of pullulan framework. For the AcPL (Exp.4), the most part of the sample became gel in DMSO. No appropriate solvent for the GPC measurement was found.

3.2. Solubility and thermal and mechanical properties of pullulan acetate

Solubility of AcPL with DS value of 1.0–3.0 is summarized in Table 2. AcPLs with DS 1.7 or above are insoluble in water. All the AcPLs except the AcPL with DS 3.0 are soluble in DMSO, DMAc and Pyridine. The AcPL with DS 3.0 was not completely soluble in common organic solvents, and became gel in polar aprotic solvents. The gel formation of AcPL with high DS has not been reported as far as we know. Jung, et al. (2003) have reported that their AcPL was soluble in polar aprotic solvents such as DMSO, DMF, THF, and so on. The highest acetylation degree of the AcPL that they prepared was 87.39% (corresponding to DS 2.6). It is considered that the solubility of AcPL is influenced by molecular weight as well as DS. Though Jung et al. did not report molecular weight, DS of their AcPL was lower than that of our AcPL DS 3.0. The driving force for the gel insolubility of our AcPL can be noncovalent interaction and entanglement of polymer chains. Considering that our AcPL has high molecular weight, such entanglement is likely to occur in the preparation process. Complete acetylation might be responsible for reinforcement of the intermolecular interaction between the polymer chains caused by ordered molecular packing.

The glass transition temperature (T_g) and decomposition temperature (T_d) of AcPL are summarized in Table 3. All the AcPLs exhibited a clear T_g, while the native pullulan showed no clear T_g in our DSC experimental condition. The T_g decreased with increasing DS value in the range of DS 1.0–2.4. The AcPL with DS 2.4 showed the lowest T_g (153 °C), and the AcPL with DS 3.0 had a slightly higher T_g (163 °C). This might be due to the intermolecular interaction described above. The T_d increased with increasing DS value. The similar tendency was also reported in our previous papers involved in alkyl-etherified amylose (Teramoto, Motoyama, Yosomiya, & Shibata, 2002) and starch (Teramoto, Motoyama, Yosomiya, & Shibata, 2003). The AcPL with DS 3.0 had the highest T_d (363 °C), which is much higher than the literature value (203–227 °C) of AcPL synthesized by the reaction with acetic anhydride in DMSO (Donabedian, et al., 1992). The difference might be attributed to the difference in molecular weights of AcPLs. In fact, AcPL by acetic anhydride method has much lower molecular weight than AcPL by acetyl chloride method (unpublished data).

Table 2
Solubility of AcPLs in various solvents

	Sample				
	Pullulan	AcPL			
Solvent	DS: 0	Exp. 1 DS: 1.0	Exp. 2 DS: 1.7	Exp. 3 DS:2.4	Exp. 4 DS: 3.0
Water	○	Δ	×	×	×
DMSO	○	○	○	○	Δ
DMF	Δ	○	○	○	Δ
Pyridine	Δ	○	○	○	Δ
Acetone	×	×	×	Δ	Δ
Ethyl acetate	×	×	×	Δ	Δ
Tetrahydro-furan	×	×	×	Δ	Δ
Chloroform	×	×	×	Δ	Δ
Toluene	×	×	×	×	Δ

Solubility was determined after immersing the sample 20 mg in each solvent 1. 0 mL for 24 h. ○, Soluble; Δ, Partially soluble or swollen; ×, Insoluble.

Table 3
Thermal properties of AcPLs

Sample	T_g (°C)[a]	T_d (°C)[b]
Pullulan	–	295
AcPL (DS: 1.0)	193	306
AcPL (DS: 1.7)	189	327
AcPL (DS: 2.4)	153	353
AcPL (DS: 3.0)	163	363

[a] Measured by DSC at a heating rate of 20 °C/min under nitrogen.
[b] Measured by TGA at a heating rate of 20 °C/min under nitrogen.

Tensile properties of AcPL (DS 1.0, 1.7, and 2.4) films prepared by casting from solution are summarized in Table 4. AcPL with DS 2.4 has a higher tensile modulus and a lower elongation at break than cellulose acetate (DS 2.4). The elongation at break of AcPL was lower than that of native pullulan. All the AcPLs have a lower tensile strength than native pullulan. However, the tensile strength of the native pullulan film became 4 MPa after standing outside a desiccator for three days, because of the moisture absorption. On the other hand, the tensile strength of all the AcPLs did change little after standing for several days. Apparently, acetylation of pullulan reduced elongation at break. Judging from the large standard deviation of elongation at break of native pullulan, it seems very sensitive to moisture. Acetylation is not the best way for

Table 4
Tensile properties of pullulan, AcPL, and cellulose acetate films (±SD)

Sample	Tensile strength (MPa)	Tensile modulus (GPa)	Elongation at break (%)
Pullulan[a]	32±9	1.8±0.5	3.2±2.1
AcPL (DS: 1.0)	14±1	1.7±0.2	1.0±0.1
AcPL (DS: 1.7)	19±2	1.3±0.1	1.8±0.1
AcPL (DS: 2.4)	21±3	1.9±0.2	1.3±0.1
Cellulose acetate (DS: 2.4)[b]	31±5	1.6±0.2	2.2±0.8

[a] Pullulan films were prepared by casting from the aqueous solution and drying at 50 °C for 24 h under ambient pressure, followed by further drying at 40 °C for 48 h in vacuo.
[b] Cellulose acetate films were prepared by casting from the acetone solution and drying at 50 °C for 1 h under ambient pressure, followed by further drying at 40 °C for 12 h in vacuo.

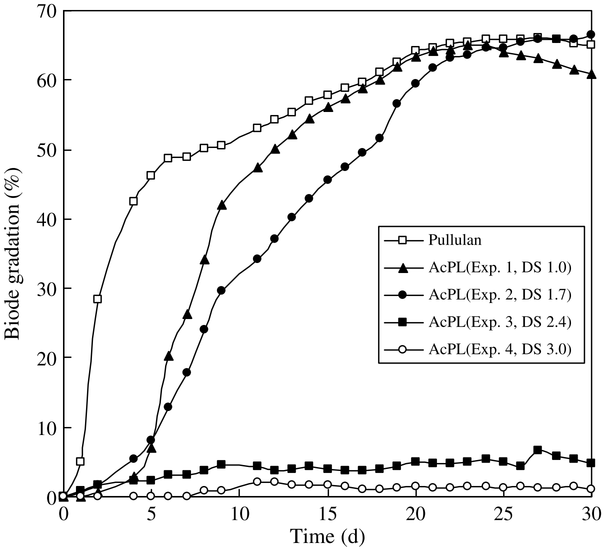

Fig. 4. Biodegradability of AcPLs with various DS values. Biodegradability was measured by BOD tests at 25 °C using activated sludge.

improvement of elongation. Indeed, acetylation of cellulose and starch did not significantly increase elongation at break. Though acetylation decreases hydrogen bonds between carbohydrate chains, polarization which comes from C=O carbonyl groups remains. Acetylation is effective for enhancing thermoplasticity, compatibility with other thermoplastic polymers, and water-resistance without serious decrease of strength and modulus.

3.3. Biodegradability of pullulan acetate

Fig. 4 shows biodegradability of AcPL measured by BOD method. AcPLs with DS 1.0 and 1.7 have high biodegradability which is comparable to pullulan. On the contrary, AcPL with DS 2.4 has a slight biodegradability, and AcPL with DS 3.0 did not show biodegradability until 30 days later. The high biodegradability of AcPL with DS 1.0 and 1.7 may be attributed to their hydrophilicity. This tendency is very similar to that of cellulose acetate reported in several researches (Buchanan, Gardner, & Komarek, 1993; Reese, 1957), in which biodegradability becomes very low when DS is over 2.4. It is expected that the biodegradation mechanism of pullulan acetate is similar to that of cellulose acetate: the acetyl groups are hydrolyzed first, followed by hydrolysis of glycoside bonds and metabolism of glucose.

3.4. Swelling behavior of pullulan acetate DS 3.0 in organic solvents

AcPL DS 3.0 was not completely dissolved in any organic solvent that we tested, including polar aprotic solvents. Furthermore, it forms a semi-clear gel in some polar aprotic solvents such as DMSO, DMF, 2-methy-1-pyrrolidinone (NMP), and 1,4-dioxane, while it forms an opaque gel in other polar solvents such as propylene carbonate (PC), methyl ethyl ketone (MEK), 1-acetoxy-3-methoxypropane, and dimethyl succinate (DMS). Fig. 5 shows photographs of the gels swollen by DMF, 1,4-dioxane, PC, and DMS. Some of these gels were disintegrated in the excess amount of the solvent over long periods of time (>4 weeks), implying that the gel is physical gel formed by entanglements of polymer chains. The disintegrated gels hardly went through a filter membrane (pore size: 0.22 μm). Swelling degree (S_w) of AcPL DS 3.0 in organic solvents is plotted as a function of swelling time in Fig. 6. Among semi-clear gels, there are almost no significant differences in swelling behaviors. The weight of the solvent absorbed in these semi-clear gels was about fifty times as large as that of the dry polymer. Chemically crosslinked hydrogel of pullulan (Mocanu, Mihai, Picton, LeCerf, &

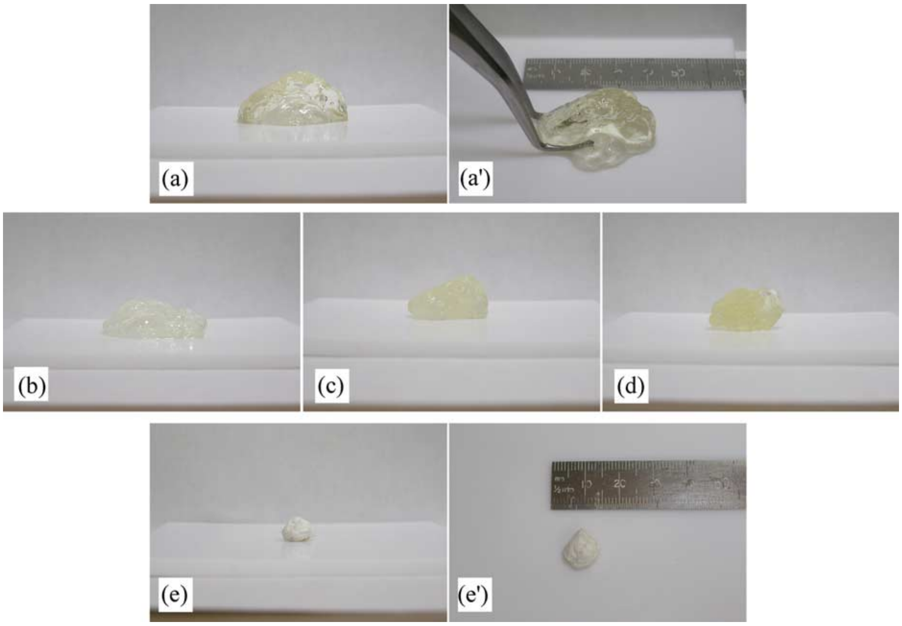

Fig. 5. Photographs of AcPL DS 3.0 swollen by (a, a′) DMF, (b) 1,4-dioxane, (c) PC, and (d) DMS. The photographs (e, e′) show the AcPL DS 3.0 before swelling.

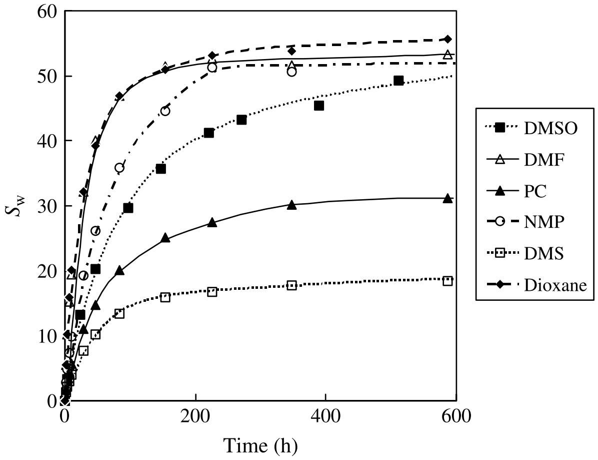

Fig. 6. Swelling degree (S_w) of AcPL DS 3.0 plotted as a function of swelling time in various solvents.

Muller, 2002) and physical gel of hydrophobized pullulan in aqueous solution (Kuroda, Fujimoto, Sunamoto, & Akiyoshi, 2002) have been reported. However, this may be the first report of organogel of pullulan acetate, and it would be applied to the gel electrolyte for an electric cell as one of potential applications.

4. Conclusions

AcPLs with high molecular weight were synthesized by the reaction of pullulan with acetyl chloride in the presence of pyridine. The DS of AcPL was varied by changing the feed ratio of acetyl chloride and pyridine. All the AcPLs have a higher decomposition temperature than unmodified pullulan, and exhibit a clear T_g which decreased with increasing DS value in the range of DS 1.0–2.4. AcPL with DS 2.4 showed the lowest T_g (153 °C), and AcPL with DS 3.0 had a slightly higher T_g (163 °C). Tensile modulus of AcPL films was comparable to that of a popular cellulose acetate film. AcPLs with DS 1.0 and 1.7 showed excellent biodegradability. AcPL with DS 3.0 was swollen in organic polar solvents to form a semi-clear gel or an opaque gel. It was found that the weight of the solvent absorbed in the semi-clear gels is about fifty times as large as that of the dry polymer.

Acknowledgements

This study was supported by Industrial Technology Research Grant Program in '03 from New Energy and Industrial Technology Development Organization (NEDO) of Japan. The authors wish to thank Professor Ryutoku Yosomiya and Professor Hiroyuki Takeishi for the helpful discussions.

References

Akiyoshi, K., Deguchi, S., Moriguchi, N., Yamaguchi, S., & Sunamoto, J. (1993). Self-aggregates of hydrophobized polysaccharides in water. Formation and characteristics of nanoparticles. *Macromolecules*, *26*, 3062–3068.

Akiyoshi, K., & Sunamoto, J. (1996). Supramolecular assembly of hydrophobized polysaccharide. *Supramolecular Science*, *3*, 157–163.

Akiyoshi K., Yamaguchi S., & Sunamoto J. (1991). Self-aggregates of hydrophobic polysaccharide derivatives. *Chemistry Letters*, 1263–1266.

Buchanan, C. M., Gardner, R. M., & Komarek, R. J. (1993). Aerobic biodegradation of cellulose acetate. *Journal of Applied Polymer Science*, *47*, 1709–1719.

Donabedian, D. H., Gross, R. A., & McCarthy, S. P. (1992). Plasticization and reactive processing of pullulan. *Polymeric Materials: Science and Engineering*, *67*, 301–302.

Heeres, A., Spoelma, F. F., van Doren, H. A., Gotlieb, K. F., Bleeker, I. P., & Kellogg, R. M. (2000). Synthesis and reduction of 2-nitroalkyl polysaccharide ethers. *Carbohydrate Polymers*, *42*, 33–43.

Hijiya, H., & Shiosaka, M. (1975). Adhesive and pastes. US Patent 3,873,333.

Jung, S.-W., Jeong, Y.-I., & Kim, S.-H. (2003). Characterization of hydrophobized pullulan with various hydrophobicities. *International Journal of Pharmaceutics*, *254*, 109–121.

Kaplan, D. L. (1998). *Biopolymers from renewable resources: Macromolecular systems—material approach*. Berlin: Springer.

Kuroda, K., Fujimoto, K., Sunamoto, J., & Akiyoshi, K. (2002). Hierarchical self-assembly of hydrophobically modified pullulan in water: Gelation by networks of nanoparticles. *Langmuir*, *18*, 3780–3786.

Mocanu, G., Carpov, A., Chapelle, S., Merle, L., & Muller, G. (1995). Chemically modified pullulans. II. New hydrophobically substituted derivatives. *Canadian Journal of Chemistry*, *73*, 1933–1940.

Mocanu, G., Constantin, M., & Carpov, A. (1996). Chloroacetylated derivatives of dextran. Chemical reactions on polysaccharides. 5. Reaction of mesyl chloride with pullulan. *Die Angewandte Makromolekulare Chemie*, *241*, 1–10.

Mocanu, G., Mihai, D., Picton, L., LeCerf, D., & Muller, G. (2002). Associative pullulan gels and their interaction with biological active substances. *Journal of Controlled Release*, *83*, 41–51.

Mocanu, G., Vizitiu, D., Mihai, D., & Carpov, A. (1999). Chemical reaction on polysaccharides. V. Pullulan chloroalkylation. *Carbohydrate Polymers*, *39*, 283–288.

Reese, E. T. (1957). Biological degradation of cellulose derivatives. *Industrial and Engineering Chemistry*, *49*, 89–93.

Shibata, M., Asahina, M., Teramoto, N., & Yosomiya, R. (2001). Chemical modification of pullulan by isocyanate compounds. *Polymer*, *42*, 59–64.

Shibata, M., Nozawa, R., Teramoto, N., & Yosomiya, R. (2002). Synthesis and properties of etherified pullulans. *European Polymer Journal*, *38*, 497–501.

Shingel, K. I. (2004). Current knowledge on biosynthesis, biological activity, and chemical modification of the exopolysaccharide, pullulan. *Carbohydrate Research*, *339*, 447–460.

Simon, J., Muller, H. P., Koch, R., & Muller, V. (1998). Thermoplastic and biodegradable polymers of cellulose. *Polymer Degradation and Stability*, *59*, 107–115.

Teramoto, N., Motoyama, T., Yosomiya, R., & Shibata, M. (2002). Synthesis and properties of thermoplastic propyl-etherified amylose. *European Polymer Journal*, *38*, 1365–1369.

Teramoto, N., Motoyama, T., Yosomiya, R., & Shibata, M. (2003). Synthesis, thermal properties, and biodegradability of propyl-etherified starch. *European Polymer Journal*, *39*, 255–261.