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Acylation of Pullulan by Ring-Opening of Lactones

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ABSTRACT: In this paper, we report on the production of modified pullulan derivatives that have been produced in solution by ring-opening ϵ-caprolactone and [L]-lactide using a tin octanoate [$Sn(Oct)_2$] catalyst system in DMSO. These derivatives designated as hexanoates (**1** and **2**) and dilactates (**4**−**7**) have been characterized and have varying degrees of substitution (DS). **1** and **2** were characterized by interesting thermal properties as well as crystalline morphology. From DSC analysis, **2**, unlike the parent polysaccharide, appears to melt at 114 °C. The crystalline morphology of this polymer was characterized by X-ray powder diffraction and *d* spacings were calculated. This pullulan derivative possessed new crystallographic reflections not seen in either pullulan or poly (ϵ-caprolactone). The pullulan-6 dilactate samples (**4**−**7**), produced had DS values ranging from 0.1 to 0.75. Depending upon the DS, these samples were soluble in a wide range of organic solvents including acetone and methanol. Although these materials did not exhibit any observable melting transitions from inspection of DSC, glass transition temperatures were detected for **5** [DS = 0.21], **6** [DS = 0.44], and **7** [DS = 0.80], at 130, 120, and 90 °C, respectively. As confirmed by ^{13}C NMR, the ring-opening process for all of these pullulan derivatives appears to be taking place through an acyl−oxygen cleavage, producing an ester linkage to the backbone with a hydroxy terminus.

Introduction

Pullulan, first described in 1959,[1] is a water-soluble, extracellular neutral glucan synthesized by the fungus *Aureobasidium pullulans.* The polymer consists of linear chains of D-glucopyranosyl units that alternate regularly between one (1→6)-α-D and two (1→4)-α-D linkages, or a linear polymer of maltotriosyl units connected by (1→6)-α-D linkages, Figure 1. Pullulan is different from amylose in that the former does not form micelles and is specifically characterized as being freely water-soluble. In its purified form, pullulan is tasteless, odorless, and nontoxic and forms films from aqueous media with excellent oxygen resistance.[2] Pullulan, like most polysaccharides, is difficult to process by conventional melt processing techniques without the use of processing aids. Another technique for imparting thermoplastic qualities to this material is to modify the polymer by acylation with acetic anhydride to produce materials capable of being extruded.[3]

Chemical modification, for example graft copolymerization, of natural polymers is one valuable method to improve the properties of a polymer and has been extensively used in the graft modification of gelatin, cellulose, and starch. Arranz et al. modified dextran with succinic anhydride to produce a carboxylic acid functionalized polymer. McCormick et al. reported on the ring-opening pathways of ϵ-caprolactone with cellulose using a LiCl/DMAc solvent system with triethylamine as catalyst. In their investigation, ring opening occurred through acyl-oxygen cleavage producing a cellulose ester.[4−9]

Unlike starch and cellulose, little has been reported in the literature about the chemical derivatization of pullulan. Bruneel et al. reported using pullulan as a prodrug carrier, by chemically modifying the backbone to accept the drug. They produced two different derivatives, a polyaldehyde and a chloroformate derivative with differing substitutions, and structurally characterized these materials.[10,11] Masahario et al. grafted ethyl acrylate or styrene onto pullulan using $K_2S_2O_8$ as the initiator and used the products for bonding of rayon−polyester nonwoven fabrics.[12] Other monomers such as butyl-2-propenoate and ethenyl acetate are also capable of being grafted onto pullulan.[13] The chemical modification of pullulan with synthetic degradable polymers offers another technique for modifying pullulan's high water-solubility. Moreover, this modification could impart thermoplastic properties to these materials producing articles useful for biodegradable applications.

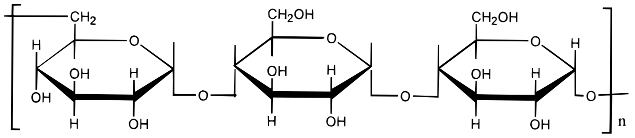

Figure 1. Structure of pullulan produced from *Pullularia pullulans.*

In this paper, acylation by ring-opening of ϵ-caprolactone and [L]-lactide is reported. The process of grafting lactones onto pullulan is shown in Scheme 1. The ring-opening of ϵ-caprolactone and [L]-lactide with stannous 2-ethylhexanoate $Sn(Oct)_2$ and alcohol initiators is well-known.[14] Pullulan, a multihydroxyl-containing polymer, could serve as a useful substrate for grafting onto reactions.

In this study, the graft reactions were conducted in dimethyl sulfoxide with stannous 2-ethyl hexanoate $Sn(Oct)_2$ as the catalyst. Modified pullulan derivatives have been produced with varying ranges of modification, exhibiting interesting thermal properties as well as showing some degree of crystallinity.

Experimental Section

1. Reagents and Procedure. (A) Polymer and Monomer Purification. Pullulan (10 g, 2.06×10^{-2} mol), PF-20 grade supplied by Hayashibara Biochemical Labs, Ltd., (average $M_n = 96\,000$ g/mol, GPC, water, pullulan standards), was purified two times by precipitation from a water (10 wt %/v)

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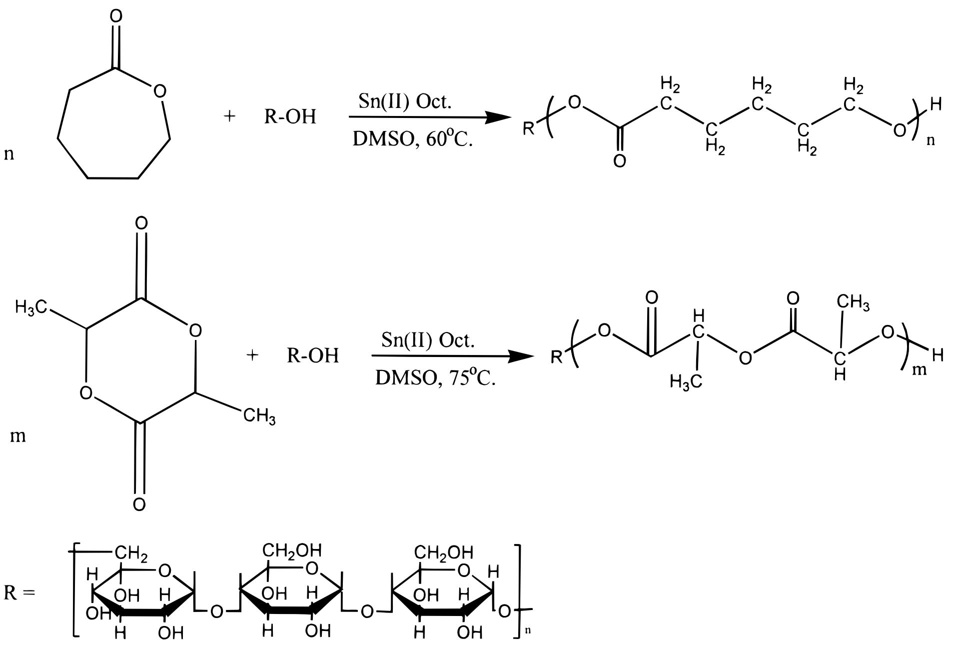
Scheme 1



solution (100 mL) into a 10:1 excess of methanol (1 L) (Fisher Scientific, reagent grade). The stringy polymer was then dried under vacuum (1 mm Hg) at 50 °C for 24 h under P_2O_5. The contents were further dried on a diffusion pump at a temperature of 100 °C under a vacuum of 30 mmHg. The average molecular weight for this purified pullulan was slightly higher than the native material (106 000). ϵ-Caprolactone, obtained from Aldrich Chemical Co., was distilled before use under reduced pressure over CaH_2. The [L]-lactide (polymerization grade, Purac America), Stannous 2-ethyl hexanoate (Sigma Chemical Co., 97%), dimethyl sulfoxide (DMSO) (Aldrich Chemical Co., anhydrous, 99% +), and Chlorotrimethylsilane (Aldrich Chemical Co., 98%) were used as received.

(B) Pullulan 6-Hydroxyhexanoates (1–3). A 0.5 g (1.03×10^{-3} mol) sample of purified pullulan was placed into two separate previously silanized ampules. The ampules, containing stir bars, were silanized with chlorotrimethylsilane following a modified procedure from Corey et al. and dried for 24 h on a drying pistol (50 °C, 10 mmHg).[15] The ampules were then removed under an argon blanket and covered with rubber septa. Then 14 mL of DMSO was added to each ampule via syringe under an inert atmosphere to produce a 3.5% (w/v) solution. The ampules were placed in an oil bath at 55 °C for 35 min to dissolve the polymer. After homogenization, 410 μL (8.0×10^{-5} mol) of stannous octanoate was added from a 0.19 M DMSO stock solution in DMSO followed by 2.0 mL (1.8×10^{-2} mol) of distilled ϵ-caprolactone. The ampules were sealed under vacuum and placed into the oil bath for 6 and 10 days, respectively, at 60 °C. The resulting contents were precipitated into cold methanol (150 mL), filtered, and Soxhlet extracted with chloroform for 48 h to remove any homopolymer that may have formed. Further purification was conducted by taking 0.100 g of the modified polysaccharide and dialyzing against distilled water using a cellulose acetate membrane (Spectrum Medical, $M_n = 3000$ g/mol cutoff) for 18 h. The recovered material (0.0950 g) was analyzed by ^{13}C NMR. All products were dried for 24 h under vacuum (1 mmHg) at 50 °C using P_2O_5 as a desiccant. To determine the degree of substitution (DS) for these polymers, the integration value at 5.2 ppm (1H) was compared to the caprolactone methylene (6H) protons at 1.7–1.2 ppm.

(C) Pullulan 6-Dilactates (4–7). A 1.5 g (3.08×10^{-3} mol) sample of purified pullulan was introduced into five silanized glass ampules with an internal volume of 25 mL. The 12 mL of DMSO was cannulated into each ampule to make a 12.5% (w/v) solution, and the ampules were placed in an oil bath at 75 °C for 2 h under an argon atmosphere. Separately, five ampules containing solution of [L]-lactide dissolved in 5 mL of distilled DMSO were also prepared by gentle heating under an argon atmosphere for 20 min. To each of the pullulan/DMSO ampules, the entire [L]-lactide solution was introduced by syringe under an inert atmosphere. After homogenization in an oil bath for 20 min, 1.4×10^{-5} mol of stannous octanoate was added to each ampule. The amount of [L]-lactide added varied, to produce a range of pullulan grafts with different amounts of substitution. The ampules were then sealed under vacuum and placed back in the oil bath for 6 days at 75 °C.

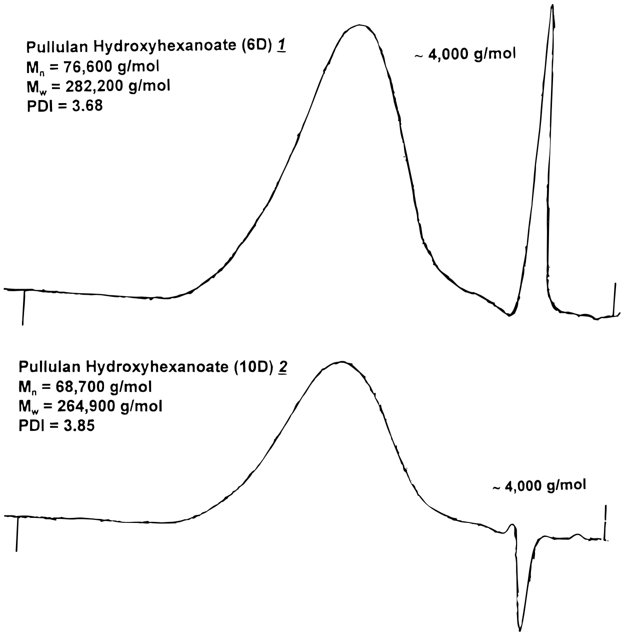


Figure 2. Gel permeation chromatograms of **1** and **2** in water prior to dialysis.

Depending upon the degree of polysaccharide modification, different nonsolvents were used to recover the polymers. Materials with higher amounts of lactide modification became water insoluble and were precipitated into an (150 mL) ethyl acetate–water (40:60) mixture. These materials were then further purified to remove unreacted monomer as well as any poly(lactide) homopolymer that may have formed. Soxhlet extraction with hexane (24 h) was conducted twice to remove any nonreacted [L]-lactide monomer. The material was then

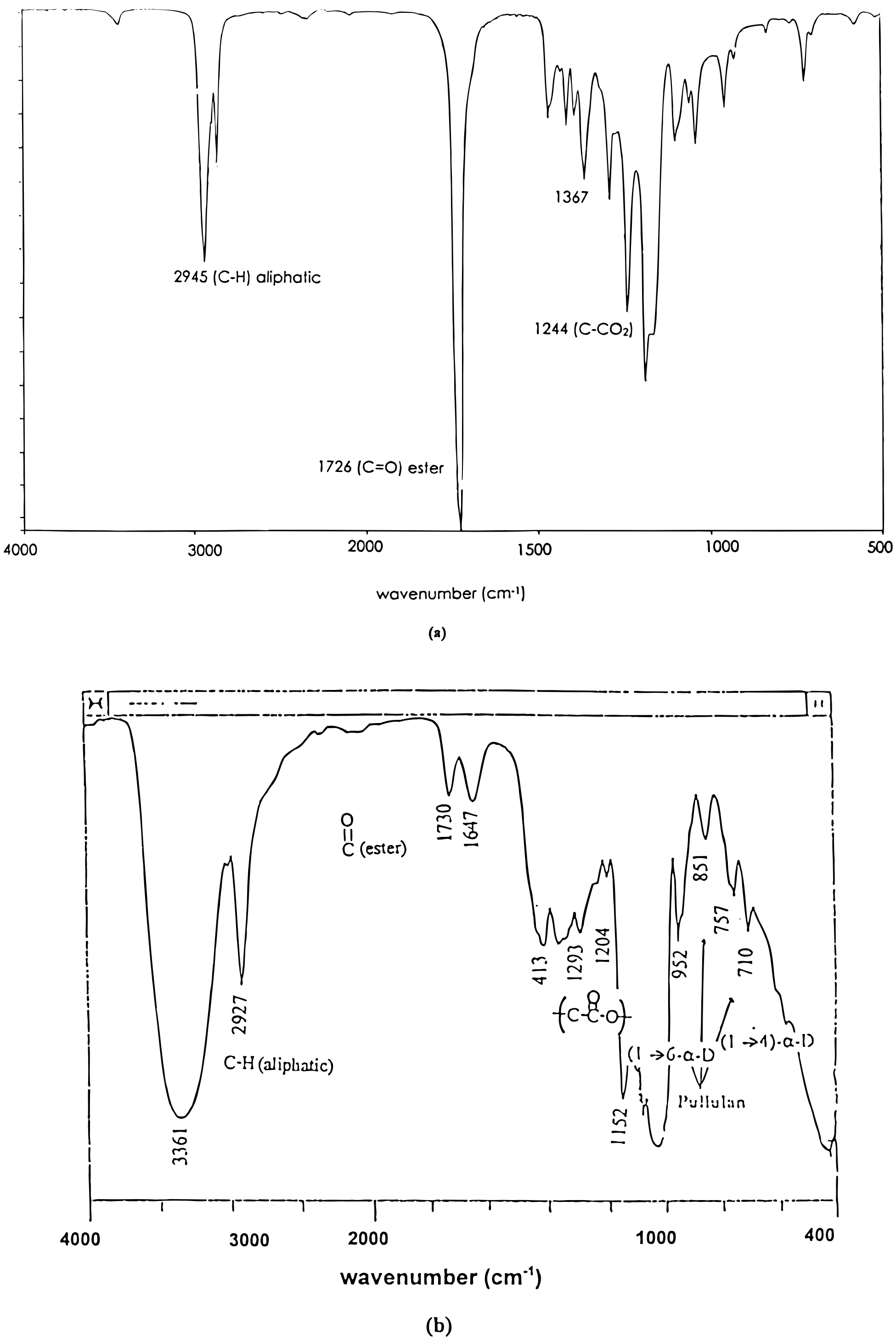


Figure 3. (a) FT-IR spectrum of TonePCL P-787 (M_n = 80 000 g/mol) solution cast from CH_2Cl_2 on a NaCl plate. (b) FT-IR spectrum of **1**, solution cast from DMSO on a NaCl plate.

washed in chloroform using a 10:1 excess of solvent to polymer. Finally, the product was redissolved in acetone and precipitated into a 10:1 excess of hexane. These products were dried at 60 °C (1 mmHg) for 24 h. To determine the DS for these polymers, the integration value at 5.2 ppm (3H) was compared to the lactide methyl (6H) protons at 1.1 ppm.

2. Measurements. (A) NMR Spectroscopy. ^1H NMR spectra were recorded on a Brucker WP-270 SY spectrometer at 270 MHz or a Varian Gemini 200 MHz spectrometer. Chemical shifts in parts per million (ppm) were reported downfield from 0.00 ppm using tetramethylsilane (TMS) as an internal reference. Residual undeuterated protons in DMSO-d_6 was also used as an internal reference giving a clear singlet at 2.49 ppm. The native polymer as well as the synthetic derivatives was run in 5 mm tubes as DMSO-d_6 solutions, using 50−100 transients.

Carbon (^{13}C) NMR spectra for all polymers were recorded at 67.9 MHz on a Brucker WP-270 SY spectrometer in 10 mm tubes as either DMSO-d_6, $CDCl_3$, or D_2O solutions, using 25 000−35 000 transients with chemical shifts in ppm referenced relative to either DMSO, $CHCl_3$, or TMS. DMSO is an internal reference with a septet centered at 39.5 ppm.

(B) Infrared Spectroscopy. Fourier transform infrared (FT-IR) spectra were recorded on either a Brucker IFS 113v or a Perkin-Elmer 1600 Series FT-IR spectrometer at 25 °C. FT-IR spectra were recorded for the derivatives as films cast

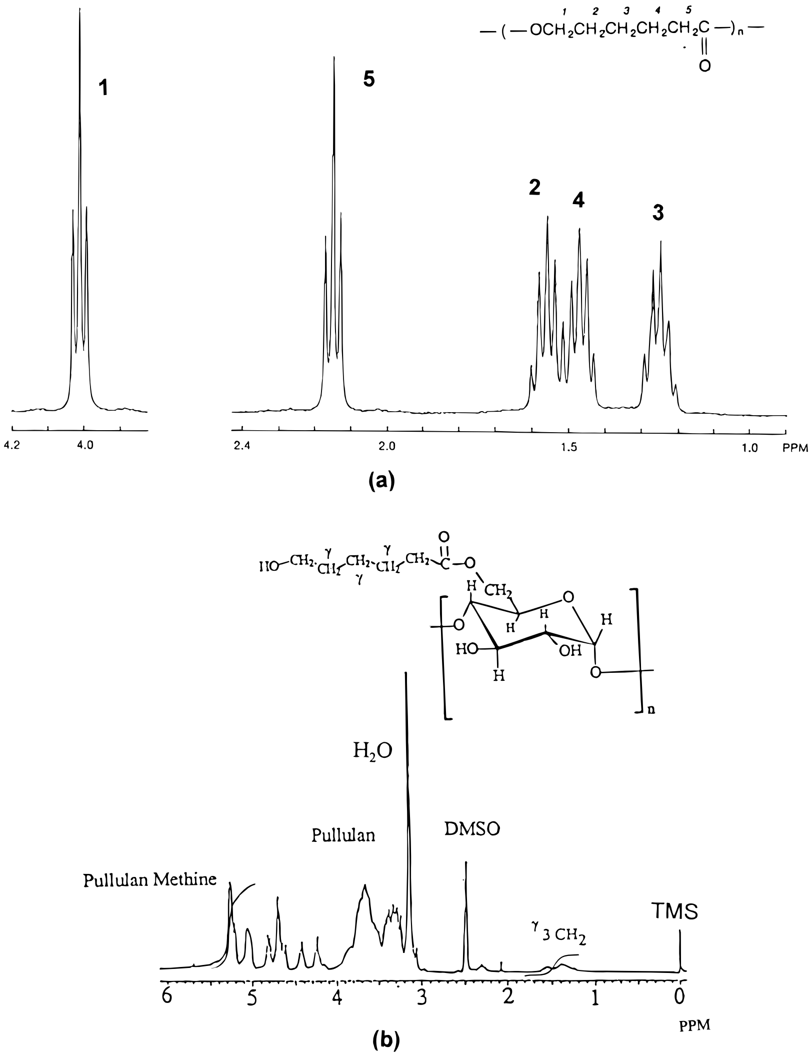


Figure 4. (a) ^{1}H NMR spectrum (350 MHz) of poly(ϵ-caprolactone) in benzene-d_6 at 50 °C. (b) ^{1}H NMR spectrum (200 MHz) of **1** in DMSO-d_6 at 70 °C.

directly on NaCl plates or as KBr pellets as indicated. The spectral position of the sample IR absorbances are given in units of reciprocal centimeters (cm^{-1}).

(C) Molecular Weight Determinations. For water insoluble samples, molecular weight determinations by gel permeation chromatography (GPC) were carried out using a Waters Model 510 pump, Model 410 refractive index detector, and Model 730 data module with 10^3, 10^4, 10^5, and 10^6 Å Ultrastyragel columns in series. Either chloroform or DMF (containing 0.1% w/v LiBr) was used as the eluent at a flow rate of 1.0 mL/min. Sample concentrations of 0.5% w/v and injection volumes of 100 μL were used. Polystyrene standards with a low polydispersity (Polysciences) were used to generate a calibration curve.

For water soluble samples, molecular weight determinations were carried out using a Waters Model 510 pump, a Model 410 refractive index detector, and a Model 730 data module. Shodex KB-800 series columns were used in series, with water was used as the eluent (0.3% Na_2SO_3) at a flow rate of 1.0 mL/min. Injection volumes were the same as described above, Pullulan standards with a low polydispersity (Polysciences) were used to generate a calibration curve.

(D) Thermal Analysis. Differential scanning calorimetry (DSC) was conducted on a TA Instruments DSC 2910 equipped with a TA 2000 data station, using between 5 and 10.0 mg of sample sealed in either hermetic or nonhermetic aluminum pans. A heating rate of 10 °C/min was employed along with a nitrogen purge. Thermogravimetric analysis was conducted on a TA instrument, model Hi Res TGA 2950, using between 1 and 4.0 mg of sample under either a nitrogen or compressed air atmosphere. The heating rate was the same for that employed for DSC.

(E) X-ray Diffraction. X-ray diffraction measurements were made on packed powder samples at 25 °C using a Rigaku Geigerflex camera operating at a voltage of 40kV and a current of 25mA. Nickel-filtered Cu Kα radiation (l = 1.542 Å) was used. The sample exposure time ranged from 6 to 12 h and the sample-to-film distance was 4.96 cm.

Wide-angle X-ray scattering (WAXS) measurements were made using a Norelco Vertical Defractometer using Bragg–Brentano focusing geometry, 3 kW constant potential generator, Cu Kα radiation (diffracted beam monochromator). All samples were mounted on a zero background substrate. The diffraction patterns were collected using a counting rate of 500 counts/s at a power setting of 22 kV and 12 mA at room temperature.

3. Synthesis and Characterization. ^{1}H NMR and FT-IR. Acylations of pullulan with ϵ-caprolactone and [L]-lactide were conducted using a stannous octanoate catalyst system. The catalyst content and reaction conditions employed were similar for both modifications. For the caprolactone modified derivatives, the reaction proceeded for 6 and 10 days respectively at 60 °C. For the lactide-based modifications, the monomer content was varied and the reaction time was 6 days at 75 °C.

At the end of the reaction, the caprolactone-modified polymers were isolated by precipitating into methanol. The percent yields for these products was low, 25%. These crude polymers were Soxhlet extracted with chloroform for 2 days prior to characterization to remove any homopolymer that may

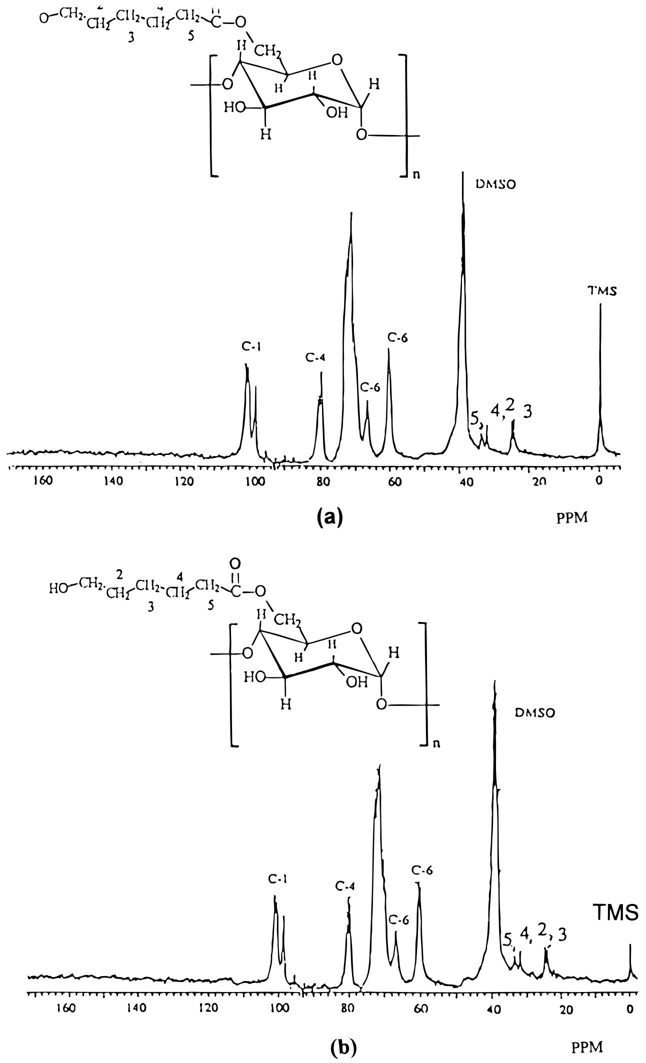


Figure 5. (a) ^{13}C NMR spectrum (55 MHz) of **2** in DMSO-d_6 at 25 °C prior to dialysis. (b) ^{13}C NMR spectrum (55 MHz) of **2** in DMSO-d_6 at 25 °C after dialysis.

have formed. From this extraction process, ~70% of the chloroform-soluble byproducts were removed. These materials were then characterized by FT-IR, NMR, DSC, and X-ray diffraction.

IR analysis shows Tone PCL P-787 (M_n = 80 000 g/mol by GPC, chloroform, Polystyrene standards) and the product **1**, after reaction for 6 days, Figure 3. The weak absorption at 1730 cm^{-1} is indicative of the carbonyl absorption for the graft. The molecular weight for this material was determined by GPC and was 76 600 g/mol ([0.3% Na_2SO_3] H_2O). The molecular weight of the graft is somewhat lower than that of the native material, which is attributed to the harsh nature of the solvent. From this GPC trace, a large low molecular weight peak was also observed at ~ 4000 g/mol, Figure 2. The molecular weight for the sample obtained after 10 days, **2**, was 68,700 g/mol with a similar low molecular weight peak present. This low MW peak could be a result of byproduct from the grafting onto reaction. These materials, like pullulan, were soluble in dimethyl sulfoxide and water. To provide further evidence of successful acylation, **2** was further purified by dialysis against distilled water for 18 h. After dialysis, the molecular weight of the dialyzed material was M_n = 59 900 g/mol with PDI = 4.13, and the low molecular weight peak was no longer evident. The amount of material recovered after dialysis was 95%. It appears that the dialysis was successful in removing any low molecular weight water-soluble byproducts that may have formed during the reaction. When cast as aqueous solutions, both materials formed clear, colorless, tenacious films.

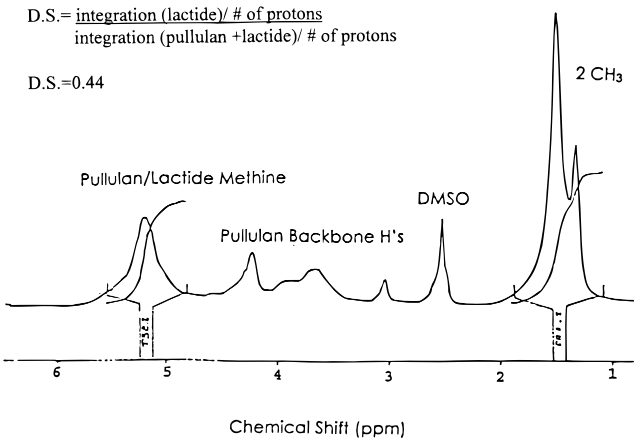
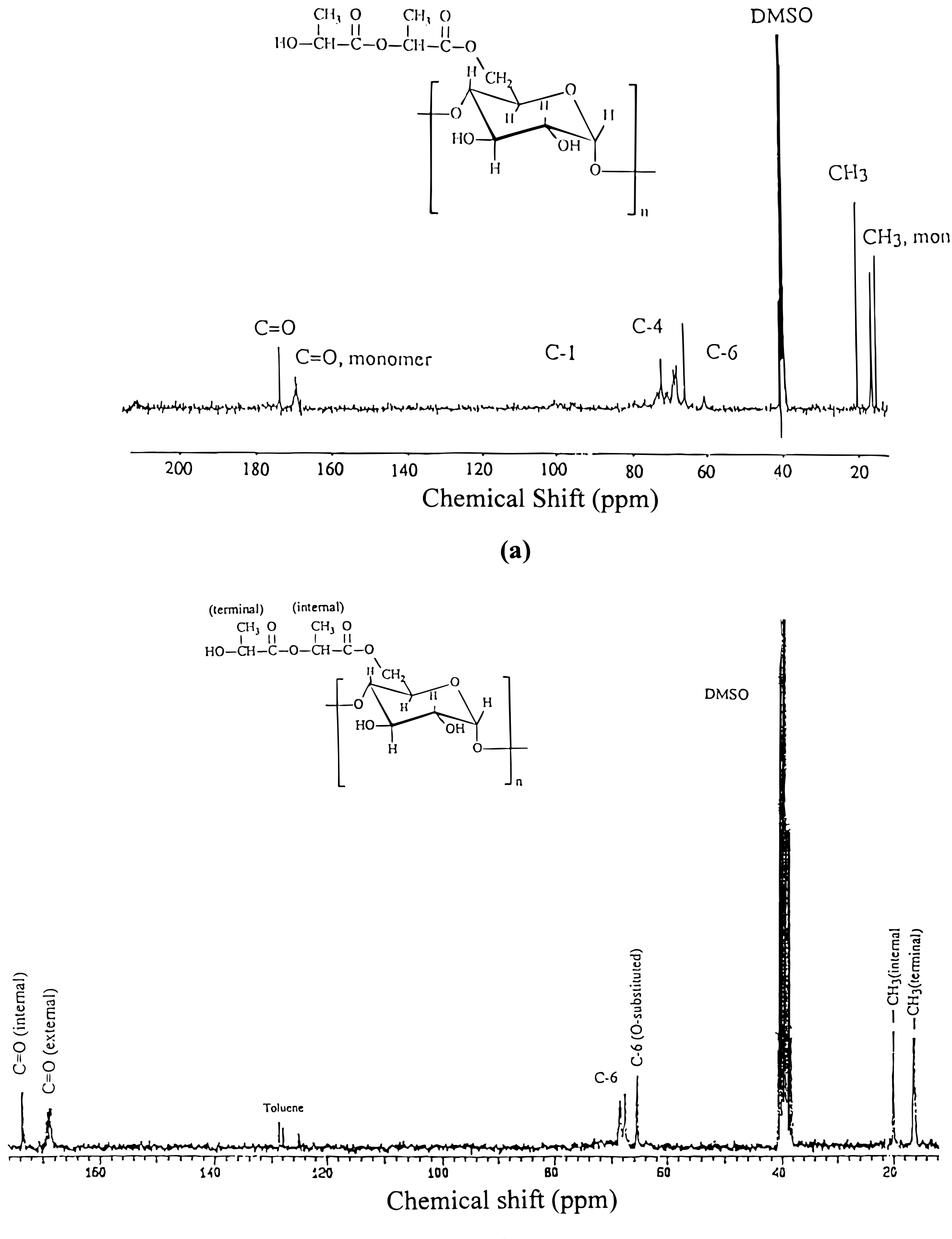


Figure 6. ^{1}H NMR spectrum (270 MHz) of **6** in DMSO-d_6 at 70 °C.

From inspection of the ^{1}H NMR spectra, we see evidence of 6-hydroxycaproate structural elements in the region of 1.7−1.2 ppm and at 4.2 ppm, Figure 4. The manner in which ϵ-caprolactone is added to pullulan can be described in either terms of degree of substitution (DS) or molar substitution (MS). The degree of substitution designates the average number of hydroxyl groups on the anhydroglucose ring that have been reacted with ϵ-caprolactone. The molar substitution is defined as the average number of ϵ-caprolactone molecules that have reacted with each anhydroglucose unit. Once a hydroxy terminated caprolactone group is attached, it can further react with additional groups in an end-to end formation; as long ϵ-caprolactone is available this reaction can continue theoretically without limit. The degree of substitution (DS) for these materials was low, and was determined by taking the integration values in the region of 1.7−1.2 (6H) and comparing it to the pullulan methine peak at 5.2 ppm. The degree of substitution for **1** and **2** were 0.10 and 0.25, respectively. The ^{13}C NMR analysis was performed on this sample both before and after dialysis, Figure 5. The peaks in the region of 38.4 ppm-24.5 ppm are indicative of methylene units from the caprolactone side chain that is grafted onto the pullulan backbone. The absence of an observable carbonyl absorption is most likely due to the long relaxation times associated with the polymer. These data, along with the disappearance of the low molecular weight peak from the GPC trace, is a strong indication that acylation of ϵ-caprolactone and subsequent grafting onto the pullulan backbone has occurred.

Pullulan was also modified by acylating with [L]-lactide in DMSO at 75 °C using the stannous octanoate catalyst system. The very low substituted materials **4** and **5**, like the parent polysaccharide, were soluble in water and insoluble in organic solvents. The other two samples, **6** and **7**, will be discussed in detail because they have the most interesting structural as well as thermal properties. After acylation, these materials were precipitated into an ethyl acetate/water mixture. Unlike the parent polysaccharide, the precipitates were no longer soluble in water but are now soluble in acetone, and the highest substituted product was soluble in methanol. Since homopolymer formation and residual monomer are a concern in reactions of this type, a stringent purification procedure was employed. The product was Soxhlet extracted for over 18 h in hexane to remove any unreacted [L]-lactide monomer. This procedure was conducted twice and resulted in removing approximately 60% of unreacted lactide that was charged to the ampule. This material was identified as unreacted monomer by FT-IR and ^{1}H NMR. Further unreacted monomer and homopolymer were removed by washing this material in chloroform for 16 h at room temperature. This washing procedure resulted in removal of another 10% of byproduct. Finally, this modified polysaccharide was redissolved in acetone and precipitated into hexane. The percent yield for this grafting reaction, after recalculating for the amount of lactide actually consumed was 50%.

The manner in which [L]-lactide is added to pullulan can also be described in terms of degree of substitution (DS). The



(a)

(b)

Figure 7. (a) ^{13}C NMR spectrum (67.9 MHz) of **7** in DMSO-d_6 at 70 °C prior to the second Soxhlet extraction with hexane. (b) ^{13}C NMR spectrum (67.9 MHz) of **7** in DMSO-d_6 at 30 °C after the second Soxhlet extraction with hexane and chloroform washing (spiked with toluene).

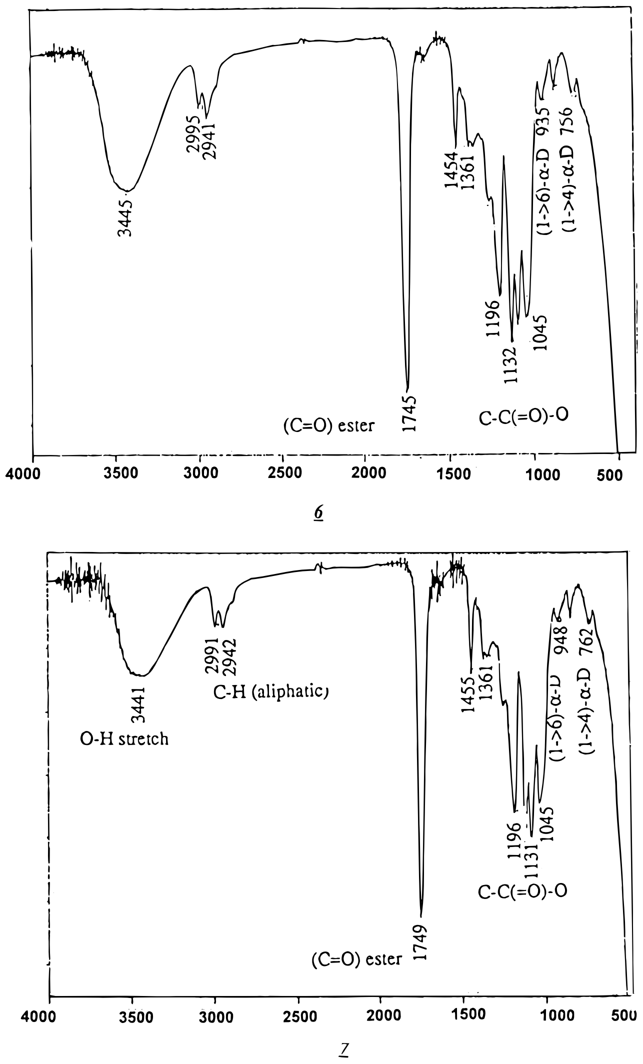
^{1}H NMR of **6** in DMSO-d_6 at 50 °C is in Figure 6. The characteristic pullulan protons are seen in the region from 5.0 to 3.0 ppm. Also, the appearance of a doublet at 1.5 ppm was indicative of the methyl group of the lactide moiety. The methine proton for the lactide is normally found in this region as a quartet. To determine the DS for these polymers, integration at 5.2 ppm (3H) was compared to the lactide methyl (6H) protons. The DS for these derivatives ranged from 0.1 to 0.75 per residue.

Inspection of the ^{13}C NMR after the first Soxhlet extraction in hexane reveals three peaks in the region of 169.8 ppm-169.0 ppm and one peak at 173.7 ppm, Table 1.[17] This table

Table 1. Chemical Shifts for 6, 7, [L]-Lactide, and Poly [L]-Lactide

	Chem shifts, δ				
	^{13}C			^{1}H	
sample	C=O	Cα	Cβ[c]	CHα	CH$_3$
[L]-lactide[a]	167.9	15.7	72.5	1.66 (d)[d]	5.09 (q)
poly([L]-lactide)[a]	169.5	16.7	69.1	1.56 (d)	5.17 (q)
6[b]	173.7, 169.8, 169.5, 169.0	20.2, 16.5, *16.4*, 16.2		1.55 (d), br	5.25 (s) br
7[b]	173.7, 169.9, 169.5, 169.0	20.2, 16.4, 15.2		1.55 (d), br	5.25 (s) br

[a] Data from ref 17; chemical shifts relative to internal TMS in CDCl$_3$. [b] Chemical shifts relative to internal TMS in DMSO at 70 °C. [c] Unable to determine Cβ due to overlap of pullulan C-2 and C-3 absorptions. [d] Key: (s), singlet, (d), doublet; (q), quartet, br, broad.



6

7

Figure 8. FT-IR spectrum of **6** and **7** cast from DMSO on a NaCl plate.

summarizes the relative absorption of [L]-lactide, poly([L]-lactide), and the two derivatives, **6** and **7**. The carbonyl absorption from poly [L]-lactide is evident in both pullulan derivatives; however, the carbonyl absorption of the lactide monomer is not evident. There is also the appearance of a strong absorption in the region of 173 ppm for all the derivatives, not seen in either the lactide monomer or polymer, indicative of successful acylation. Inspection of the C_α region shows the appearance of a new peak at 20 ppm that is not evident in either the lactide monomer or polymer, Figure 7a. There are, however, peaks at 16.5 and 16.4 ppm that are representative of the internal methyl group of the lactide graft. The peak at 15.2 ppm is attributed to residual monomer. The second Soxhlet extraction of **7** with hexane, washing with chloroform, and final recovery in hexane yielded the following ^{13}C NMR shown in Figure 7b. We no longer observe the peak at 15.2 ppm which was a result of unreacted [L]-lactide monomer, Table 1. We still see evidence of grafted lactide moieties, and it appears the ring-opening event is producing a dilactate side group that is grafted off the pullulan backbone. The carbonyl absorptions are heterogeneous in nature, probably due to the random substitution of the lactate groups on the polymer.

FT-IR analysis of **6** and **7** was performed and the samples showed a strong absorption band in the region 1745−1749 cm^{-1} indicative of a carbonyl stretch along with a decrease in the OH absorption at 3400 cm,$^{-1}$ Figure 8. These data suggest that successful modification has taken place with a decrease in OH absorption and the appearance of ester absorptions. The carbonyl absorption for pure poly [L]-lactide is generally seen at 1759 cm.$^{-1}$ [18,19] Taken together, this evidence supports the premise of acylation of pullulan by ring-opening of [L]-lactide onto the pullulan backbone.

Table 2. Monomer Concentration, DS, and Thermal Properties for Pullulan Dilactates, 4−7

sample	lactide: OH (mol)/per anhydroglucose	H_2O solubility	DS[a]	T_g (°C)	T_d[c] (°C)
P6-Dilact **4**	0.15:1.00	+	0.08	nd[b]	>200
P6-Dilact **5**	0.33:1.00	+	0.21	130	>200
P6-Dilact **6**	0.66:1.00	−	0.44	120	>200
P6-Dilact **7**	1.65:1.00	−	0.75	90	185

[a] DS determined from ^1H NMR. [b] Not detected by DSC analysis. [c] T_d is onset of decomposition determined by TGA under N_2.

Table 3. Thermal Properties for 1−3

	1st heat (°C)		2nd heat (°C)		3rd heat (°C)		
sample	H_2O	T_c	T_c	T_m	T_c	T_m	T_d (°C)
1[a]	yes	25	30	115	23	110	240
2[b]	yes	25	38	120	24	127	240
3[b,c]	yes	25	37	114			240

[a] Reaction time for this sample was 6 days. [b] Reaction time for this sample was 10 days. [c] This sample was held isothermally for 1 h at 180 °C, quenched to −100 °C, and then reheated.

The corrected monomer concentration, DS, and thermal properties are summarized in Table 2 for all of the samples. The lactide grafted samples **4**−**7**, had varying ranges of solubility. The lower substituted products, **4** and **5** were still soluble in water. The higher substituted products were no longer soluble in water with **6** now being soluble in methanol and **7** being soluble in both methanol and acetone.

Results and Discussion

Thermal Analysis. The thermal properties of **1** and **2** were quite different from native pullulan as can be seen in Table 3. Pullulan, like most unmodified polysaccharides, degrades before it melts and cannot flow without the use of processing aids such as plasticizers, extenders, etc. Unlike the native material, we observed that upon a first heating cycle for **1** and **2**, a large water endotherm is apparent along with a small crystallization peak. Upon quenching and reheating, a more observable crystallization peak appears at ∼ 30 °C. There is also a melting phenomen associated with both **1** and **2** at ∼ 115 °C. On a third heating cycle, this behavior is again replicated. To remove any residual water that still may be present in the material, a new sample was prepared from **2**, designated as **3**, and was first heated to 180 °C, and held isothermally for 1 h. However, after this thermal treatment, a water absorption peak is still apparent, Figure 9, most likely a result of bound water. After quenching, the second heat obtained again shows a crystallization peak at 37 °C and a melting peak at 114 °C, Figure 10. This result indicates that we have been able to produce a modified pullulan derivative with a relatively low melting point. The crystalline morphology of these materials will be elucidated by X-ray diffraction.

The thermal properties of **5**, **6**, and **7** are interesting in that all exhibited a T_g, with the highest substituted product having the lowest glass transition temperature. The highest substituted material had a T_g of 90 °C and the lower substituted graft had a T_g of 130 °C. Neither material exhibited any melting phenomenon up to 200 °C. The onset of the decomposition temperature (T_d) for the lower substituted products was slightly higher than that for the higher substituted materials. This result is expected since the lower substituted materials are more pullulan-like in structure. Due to the relatively low amount of grafting and side chain length, it

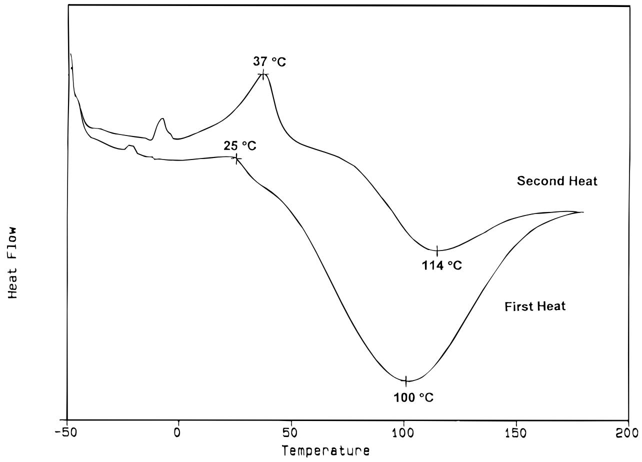


Figure 9. DSC thermogram for the first heating scan of **3** held at 180 °C for 1 h. DSC thermogram for the second heating scan of **3** recorded after a first heat and a rapid quench in liquid nitrogen.

Table 4. Interplanar *d* Spacings (Å) Obtained by X-ray Diffraction Analysis for 1, 2, and the Respective Homopolymers

pullulan	Tone PCL P−787	**1**	**2**
6.16 (w)[a]	5.49 (w)[b]	3.06 (m)	3.06 (m)
3.50 (w)	4.50 (w)	2.37 (s)	2.84 (m)
3.06 (m)	4.05 (s)	2.05 (s)	2.60 (m)
2.82 (m)	3.93 (m)		2.37 (s)
3.65 (m)	2.30 (w)		2.92 (w)
	2.05 (s)		

[a] Intensities: w = weak, m = medium, and s = strong. [b] *d* spacings determined using film-to-sample distance of 4.96 cm and counting rates of 500 counts/s. The samples were mounted on a zero background substrate.

is not surprising that these materials did not exhibit any crystalline melting behavior.

Morphological Characterization. **1** and **2** were further analyzed by calculating their *d* spacings from X-ray analysis. Both materials were similar in their crystalline structure, showing reflections at 2.05, 2.37, and 3.06 Å. The reflection at 3.06 Å is consistent with that seen in native pullulan. A second run was conducted on **2** in which it was melted in an oven at 180 °C for 1 h and then allowed to cool isothermally to room temperature. X-ray analysis showed the same peaks observed without the thermal treatment along with new reflections that can be seen in Table 4, (2.30, 2.60, and 2.84 Å). Once again, the pullulan reflection at 3.06 Å is evident, along with an additional pullulan reflection at 2.84 Å. The reflections at 2.30 and 2.60 Å are new reflections that are not evident in the Tone PCL-300 samples. These new reflections indicate that the thermal treatment applied to this sample may be causing a change in the crystalline morphology of this sample.

X-ray analysis of **5**, **6**, and **7** revealed a large amorphous halo and little evidence of any crystallographic reflections. This result is consistent with the lack of any observable melting transition observed from thermal analysis.

Conclusion

Chemical modification of pullulan by ring-opening ϵ-caprolactone and [L]-lactide produces modified pullulan derivatives with a wide range of thermal as well as morphological properties. These reactions have successfully been carried out using a $Sn(Oct)_2$ catalyst system. The pullulan derivatives produced by caprolactone modification appear to have a low degree of grafted caprolactone character. The solubility of the grafted polysaccharides is similar to the native material, being soluble in DMSO and water but insoluble in chlorinated solvents. However, the morphological and thermal properties of this material have changed considerably, as was structurally confirmed by ^{13}C NMR, DSC, and X-ray diffraction. These modified derivatives have shown melting behavior, which would indicate that thermal processing techniques such as extrusion, injection, and compression molding may be a viable technique to produce films and tapes for biodegradable applications.

The pullulan derivatives produced by [L]-lactide ring-opening were no longer soluble in water, but showed solubility in both acetone and methanol. After considerable extraction of unreacted monomer and homopolymer, it appears that successful acylation of [L]-lactide onto pullulan has been confirmed by FT-IR and ^{13}C NMR. These materials did not exhibit any melting behavior, which was confirmed by DSC.

As confirmed by ^{13}C NMR, it appears that the grafting of these lactones onto pullulan occurs through a ring-opening process, which is taking place through an acyl−oxygen cleavage, producing an ester linkage to the backbone with a hydroxy terminus.

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