Preparation of transparent self-standing thin films made from acetylated euglenoid β -1,3-glucans

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Abstract

The feasibility of using acetylparamylon to prepare self-standing optical films was investigated. Its thermal stability, represented by the glass transition temperature, and 5% weight loss temperature are equivalent to those of acetylcellulose. The mechanical properties (maximum stress, elongation at break, and elastic modulus) of acetylparamylon films prepared by solution casting were comparable to those of acetylcellulose films. The degrees of their transparency and birefringence were the same as those of the acetylcellulose film. These results, together with the finding that acetylparamylon with a higher degree of acetylation formed a thin film with fewer micrometer-sized defects and that acetylparamylon with a lower degree of acetylation formed a thin film with a nanometer-level flat surface, suggest that acetylparamylon is a feasible material for self-standing optical film.

1. Introduction

There is a growing environmental demand in the area of material science to replace

synthetic, petroleum-derived materials with naturally derived products. Polysaccharides are a promising candidate and have been well studied over the past decade or two. The most extensively studied one has been cellulose, the most abundant biomass on the globe (Edgar et al., 2001; Isogai, Saito, & Fukuzumi, 2011; Iwatake, Nogi, & Yano, 2008; Kim, Kim, Kang, Marquez, & Joo, 2006; Teramoto, Yoshioka, Shiraishi, & Nishio, 2002). Chitin and chitosan derived from Crustacea, the second most abundant biomass, have also been well studied (Ifuku et al., 2013; Ifuku & Saimoto, 2012). Their characteristics differ from those of other polysaccharides primarily because of the presence of acetamide and amino groups.

Our efforts in this area have focused on *Euglena*-derived β -1,3-glucan, which is referred to as paramylon (Shibakami, Sohma, & Hayashi, 2012; Shibakami, Tsubouchi, & Hayashi, 2014; Shibakami, Tsubouchi, Nakamura, & Hayashi, 2013a, 2013b; Shibakami, Tsubouchi, Sohma, & Hayashi, 2015). The high productivity of paramylon (Barsanti, Vismara, Passarelli, & Gualtieri, 2001; Santek, Friehs, Lotz, & Flaschel, 2012) as well as its use of a “helix-formable” β -1,3-bond as a glucose linkage by *Euglena* (Booy, Chanzy, & Boudet, 1981; Clarke & Stone, 1960) prompted us to explore the feasibility of this polysaccharide as a biomass with the expectation of it having functions differing from those of cellulose, chitin, and chitosan. Our series of studies exploring the feasibility of using paramylon as a raw material started with the preparation of “paramylon doughnuts” and then moved on to the construction of nanofibers. We recently created *Euglena*-based thermoplastics made from mixed paramylon esters (Shibakami et al., 2014).

In our previous studies, we performed acylation, especially acetylation, on paramylon (Shibakami et al., 2012; Shibakami et al., 2014). Acetylation provides paramylon with good solubility in an organic solvent and thermoplasticity. It has been a frequently used chemical reaction in the field of polysaccharide material chemistry over the past half-century (Malm, Tanghe, & Laird, 1946; Rodrigues et al., 2008). A major application of transparent self-standing film made from acetylcellulose was as a photograph film and it is currently as a transparent protective film for liquid crystal polarization plates (Nakayama et al., 2006; Sata, Murayama, & Shimamoto, 2004; Yamaguchi, Abd Manaf, Songsurang, & Nobukawa, 2012). The similarity of the chemical structures of paramylon and cellulose means that films made from acetylparamylon should have protective characteristics comparable to those of cellulose films.

In this paper, we describe the synthesis of two types of acetylparamylon, their use in preparing transparent self-standing thin films, and their evaluation in terms of thermal, physical, mechanical, and optical properties by comparing acetylparamylon films with acetylcellulose-based films.

2. Experimental

2.1 General methods

All chemicals and reagents were commercially available and used without further purification. Paramylon was obtained as micrometer-sized particles in accordance with a previously reported method (Shibakami et al., 2012). Dissolving pulp (α -cellulose >92%, NDPS) was obtained from Nippon Paper Industries Co., Ltd. Microcrystalline cellulose powder (particle size ca. 20 μ m) was obtained from Aldrich. ^1H nuclear magnetic resonance (NMR) spectra were recorded using a Bruker AVANCE 500 spectrometer. Degree of substitution (DS) values, i.e., the average number of functional groups attached to a glucose unit, were determined by comparing the integral values of the methyl protons of the long alkyl chains and acetyl groups with those of the glucosidic protons in the ^1H NMR spectrum; DS_{lac} and DS_{ace} in the text represent the DS of the long alkyl chain (i.e., myristoyl) and acetyl group, respectively. Fourier transform infrared (FT-IR) spectra were recorded using a JASCO FT/IR-480ST spectrophotometer equipped with an attenuated total reflectance accessory (ATR Pro 400-S, ZnSe prism, JASCO). All FT-IR spectra were recorded between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1} .

2.2 Synthesis of acetylparamylons (1 and 2)

A mixture made from paramylon (10.00 g, 61.70 mmol based on glucose unit), N,N-dimethylacetamide (DMAc) (500 mL), and lithium chloride (LiCl) (7.85 g, 185.19 mmol) was stirred at 120°C under a nitrogen atmosphere for 0.5 h to obtain a homogeneous solution. DMAc (1000 mL), pyridine (168 mL, 2.09 mol), and acetic anhydride (240 mL, 2.54 mol) were added to this solution after it was cooled to 70°C. Intermediate solids were obtained from the reaction mixture as follows. A portion (30 mL) was withdrawn from the mixture at 1.5, 3, and 4 h after the start. Addition of water (30 mL) to each portion precipitated a white solid. After separation by centrifugation, the solid was washed with methanol (60 mL) by mechanical stirring for 15 min. This purification procedure was repeated in triplicate. Air drying at ambient temperature for 21 h and vacuum drying at 90°C for 4 h resulted in a purified intermediate solid. The intermediate solid at 5 h was obtained from a portion (300 mL) using a similar procedure. Further purification was performed as follows. The solid (2.27 g) was dissolved in chloroform (125 mL). Methanol (450 mL) was then added to the homogeneous solution. The precipitated solid was obtained by centrifugation. This purification procedure was repeated in triplicate. Air drying at ambient temperature for 21 h and vacuum drying at 90°C for 2 h resulted in the desired product, a pale yellowish opaque solid (**1**) (1.44 g, yield 55.3 %). Successful preparation was confirmed by ^1H NMR and FT-IR measurements; ^1H NMR (CD_2Cl_2) δ 5.03–4.58 (m), 4.51–4.12 (m), 4.11–3.91 (m), 3.88–3.36 (m), 2.12 (s), 2.06 (s), 2.00 (s). DS_{ace} 2.01. FT-IR (cm^{-1}) 1737, 1631, 1434, 1367, 1213, 1077, 1038, 1026, 892.

The residual reaction mixture was mechanically stirred for an additional 16 h. Sequential addition of dichloromethane (800 mL), methanol (1600 mL), and water (1600 mL) precipitated a white solid. After removal of the supernatant by centrifugation, the wet solid was washed with

methanol (500 mL) by mechanical stirring for 1 h. This purification process was repeated in triplicate. After air drying at ambient temperature for 16 h and vacuum drying at 90°C for 6 h, the solid was further purified by precipitation: methanol (1800 mL) was added to a homogeneous solution of the resulting solid in dichloromethane (900 mL). The precipitated solid was obtained by centrifugation. This purification process was repeated in triplicate. Air drying at ambient temperature for 16 h and vacuum drying at 90°C for 3 h resulted in the desired product, a pale yellowish opaque solid (**2**) (7.78 g, yield 60.2 %). Successful preparation was confirmed by ¹H NMR and FT-IR measurements; ¹H NMR (CD₂Cl₂) δ 4.97–4.70 (m), 4.41–4.21 (m), 4.05–3.91 (m), 3.74–3.47 (m), 2.11 (s), 2.06 (s), 2.00 (s). DS_{ace} 2.37. FT-IR (cm⁻¹) 1736, 1629, 1434, 1368, 1211, 1029, 891, 834, 669.

2.3 Synthesis of acetylcellulose from dissolving pulp (**3**)

A mixture made from dissolving pulp (2.00 g, 12.33 mmol based on glucose unit) and DMAc (75 mL) was stirred at 150°C under a nitrogen atmosphere for 1 h. LiCl (3.50 g, 82.6 mmol) was then added to the heterogeneous mixture. After stirring for an additional 1 h, the mixture was allowed to cool to ambient temperature over a period of 22 h to create a homogeneous solution. To this solution was added a mixture of DMAc (75 mL), pyridine (34 mL, 422 mmol), and acetic anhydride (48 mL, 508 mmol). The mixture was then stirred at 70°C for 6 h and at ambient temperature for 15 h. Addition of methanol (300 mL) precipitated a white solid. After removal of the supernatant by centrifugation, the wet solid was washed with methanol (100 mL) by mechanical stirring for 15 min. After air drying at ambient temperature for 16 h, the resulting yellowish solid was further purified by precipitation: methanol (600 mL) was added to a solution of the solid in dichloromethane (200 mL). The precipitated solid was obtained by centrifugation. This purification process was repeated in triplicate. Air drying for 16 h and vacuum drying at 90°C for 3 h resulted in the desired product, a white solid (**3**) (2.53 g, yield 73.9 %). Successful preparation was confirmed by ¹H NMR and FT-IR measurements; ¹H NMR (CD₂Cl₂) δ 5.12–4.97 (m), 4.79–4.68 (m), 4.47–4.26 (m), 4.13–3.93 (m), 3.78–3.65 (m), 3.57–3.46 (m), 2.07 (s), 1.98 (s), 1.92 (s). DS_{ace} 2.75. FT-IR (cm⁻¹) 1733, 1362, 1208, 1030, 895.

2.4 Synthesis of cellulose acetate myristate from dissolving pulp (**4**)

To a round-bottomed flask containing trifluoroacetic anhydride (TFAA) (4.0 mL) were added acetic acid (420 μL, 7.34 mmol) and myristic acid (567 mg, 2.48 mmol) at 50°C under a nitrogen atmosphere. After stirring for 5 min, dissolving pulp (100 mg, 0.62 mmol based on glucose unit) was added to the solution, and the mixture was stirred for an additional 1 h. The resulting homogeneous solution was poured into a saturated sodium hydrogen carbonate aqueous solution (200 mL). The resulting white solid was separated from this solution by filtration. This solid was

then washed with water (90 mL) for 15 min and methanol (90 mL) for 15 min by mechanical stirring (three times each), followed by vacuum drying at 90°C for 2 h, resulting in the desired product, a white solid (**5**) (207 mg, yield 93.4 %). Successful preparation was confirmed by ¹H NMR and FT-IR measurements. ¹H NMR (CDCl₃) δ 5.23–4.97 (m), 4.83–4.21 (m), 4.13–3.92 (m), 3.89–3.23 (m), 2.41–2.21 (m), 2.12 (s), 2.00 (s), 1.93 (s), 1.61 (m), 1.25 (s), 0.88 (t, J = 6.0). DS_{ace} 2.15, DS_{lac} 0.50. FT-IR (cm⁻¹) 2923, 2851, 1748, 1467, 1427, 1365, 1231, 1156, 1082, 1038, 900, 627, 602.

2.5 Synthesis of acetylcellulose from microcrystalline cellulose (**5**)

A mixture made from microcrystalline cellulose (2.00 g, 12.33 mmol based on glucose unit) and DMAc (75 mL) was stirred at 150°C under a nitrogen atmosphere for 2 h. LiCl (3.51 g, 82.8 mmol) was then added to the heterogeneous mixture. After stirring for an additional 1 h, the mixture was allowed to cool to ambient temperature over a period of 22 h to create a homogeneous solution. To this solution was added a mixture of DMAc (75 mL), pyridine (34 mL, 422 mmol), and acetic anhydride (48 mL, 508 mmol). The mixture was then stirred at 70°C for 6 h and at ambient temperature for 15 h. Addition of methanol (300 mL) precipitated a white solid. After removal of the supernatant by centrifugation, the wet solid was washed with methanol (100 mL) by mechanical stirring for 15 min. After air drying at ambient temperature for 16 h, the resulting brownish solid was further purified by precipitation: methanol (300 mL) was added to a solution of the solid in dichloromethane (100 mL). The precipitated solid was obtained by centrifugation. This purification process was repeated in triplicate. Air drying for 16 h and vacuum drying at 90°C for 3 h resulted in the desired product, a white solid (**4**) (2.33 g, yield 65.6 %). Successful preparation was confirmed by ¹H NMR and FT-IR measurements; ¹H NMR (CD₂Cl₂) δ 5.06–4.97 (m), 4.76–4.70 (m), 4.44–4.26 (m), 4.12–4.00 (m), 3.77–3.67 (m), 3.57–3.47 (m), 2.07 (s), 1.98 (s), 1.92 (s). DS_{ace} 2.82. FT-IR (cm⁻¹) 1733, 1623, 1420, 1363, 1209, 1076, 1038, 1024, 967, 894.

2.6 Synthesis of cellulose acetate myristate from microcrystalline cellulose (**6**)

To a round-bottomed flask containing TFAA (4.0 mL) were added acetic acid (420 μL, 7.34 mmol) and myristic acid (569 mg, 2.49 mmol) at 50°C under a nitrogen atmosphere. After stirring for 5 min, microcrystalline cellulose (100 mg, 0.62 mmol) was added to the solution, and the mixture was stirred for an additional 1 h. The resulting homogeneous solution was poured into a saturated sodium hydrogen carbonate aqueous solution (200 mL). The resulting white solid was separated from this solution by filtration. This solid was then washed with water (90 mL) for 15 min and methanol (90 mL) for 15 min by mechanical stirring (three times each), followed by vacuum drying at 90°C for 2 h, resulting in the desired product, a white solid (**6**) (155 mg, yield 65.4 %). Successful preparation was confirmed by ¹H NMR and FT-IR measurements. ¹H NMR (CDCl₃) δ 5.15–4.95 (m), 4.83–4.68 (m), 4.50–4.23 (m), 4.11–3.92 (m), 3.79–3.60 (m), 3.58–3.42 (m),

2.41–2.21 (m), 2.12 (s), 1.99 (s), 1.93 (s), 1.62 (m), 1.25 (s), 0.88 (t, $J = 6.0$). DS_{ace} 2.42, DS_{lac} 0.56.
FT-IR (cm^{-1}) 2923, 2853, 1748, 1365, 1224, 1156, 1047, 897, 668.

2.7 Film preparation and observation

Transparent self-standing thin films were prepared by solution casting. A homogeneous solution of polysaccharides (250 mg) in chloroform (30 mL for **1**) or dichloromethane (30 mL for **2**, **3**, and **5**) was placed on a Teflon dish (75×100 mm) covered with aluminum foil having five holes (diameter of ca. 1 mm). Gradual removal of the solvent at ambient temperature over 8 h resulted in a transparent thin film. Optical microscopic observations were performed on the films using a Leica DMRE optical microscope. Viscometry on dichloromethane solutions containing the polysaccharide (8.5 mg/mL) was performed using an A&D SV-1A vibratory viscometer at $22.3 \pm 2.0^\circ C$. Atomic force microscopic observations were carried out on the films using a Seiko SPI4000 instrument equipped with SPA-400 containing a $20 \mu m$ scanner at ambient temperature. The scan speed was 1 Hz using an Olympus SN-AF01 cantilever (length, $100 \mu m$; spring constant, 0.080 N/m; frequency, 34 kHz) at ambient temperature.

2.8 Size-exclusion chromatography

Weight average molecular weight (M_w) and number average molecular weight (M_n) were determined by size-exclusion chromatography with multiangle laser light scattering (SEC-MALLS). The SEC-MALLS measurements were performed using a miniDAWN multiangle laser photometer (Wyatt Technology), a quasi-electric light scattering (QELS) dynamic light scattering module (Wyatt Technology), and an Optilab rEX refractive index detector (Wyatt Technology) equipped with a gel permeation chromatography column (KD-805, Shodex) (chloroform, $40^\circ C$, 1.0 mL/min). The cellulose acetate myristate solutions were purified using a $0.20\text{-}\mu m$ filter. The injection volume was $100 \mu L$ with a concentration of approximately 4.0 mg/mL. The dn/dc value was 0.0372.

2.9 Thermal analyses

Differential scanning calorimetry (DSC) was performed using a Rigaku Thermo plus EVO II DSC8230 calorimeter. The sample was heated from 25 to $230^\circ C$ at a scan rate of $10.0^\circ C/min$ and held at $230^\circ C$ for 3 min. After being cooled from 230 to $25^\circ C$ and being held for 5 min at this temperature, the sample was heated to $250^\circ C$ at the same scan rate. Thermograms obtained during the second heating were used for determining the glass transition temperature (T_g). Thermogravimetry (TG) analysis was performed using a Rigaku Thermo plus EVO II TG8120 thermogravimetric analyzer to determine the 5% weight loss temperature ($Td5$). The sample was heated from 25 to $500^\circ C$ at a scan rate of $10.0^\circ C/min$ under a nitrogen flow of 100 mL/min.

2.10 X-ray diffractometry

X-ray diffractometry (XRD) of the polysaccharide solids was performed using a MAC Science MFX-HP diffractometer with monochromatic Cu-K α radiation ($\lambda = 0.15418$ nm) generated at 40 kV and 30 mA through an optical slit system: soller slit = 5° (incident side); parallel soller analyzer = 0.114° (receiving side). XRD measurement was performed for a scattering angle 2θ of 3–35° with a 2θ step of 0.05° and an accumulation time of 5 s.

XRD of the polysaccharide films was performed using a Rigaku SmartLab diffractometer with monochromatic Cu-K α radiation ($\lambda = 0.15418$ nm) generated at 45 kV and 200 mA through an optical slit system: divergence slit = 1°; scattering slit = 1°; and receiving slit = 0.3 mm. Scattering was performed for a scattering angle 2θ of 5–60° with a 2θ step of 0.04° and a scan speed of 5°/min. The thickness of the films made from **1**, **2**, **3**, and **5** were 39.3 ± 2.3 , 22.4 ± 3.8 , 81.7 ± 14.2 , and 41.3 ± 2.6 μm , respectively. Quantitative analysis was performed using integrated X-ray powder diffraction software (PDXL, Rigaku Corporation).

2.11 Mechanical strength measurement

Tensile tests were performed on the polysaccharide films using a Tensilon RTG-1225 universal tester (A&D Company, Limited) at ambient temperature. The test specimens had a rectangular shape: 50 (length) $\times$ 10 (width) $\times$ ca. 0.05 (thickness) mm. The initial distance between chucks was set to 30 mm, and the pulling rate was 3 mm/min.

2.12 Light transmittance and birefringence measurements

Light transmittance spectrometry was performed on the polysaccharide films (ca. 10 $\times$ 10 mm) using a Shimadzu UV-2500 spectrophotometer. All spectra were recorded between 400 and 800 cm^{-1} . The thicknesses of the films made from **1**, **2**, **3**, and **5** for the light transmittance measurement were 21.8 ± 2.1 , 42.3 ± 11.6 , 25.3 ± 5.3 , and 8.6 ± 1.1 μm , respectively. Birefringence measurements were performed on the films (40 $\times$ 40 mm) with a Metricon 2010/M prism coupler (632 nm). The thicknesses of the films made from **1**, **2**, and **5** for the birefringence measurement were 22.4 ± 3.8 , 49.3 ± 17.9 , and 8.6 ± 1.1 μm , respectively.

3. Results and discussion

3.1 Syntheses and crystallinity of acetylparamylons (1 and 2)

A previous X-ray study revealed that fully-acetylated β -1,3-glucan forms a well-ordered assembly in the crystalline state, in which the acetyl groups are vertically attached to a columnar β -1,3-glucan helix (Okuyama et al., 1996). This molecular assembly suggested to us that acetylparamylon films are optically isotropic regardless of their crystallinity. Our hypothesis was as follows. When paramylon is fully acetylated (i.e., highly crystalline), the acetyl group's dipole

moments are compensated by the well-ordered helical arrangement, resulting in zero birefringence. When polysaccharide is partially acetylated (i.e., weakly crystalline), the acetyl group's dipole moments are compensated by the disordered polymer arrangement, resulting in zero birefringence. To examine the validity of this hypothesis, we prepared several acetylparamylons with different crystallinities for use in preparing films that would inherit the crystallinity.

The synthesis scheme used to prepare the acetylparamylon samples is depicted in Scheme 1. The DMAc/LiCl solvent system has frequently been used to prepare polysaccharide derivatives because it can efficiently dissolve polysaccharides and prevent their depolymerization (Lima, Sierakowski, Faria-Tischer, & Tischer, 2011; Marson & El Seoud, 1999; Shibakami et al., 2014; Shigemasa et al., 1999; Tosh, Saikia, & Dass, 2000).

To determine a reaction time suitable for preparing acetylparamylon solids with the desired crystallinity, we performed XRD on several intermediate solids produced between 1.5 and 21 h from the start. Fig. 1(a) shows that the 1.5-h solid had two broad peaks around 6.8 and 19.6°. Comparison of this diagram with that for natural paramylon, which shows strong and sharp peaks at 6.8, 19.3, 20.5, and 24.0° (Shibakami et al., 2012), indicates that the 1.5-h solid retained, to some extent, the crystal structure of the natural paramylon. Fig. 1(b) shows that an additional 1.5-h reaction induced broadening and high-angle shift of the two peaks observed in the 1.5-h solid diagram. Fig 1(c) shows that the broadening increased as the acetylation progressed. These diagrams indicate that paramylon lost its crystallization ability when the acetylation time exceeded 4 h. It is notable that one sharp peak emerged at 9.8° in the diagram for the 5-h solid (1) (Fig. 1(d)), indicating that another type of polymer arrangement formed as the acetylation progressed. This observation is supported by the higher crystalline state indicated by the diagram for the 21-h solid (2), which shows relatively sharp peaks at 9.8, 11.8, 14.8, 18.2, 19.9, 21.3, 21.8, 25.1, 27.3, and 31.7° (Fig. 1(e)).

Figure 1

In a separate experiment, we found by naked-eye observation that **1** dissolved completely in chloroform and dissolved partially in dichloromethane and that **2** dissolved completely in dichloromethane and dissolved partially in chloroform. We also found that the 1.5–4 h solids dissolved partially in both dichloromethane and chloroform. A series of XRD diagrams indicated that the 4-h solid had the lowest crystallinity (i.e., disordered polymer arrangement). The insolubility of this solid in low-boiling solvents makes it inappropriate for solution casting because this film preparation method requires good solubility of solids in low-boiling solvents. We thus used **1** and **2** as typical amorphous and crystalline film materials, respectively, in this study.

The ^1H NMR measurements revealed that the DS_{ace} values of **1** and **2** were 2.01 and 2.37, respectively. These values indicate that replacement of two-thirds of the hydroxyl with acetyl group brings about the onset of crystallization (**1**) and that an ordered polymer arrangement is attained by 80% replacement (**2**). While the M_w values for **1** and **2** were not determined due to column clogging induced upon injection of solutions containing these polysaccharides, which are similar to other acetylated glucans (Marubayashi, Yukinaka, Enomoto-Rogers, Takemura, & Iwata, 2014), their M_w values should be comparable to that of paramylon (M_w of 2.959×10^5 Da, degree of polymerization (DP) of 1827) because of the DMAc/LiCl nature of preventing significant depolymerization.

3.2 Synthesis of acetylcellulose

As a control for **1** and **2**, acetylcellulose (**3**) was prepared using a process similar to that used for the acetylparamylons (Scheme 1). The major differences included the duration and temperature required for preparing a homogeneous solution; that is, a homogeneous solution of paramylon was prepared under a milder condition than that for cellulose. Dissolving pulp containing >92% α -cellulose was used as a starting material. The injection of a dichloromethane solution containing **3** induced severe column clogging, so we estimated the M_w and DP of the cellulose from those of cellulose acetate myristate (**4**), which is readily soluble in chloroform, prepared from the dissolving pulp. In brief, on the basis of the M_w of **4** (7.240×10^5 Da; determined by SEC-MALLS), together with its DS_{ace} and DS_{lac} values (determined by ^1H NMR), the M_w and DP of the cellulose

were estimated to be 3.285×10^5 Da and 2026, respectively. These values are comparable to those of paramylon, which validates the use of **3** as a control for **1** and **2**.

3.3 Thermal properties of acetylated polysaccharides

To investigate the suitability of acetylparamylon as a self-standing optical film material, we first examined the thermal properties of acetylparamylon samples (**1**, **2**) by comparing them with those of acetylcellulose sample (**3**). We performed DSC and TG analyses to determine their T_g and $Td5$ values. The thermograms used for determining T_g and $Td5$ are shown in Figs. S1 and S2 (see Supplementary data). The T_g and $Td5$ values are summarized in Table 1. The higher T_g value for **2** than for **1** was likely due to the well-ordered polysaccharide arrangement of **2**, as suggested by the XRD diagrams. The T_g difference between **2** and **3** was 8.1°C, which indicates that the polymer constraint of **2** in the solid state was lower than that of **3**. The non-fully acetylation degree of **2** (i.e., DS_{ace} of 2.37) is probably not enough for perfect molecular alignment. Unraveling the reason for this difference is difficult because **2** and **3** have different structural factors (i.e., glucose linkage and DS_{ace}) that possibly determine the thermal properties. In contrast to the T_g results, **2** showed a higher $Td5$ than **3**. Although this can be attributed to the number of ester bonds that are cleavable ether in **3** being larger than that in **2**, it is possible that β -1,3-glucans are inherently more thermally stable than β -1,4-glucans.

Table 1 Thermal, physical, and mechanical properties of acetylated polysaccharides

acetylated polysaccharide	T_g (°C)	$Td5$ (°C)	viscosity (mPa•s)	maximum stress value (MPa)	elongation at break (%)	Young's modulus (MPa)
1	172.2	322.4	–	30.1 ± 4.2	14.2 ± 9.5	848.1 ± 76.5
2	176.3	336.1	34.08 ± 1.52	39.4 ± 10.1	5.4 ± 2.4	1107.0 ± 172.8
3	184.4	330.0	25.46 ± 0.61	38.4 ± 19.5	4.6 ± 2.7	1315.5 ± 335.4

3.4 Crystallinity of acetylated polysaccharide films

We prepared self-standing acetylparamylon films from **1** and **2** with the expectation that they would inherit the polymer alignment in the solids. As is evident in Fig. 2(a, b), the solution casting method produced self-standing films from **1** and **2** (“film 1” and “film 2,” respectively). The corrugated shape of film **1** is plausibly due to uneven evaporation of chloroform from the solution. To determine whether these films had inherited the crystallinity of **1** and **2** as we had expected, we performed XRD on them. We also measured the diffraction of the acetylcellulose film prepared from **3** (“film 3,” Fig. 2(c)) as a control.

The pattern for film **1** (Fig. 3(a)) showed a broad peak around 9.5°. Since XRD measurement of the sample holder (blank measurement) revealed that neither scattering nor

diffraction appeared in this region, this broad scattering must have been an amorphous halo due to a low crystallinity region in film **1** (Marubayashi et al., 2014). The pattern for film **1** also showed a slight halo around 16–18°, which was plausibly derived from the amorphous region.

Figure 2

Figure 3

In contrast, the pattern for film **2** showed sharp peaks at 9.3, 9.5, 16.2, 16.5, 18.7, and 25.2° and halos overlapping these peaks (Fig. 3(b)). Thus, both films were composed of crystalline and amorphous regions, and the crystallinity of film **2** was much higher than that of film **1**. Although the diffraction diagram for film **2** was not the same as the powder XRD diagram for solid **2** (Fig. 1(e)), some peak positions did coincide (approximately 9–10, 18.5, and 25.0°). This indicates that film **2** inherited to some extent the molecular alignment of solid **1**.

The pattern for film **3** showed broad peaks at 8.0, 16.5, and 21.3° (Fig. 3(c)), indicating that this film contained both crystalline and amorphous regions. Quantitative analysis indicated that the crystalline characteristic of film **3** was due to triacetylcellulose II. A comparison of the patterns for films **2** and **3** indicates that acetylparamylon has a higher ability to align itself than acetylcellulose in the film state.

To gain insight into the molecular alignment ability of **2**, we compared the viscosity of dichloromethane for **2** with that for **3**. As shown in Table 1, the solution of **2** had higher viscosity

than that of **3**, suggesting that **2** formed a much more ordered polymer assembly in the solution than **3**. If we assume that this high viscosity is due to rigidity of the solute, a plausible assembly is an aggregate composed of helices of **2** that is characterized by rigidity (Okuyama et al., 1996). This pre-formed ordered polymer assembly should facilitate preparation of crystalline film.

3.5 Mechanical strength of acetylated polysaccharide films

To examine the mechanical properties of films **1–3**, we performed tensile tests. The results are shown in Table 1. In particular, film **2** showed a maximum stress value comparable to that of **3**. This high value likely accounts for the higher crystallinity of **2**. The elongation at break was the largest for film **1**, which is attributed to its amorphous state, as shown in the film XRD pattern (Fig. 3(a)). Film **3** showed the largest Young's modulus, and film **2** showed one about 15 % smaller. Film **1** had the smallest one. These results indicate that the acetylparamylon films were more deformable than the acetylcellulose one.

3.6 Optical properties of acetylated polysaccharide films

Films **1** and **2** were transparent, and film **3** was translucent to the naked eye. To quantitatively evaluate their transparencies, we measured their visible light spectra. As shown in Fig. S3 (see Supplementary data), the transmittances of films **1** and **2** were nearly 90% in the visible light region. That of film **3** was substantially lower. Given that the estimated dispersity value (M_w/M_n) of **3** was close to that of the pulp-derived cellulose (3.21), we attribute its lower transparency to a poor film state possibly caused by the polydispersity-derived inhomogeneous solidification behavior of solutes as well as impurities.

We thus prepared an alternate transparent self-standing film using highly pure microcrystalline cellulose as the starting material. We synthesized acetylcellulose (**5**) from this cellulose (Scheme 1) and successfully prepared a film made from **5** ("film **5**," Fig. 2(d)). The estimated M_w and M_n of **5** based on those of SEC-MALLS-applicable cellulose acetate myristate (**6**) were 35,000 Da and 18,500 Da, respectively, showing that the dispersity value of **5** was close to 1.89, lower than that of **3** as expected. As shown in Fig. S3(d), film **5** exhibited higher transparency, comparable to those of films **1** and **2**, as expected. The broad peaks and halos in the XRD pattern for film **5** indicate that it was composed of crystalline and amorphous regions, similar to the composition of film **3** (Fig. 3(d)).

Optical films for practical application require not only transparency but also a defect-less surface to prevent abnormal polarization. To investigate whether the current solution casting method can produce acetylparamylon films with both features, we observed the surfaces of films made from **1–3** and **5** using a polarization microscope. As is shown in Fig. 4 (a, b), film **1** contained a few defects while films **3** and **5** contained a number of defects that were considered to be impurities in

the starting materials or poorly acetylated polysaccharides. In contrast, both the number and size of the defects in the surface of film **2** were much smaller than for the other films.

Figure 4

To quantitatively evaluate the film surface states on the micrometer scale, we estimated the total area of defects in the film surfaces. In brief, we calculated the area of a perfect circle surrounding the bright spots (i.e., defects) observed by polarization microscopy ($\times 40$). The total areas per $70,000 \mu\text{m}^2$ were 606, 350, 14,137, and $8,077 \mu\text{m}^2$ for films **1**, **2**, **3**, and **5**, respectively. Note that the actual total area was much smaller than the total circle area. Comparison of these areas numerically confirmed that film **2** had the smoothest surface. Its smoothness is attributed to the near monodispersity of **2**, a characteristic inherited from the paramylon (M_w/M_n of 1.37) as well as to the 100% purity of the paramylon, both of which should contribute to the preparation of structurally homogeneous acetylparamylon.

The smooth film surface of **2** is attributed to the homogeneous solidification behavior of the solutes due to the structural homogeneity. In contrast, both **3** and **5** contained factors, such as higher dispersity, impurities, and poorly acetylated cellulose, that inhibited homogeneous solidification. This inhomogeneity plausibly accounts for the rough surface. The defects observed in the film **1** surface were likely due to the inhomogeneous solubility of the solute, as suggested by the incomplete dissolution of **1** in dichloromethane (described in section 3.1).

To evaluate the film surface on the nanometer scale, we carried out atomic force microscopic observation. The images are shown in Fig. 5. Surface roughness values represented by arithmetic average roughness (R_a) are 0.95, 3.64, 1.80, and 4.70 nm for films **1**, **2**, **3**, and **5**,

respectively. Comparison of these values indicated that film **1** has the smoothest surface and that R_a of film **2** is approximately four times as large as that of film **1**, implying that acetylparamylon **1** has a potential to form the nanometer-level flat surface over the entire surface if the dichloromethane-insoluble solute is successfully removed. Close examination of Fig. 5 reveals that the surface of film **2** contains fibrous material and that of film **1** does not have it. This surface texture difference implies that the smoothness of film **1** is mainly due to the flexibility of the polymeric molecular structure inherent to nonhelical acetylparamylon **1**.

Figure 5

To determine whether acetylparamylon films have a low birefringence value, we performed preliminary birefringence measurements on films **1**, **2**, and **5** as a control; the poor film surface state of film **3** rendered it unusable for this measurement. The refraction indices and birefringence values are shown in Table 2. The birefringence values show that films **1** and **2** were almost optically isotropic (in-plane direction) and were comparable to film **5**, which had a value of zero, indicating that acetylparamylon is usable as a material for optical films requiring low

birefringence. The finding that both films **1** and **2** had low birefringence values in spite of their large crystallinity difference is intriguing. That is, it may verify our hypothesis, described in section **3.1**, that the acetyl group's dipole moments are compensated by a disordered polymer arrangement (film **1**) and by a well-ordered helical arrangement (film **2**). Intensive measurements will be performed in the next stage of our study to further elucidate the birefringence properties.

Table 2 Optical properties of acetylated polysaccharides

acetylated polysaccharide	refraction index (n_x/n_y)	birefringence ($\Delta n = n_x - n_y $)
1	1.46660/1.46678	0.00018
2	1.46347/1.46295	0.00052
5	1.47146/1.47146	0.00000

4. Conclusion

We prepared transparent self-standing thin films from two types of acetylparamylon. The advantages of using acetylparamylon over acetylcellulose as a main component of a transparent self-standing film include unnecessary of intensive purification of the starting material (i.e., paramylon) and their use in preparing defect-less and flat surface. Acetylparamylon with a higher degree of acetylation forms a crystalline film, which is comparable to acetylcellulose film in terms of thermal, mechanical, and optical properties. This film is featured by fewer micro-sized defects. Acetylparamylon with a lower degree of acetylation forms an amorphous film, which is comparable to film made from acetylcellulose in terms of thermal and optical properties. Of particular note is the nanometer-level flat surface. Finding suitable applications for these films and elucidating how the crystallinity affects the film properties are important to advancing the creation of acetylparamylon films for practical use. The results of our efforts on both fronts will be reported in due course.

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