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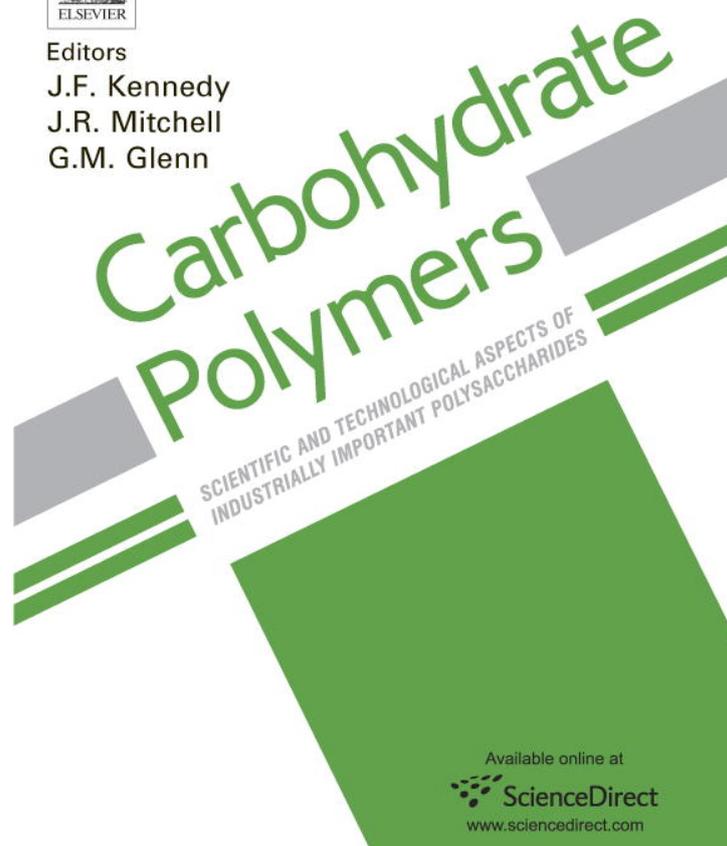
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Thermally processed levan polymers [☆]

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Abstract

Levan polysaccharide used in this study was microbially produced from *Bacillus* sp. Levan was first blended with glycerol and a concurrent change in glass transition temperature (T_g) using differential scanning calorimetry (DSC) was observed. Levan was made into films by pressing the levan/glycerol blends on a heated compression molder. It was found that films with 20–30 weight percent (wt%) glycerol made cohesive, pliable films. However, films with less than 10 wt% glycerol were too brittle to handle and test. Films with greater than 30 wt% glycerol were not cohesive and were more “liquid”-like than “solid”-like. It was found that 20 wt% glycerol was a critical point for effective levan plasticization. Based on the compression-molding data, levan was extruded using optimal glycerol contents and heating regimes. Efficient extrusion of levan/glycerol blends required at least 35% glycerol, indicating extrusion may better incorporate glycerol into the levan structure over room temperature mixing and heated compression-molding. Adding water to the blend, either by replacing glycerol or levan, greatly reduced extrusion viscosity.

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Keywords: Levan; Films; FT-IR, DSC; Mechanical properties

1. Introduction

There has been much interest over the years in utilizing biologically-derived polymers in biomedical and commodity polymers applications. Biologically-derived polymers could be biocompatible and bioresorbable for biomedical applications. For commodity polymers applications, biologically-derived polymers would be sustainable and typically biodegradable and therefore advantageous over petroleum-derived polymers. There is much literature on proteins and polysaccharides derived from plants and animals and polyesters made from fermentation of plant material. The focus here is on the thermal processing of a

polysaccharide, levan, into extruded and molded polymeric products, which have enormous commercial potential. Obtaining thermally processed, i.e., extruded, polysaccharides for food products has some similarities to extruded and molded polymers for commodity applications but the distinction is made here for polymers only. Starch has been extruded and molded successfully into thermoplastic products and the instances are too numerous to list (Finkensadt & Willett, 2004a, 2004b; Guan & Hanna, 2006). Cellulose can be dissolved and converted into “cellophane” but this process has diminished within the U.S. because of environmental concerns over the solvent (Simon, Muller, Koch, & Muller, 1998). Pectin has been combined with glycerol and extruded into films (Fishman, Coffin, Konstance, & Onwulata, 2000; Fishman, Coffin, Onwulata, & Konstance, 2004; Liu, Kerry, & Kerry, 2006). So, two preferable techniques emerge: solvent-casting and thermal-processing. Solvent casting is tedious and the use of volatile organic compounds would defeat the purpose of environmental-friendliness. Thermal processing is simpler and the method currently embraced by the polymer industry.

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If polymers from sustainable resources are to be used commercially, the polymers have to be processed through preferred processing methods.

Levan is a naturally occurring polymer of β -D-fructofuranose with $\beta(2 \rightarrow 6)$ linkages between fructose rings and branching at C-1. The molecular weight of levan, as well as the fraction of residues incorporated in side chains, depends on both the source and the growth conditions, with plant levan and microbially-produced levan having very different characteristics (Arvidson, Rinehart, & Gadal-Maria, 2006; Huber, Stayton, Viney, & Kaplan, 1994; Ingelman & Siegbahn, 1944; Kasapis & Morris, 1994; Kasapis, Morris, Gross, & Rudolph, 1994; Newburn, Lacy, & Christie, 1971; Stivala, Bahary, Long, Ehrlich, & Newburn, 1975). Plant levan typically has a molecular weight about an order of magnitude lower than microbially-produced levan. In addition, levan may have an extensively branched macromolecular structure (Kasapis et al., 1994; Stivala et al., 1975).

There are a variety of potential applications for levan. For example, Huber et al., 1994 suggest that levan may be used to emulsify, stabilize, or sweeten foods as well as to make a material for producing edible food coatings or can be used in medicine as a blood plasma extender and as an encapsulating agent for pharmaceuticals. The invention by Roberts and Garegg (1998) relates to the novel uses of levan derivatives such as levan sulfates, phosphates, and acetates as polymers that can be used in medicine and food processing. In addition, levan acetate and levan crosslinked with epichlorohydrin can be used to form thin plastic films. Combie, Steel, and Sweitzer, 2004 show that levan can be used as an adhesive. Levan has also been blended with ethylcellulose in solution and cast into films (Cavalcanti, Petenuci, Bedin, Pineda, & Hechenleitner, 2004).

In this paper, levan was combined with glycerol and de-ionized water (DI-H₂O) at various proportions. The blends were then pressed or extruded into films at typical polymer processing temperatures. Semi-transparent, cohesive films were easily obtained. The films were tested in uniaxial tension to assess mechanical properties. DSC and FT-IR showed how the molecular structure of the levan changed as a function of blending and processing conditions.

2. Experimental procedures

2.1. Materials

Levan (mol. wt $>2 \times 10^6$ g/mol, $\rho = 1.4$ g/cm³) produced from *Bacillus* sp. was obtained from Montana Polysaccharides (Rock Hill, SC). The levan was in the form of white particles. The particles were ground into a fine powder on a Retsch PM 400 ball mill. The levan particles were loaded into 500 ml stainless steel grinding vessels so that they occupied about a quarter of the volume. The grinding media was four 40 mm stainless steel spheres for a total of 1132 g grinding media. Grinding proceeded at 200 rpm for 30 min. The powder was sieved manually and the predom-

inant fraction remained above the 53 μ m sieve. Reagent grade glycerol (mol. wt = 92.1 g/mol, boiling point = 290 °C, density = 1.26 g/cm³) was obtained from Sigma-Aldrich.

2.2. Film preparation

Glycerol was added to ground levan from 5 to 40 weight percent (wt%). Mixing occurred on a Brabender mixing head. First, the levan was added into the mixing head then the glycerol was slowly added. The total material occupied 70% of the volume of the mixer. Mixing proceeded at 40 °C and 50 rpm for 15 min. The actual blend temperature at the end of mixing varied depending on the glycerol concentration. The measured blend temperature at the end of mixing was 82 °C, 80 °C, 72 °C, 65 °C, 53 °C, 46 °C, and 42 °C for 5, 10, 15, 20, 25, 30, and 40 wt% of glycerol, respectively.

Following mixing, 5 g of sample was sandwiched between Teflon-coated aluminum foil and pressed into films in a Carver Press Autofour/30 Model 4394 at 90 °C and 133,446 N of applied force for 2 min. The sample was removed from the compression-molder and allowed to air cool until it reached room temperature before it was removed from between the Teflon-coated aluminum foil. The film thickness depended on the amount of glycerol. Table 1 lists the dependence of film thickness and therefore applied compressive stress on the glycerol concentration.

2.3. Extrusion

Ground levan, glycerol, and de-ionized water were first manually mixed with a spatula, and then put into a Waring kitchen blender and mixed on high-speed for several seconds to incorporate the liquid into the solid effectively. The material was mixed for 2 min on high-speed using a plastic spatula to vigorously move material out of the stagnant areas of the blender and into the flow. After 2 min, mixing was stopped, the blender capped and shaken by hand for about a minute, and the high-speed blender mix repeated. During each break in the mixing, the mixing blade and blender were felt for heat and the equipment was, at most, warm to the touch. The mixing times were decided upon so that liquid would be incorporated into the ground levan without heating the polysaccharide.

Table 1
Pressing conditions for levan films

Glycerol (wt%)	Film thickness (cm)	Compressive stress (MPa)
5	0.022	11.1
10	0.017	8.7
15	0.015	7.7
20	0.014	7.3
25	0.009	4.6
30	0.007	3.6
40	0.009	4.7

Extrusion was performed on a 3/4" single-screw extruder (SSE) from Brabender powered by a Prep-Center® drive. The length to diameter ratio of the barrel was 25:1 and had a 3:1 compression ratio screw. A slit die was used that was $W = 2.54$ cm wide, $H = 0.05$ cm thick, and $L = 0.90$ cm long, so the aspect ratio of the die was $L/H = 18$. The extruder barrel had three temperature zones and a fourth temperature zone was located on the die, all heated with band heaters. A fifth temperature zone was added at the confluence of the barrel and die and was maintained at the same temperature as the third barrel temperature closest to the die. The most efficient extruder temperature profile found was 110 °C–110 °C–110 °C–110 °C–110 °C going from the hopper to the die. Extruder motor speed was set at 15–65 rpm and melt pressure, P , was measured at the confluence of the barrel to the die or at the die entrance. Apparent shear stress, σ_a , was defined as $\sigma_a = PH/2L$. The volumetric flow rate of material, Q , was calculated from $Q = m/\rho t$ where m/t was the mass of extrudate flowing per unit time and ρ was the density of the flowing melt. The density of the material was determined from the equation $\rho = (w_l/\rho_l + w_g/\rho_g + w_w/\rho_w)^{-1}$ where w was weight fraction, ρ was density, and l , g , and w denote levan, glycerol, and water, respectively. Apparent shear rate, $\dot{\gamma}_a$, was then defined as $\dot{\gamma}_a = 6Q/WH^2$. Apparent viscosity, η_a , was then $\eta_a = \sigma_a/\dot{\gamma}_a$.

2.4. Mechanical testing of films

Test samples were prepared according to ASTM D882 for thin plastic films. The samples were 2.54 cm wide by 10.16 cm long and a 5.08 cm gage length was employed. Mechanical testing of the films was performed at a cross-head speed of 2.54 cm/min using a Com-Ten Industries 95 RC Test System. For extruded films, samples were tested as-extruded and simply cut into sections 10.16 cm long. A minimum of 10 films was tested and results reported as average values of modulus, E , stress at break, σ_b , and strain at break, ϵ_b , with standard deviations given.

2.5. Differential scanning calorimetry (DSC)

The effect of glycerol concentration on glass transition temperature (T_g) was assessed using a TA Instruments 910s Differential Scanning Calorimeter (DSC). Sample sizes of ca. 5 mg were used in a N_2 atmosphere and small pinholes were punched into the lids to allow water to escape. Two heating cycles were employed. This was because a large water peak was observed from about 50 °C up to about 150 °C that masked some of the true thermal properties of the levan and levan/glycerol blends. Therefore, a first heating cycle from 0 °C to 160 °C was employed at a heating rate of 10 °C/min according to ASTM D3417. This evaporated the water. The sample was then cooled to 0 °C and a second heating cycle employed from 0 °C to 400 °C to assess true thermal properties. The assignment of peaks

and integration of peak areas was performed according to ASTM D3418.

2.6. Fourier transform-infrared (FT-IR) spectroscopy

FT-IR analysis was performed with a Thermo Nicolet Avatar 370 in attenuated total reflectance (ATR) mode with a flat 45° ZnSe crystal. Pressure was applied to each film to ensure good polymer/crystal contact. Spectra were obtained at 4 cm^{-1} resolution over 64 scans. Blanks were run between experiments and backgrounds run before each experiment to make it possible to quantitatively interpret the results once the spectrum was converted from transmission to absorbance data.

3. Results and discussion

3.1. Glass transition temperature

The results of the DSC analysis are shown in Fig. 1. Glass transition temperatures were discernible for all of the blends and pure levan except for 40 wt% glycerol, which seemed to have a T_g below 0 °C and was outside the scope of the instrument. The data were fit to a Couchman–Karasz function of the form

$$\ln\left(\frac{T_g}{T_{g,gly}}\right) = \frac{w_l \ln\left(\frac{T_{g,l}}{T_{g,gly}}\right)}{w_{gly}\left(\frac{T_{g,l}}{T_{g,gly}}\right) + w_l} \quad (1)$$

where $T_{g,gly} = 180$ K was the T_g of glycerol, $T_{g,l} = 414$ K was the T_g of levan, w_{gly} was the weight fraction of glycerol, and w_l was the weight fraction of levan (Fried, 1995; Win & Menon, 2006). The Couchman–Karasz equation

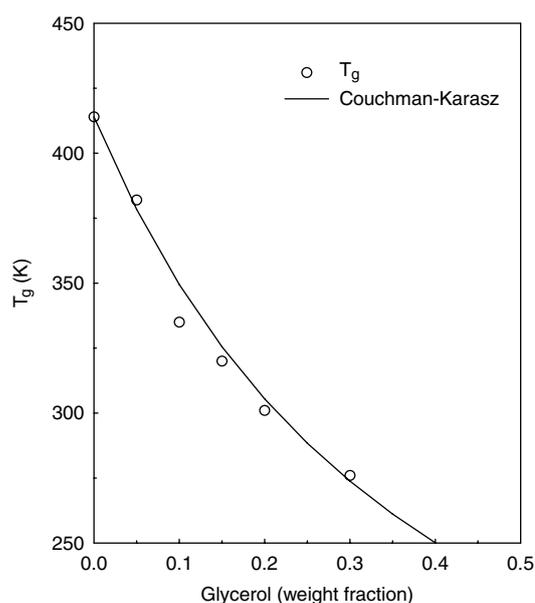


Fig. 1. DSC results for levan-glycerol blends. The line is a fit to the data using the Couchman–Karasz equation, Eq. (1).

gave a much better representation of the experimental data than the Fox equation.

3.2. Mechanical properties of films

Fig. 2 contains the mechanical properties of the levan blend films between 10 and 20 wt% glycerol that were cold-mixed and compression-molded. The 5 wt% glycerol blends, although they could be removed from the Teflon-coated aluminum foil, could not be successfully loaded into the tensile tester grips without cracking. The 10 wt% glycerol blend gave small strain data, from which modulus values could be calculated, but failed prematurely in the tensile tester grip. Only the blends of 15 and 20 wt% glycerol gave films easily capable of being tested in tension. The blends at 25 and 30 wt% glycerol were easy to remove from the Teflon-coated aluminum foil and load into the tensile tester but did not generate enough stress prior to breaking to be read by the force transducer of the instrument, which had a maximum load of 445 N. The 30 wt% glycerol sample was very uniform whereas the 25 wt% glycerol sample had observable opaque spots in the film. The 40 wt% glycerol blend was very “liquid”-like prior to pressing and produced a clear but non-cohesive film after pressing that could not be removed from the Teflon-coated aluminum foil. Pressing stress for the 40 wt% glycerol blend was inferred from what dimensions could be measured of the film remaining on the Teflon-coated aluminum foil and probably accounts for the break in the trend observed in film thickness and therefore pressing stress in Table 1.

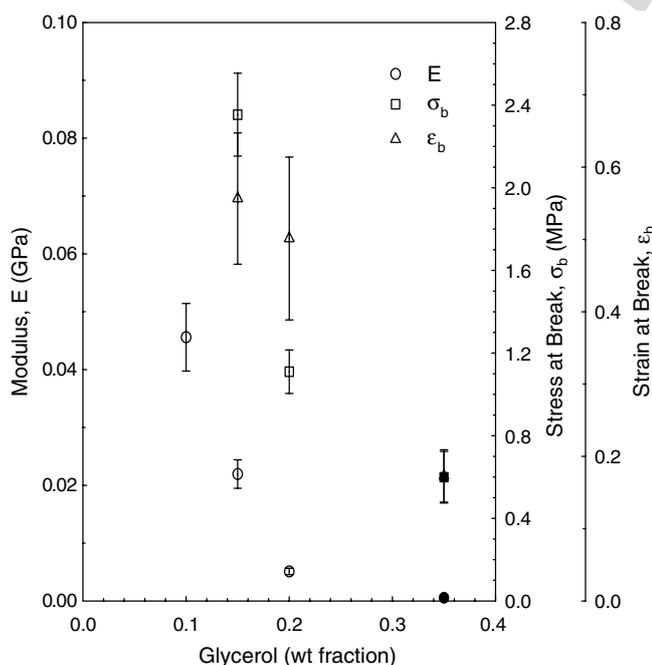


Fig. 2. Effect of glycerol concentration on compression-molded film properties. Filled symbols are the results of the 65:35 levan:glycerol blend that was extruded at 110 °C.

3.3. Extrusion results

Extrusion of levan with glycerol was more problematic than compression-molding. Using the compression-molding results as a basis, 85:15 levan:glycerol blends were prepared and extruded at an extruder profile of 90 °C at all sections. This blend failed to form a polymer in the barrel or die and very little flow occurred through the die at low extruder speed before the pressure reached the maximum of the transducer. The same held true for 75:25 levan:glycerol at 100 °C. However, a 65:35 levan:glycerol blend at an extruder temperature profile of 110 °C produced a free flowing extrudate over a wide extruder speed range. The extrudate is shown in Fig. 3 and it was opaque and not discolored. Higher extrusion temperatures and residence times produced a very dark colored extrudate. Therefore, the components were pre-blended at room temperature in a kitchen blender prior to extrusion to minimize temperature and shear exposure in the extruder and therefore keep degradation of the levan to a minimum. The extrusion rheology data are presented in Fig. 4. At 65:35 levan:glycerol, the viscosity was quite high and the flow showed a dependence of $\log \dot{\gamma}_a \approx (\log \sigma_a)^{3.7}$, which was strongly shear-thinning.

De-ionized water (DI-H₂O) was added to form a three-phase blend. The scaling of apparent shear rate with apparent shear stress was the same for the levan–glycerol–water blends as for the blend without water. The addition of DI-H₂O to the blend had a profound effect on the flow properties of the levan–glycerol. Replacing about one-third of the glycerol with water reduced the apparent viscosity by 4 times. Replacing about 15% of the levan with water resulted in an apparent viscosity decrease of almost 8 times. For the 55:35:10 levan:glycerol:DI-H₂O blend, the extrudate “split” upon exiting the die at all applied shear stress values. For the 65:25:10 levan:glycerol:DI-H₂O blend, extrudate splitting occurred at $\sigma_a > 0.3$ MPa. The addition of water made the blends much softer and therefore it was not possible to test these extrudates in tension.

Fig. 5 shows the effect of apparent shear stress on physical properties for the 65:35 levan:glycerol extruded blend. This would in principle show any effects from molecular

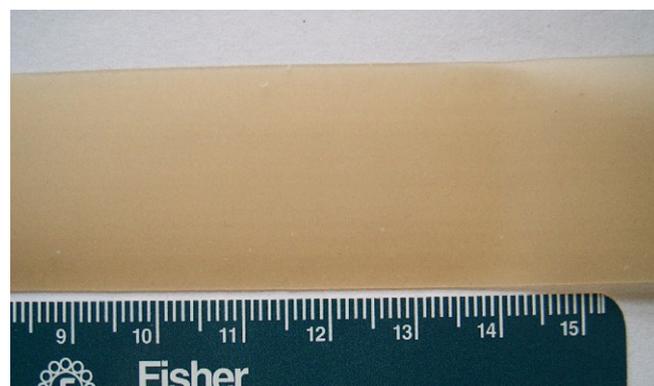


Fig. 3. Picture of an extrudate of the 65:35 levan:glycerol blend extruded at 110 °C.

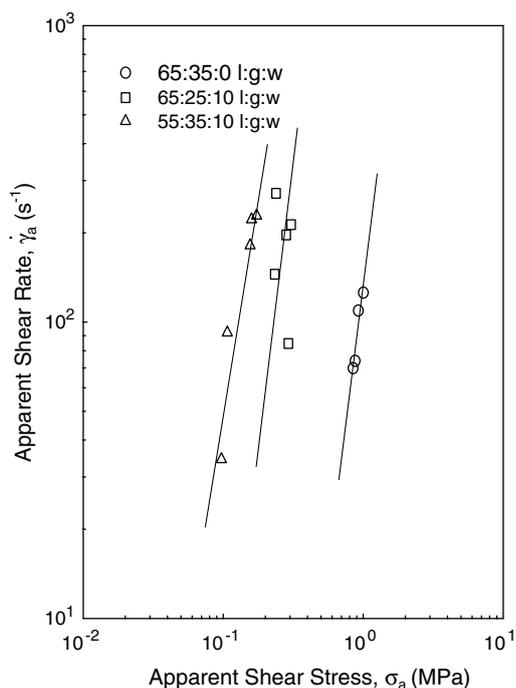


Fig. 4. Rheological data for levan:glycerol:DI-H₂O (l:g:w) blends of (open symbols) 65:35:0, (closed symbols) 65:25:10, and (dotted symbols) 55:35:10.

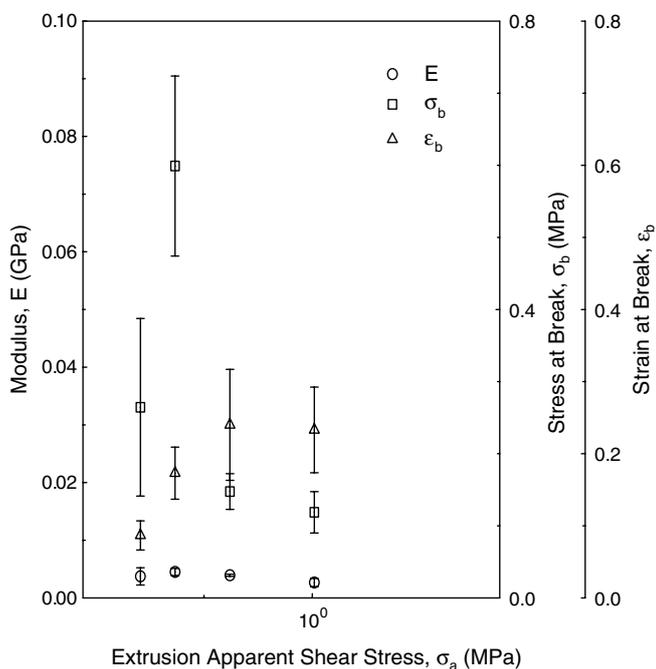


Fig. 5. Effect of applied apparent shear stress during extrusion, σ_a , on extrudate physical properties for the 65:35 levan:glycerol extruded blend.

orientation on film properties, i.e., higher σ_a translated to more molecular orientation along the tensile axis. Modulus showed no change but strain at break, ϵ_b , increased with applied apparent shear stress, possibly showing the effect of more covalent bond strain over molecular re-orientation

during tensile deformation. Stress at break, σ_b , increased considerably then decreased but may be the result of defects incurred during tensile deformation in testing, i.e., defects in the extrudates made it difficult to get reliable stress at break values.

3.4. FT-IR

Glycerol and levan, both being hydroxyl-containing hydrocarbons, have many similarities in FT-IR spectra. C–O–C vibrations in levan could be separated from the glycerol, which did not contain these groups. In addition, glycerol was a linear molecule and levan was a polymer of 5-membered ring fructose, so some information can be discerned between ring and linear hydroxyl-containing molecules. Fig. 6 shows the spectra of glycerol and a blend of levan with 30 wt% glycerol. The y-axis is not a common scale because of the large absorbance of glycerol. Comparison of peak positions allowed for the determination of peaks originating in glycerol and peaks originating in levan. Subtracting the glycerol spectrum from the levan plus glycerol spectrum revealed the major peaks associated with levan, which are summarized in Table 2 (Alpert, Keiser, & Szymanski, 1970; Kacurakova & Mathlouthi, 1996; Nakanishi & Solomon, 1977; Sivakesava & Irudayaraj, 2000; White & Powell, 1995; Wolkers, Oliver, Tablin, & Crowe, 2004). Fig. 7 shows the effect of increasing glycerol concentration on levan. In Fig. 7, the y-axis is a common scale to allow for quantitative interpretation of the data. The region from 600 cm^{-1} to 1800 cm^{-1} is highlighted to provide the most information on levan structural changes. As more glycerol was added, the CH bend on levan, $\delta(\text{CH})$, the CO stretch on inter-ring linkages between fructose monomers, $\nu(\text{CO})_{\text{link}}$, and the intra-ring CO stretch in levan, $\nu(\text{CO})_{\text{ring}}$, increased in intensity. So the levan molecule became more mobile, which was a result of plasticization.

Fig. 8 plots the peak intensity of the OH at 3282 cm^{-1} , HOH scissor of residual water at 1647 cm^{-1} , and COH bend, $\delta(\text{COH})$, at 1032 cm^{-1} as a function of glycerol concentration. The value at 35 wt% glycerol was from an extruded sample while the rest were from compression-molded samples. On a log-log plot, the peak intensity, I , at 3282 cm^{-1} and 1647 cm^{-1} increased steadily over all glycerol concentrations, c , with $\log I \sim (\log c)^{2.6}$. Therefore, these peaks increased because more glycerol was added with a concurrent rise in residual water. The role of hydrogen bonding between levan molecules and glycerol molecules appeared to be important in the properties of the blends given the large increase in the OH peak at 3282 cm^{-1} . The other peaks did not increase steadily over all glycerol concentrations. The curves all displayed discontinuities at $c = 0.2$ weight fraction glycerol with $\log I \sim (\log c)^{0.7}$ at $c \leq 0.2$ and $\log I \sim (\log c)^{3.4}$ at $c \geq 0.2$. Although the peak at 1032 cm^{-1} was present in glycerol and levan–glycerol blends, the discontinuity indicated that perhaps levan was contributing more to this peak than glycerol, which would be reasonable given its higher mass

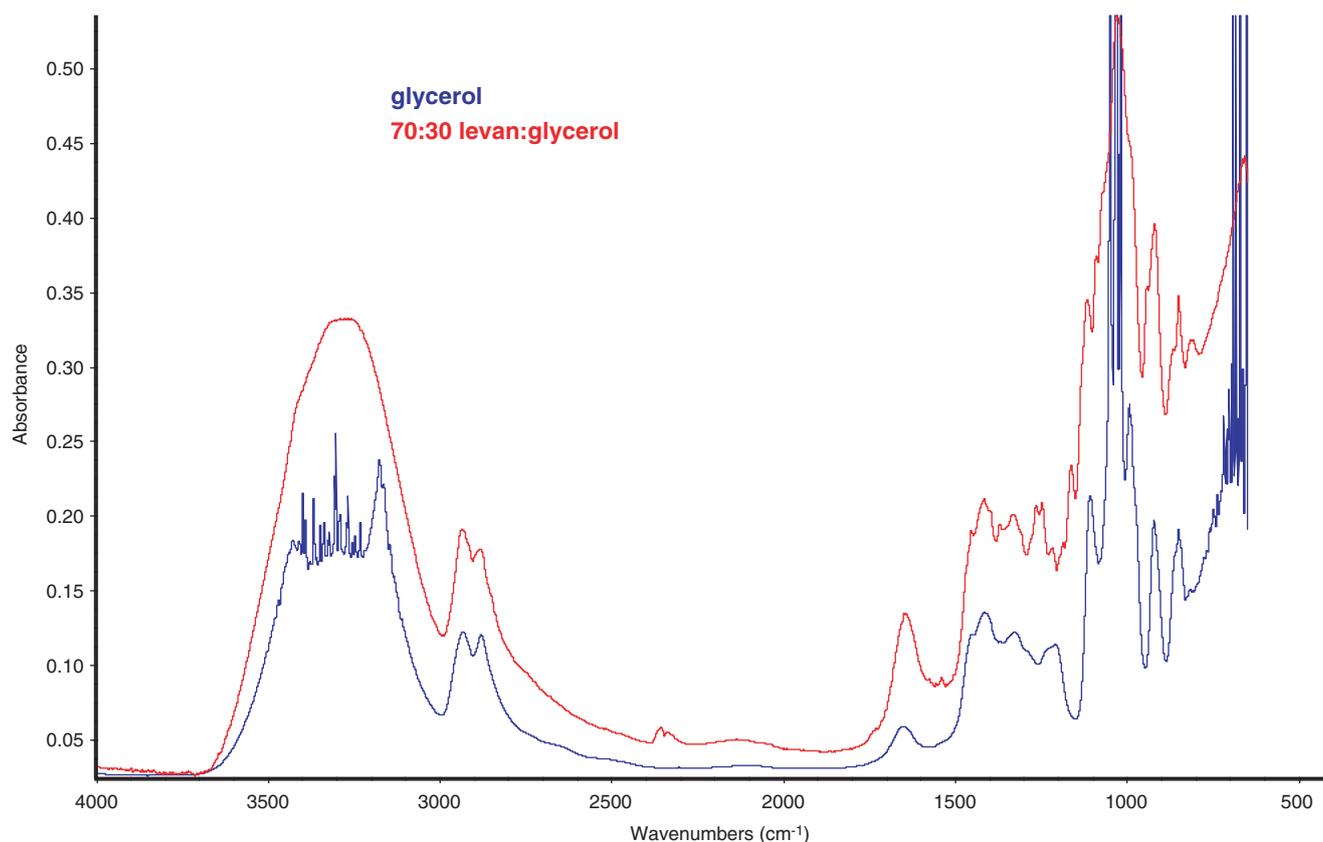


Fig. 6. Comparison of the FT-IR spectra for glycerol and 70:30 levan:glycerol blend cold-mixed and compression-molded at 90 °C. The y-axis is not a common scale because of the high absorbance of glycerol.

Table 2
Peaks originating in levan

Peak position cm^{-1}	Peak assignment
1398	$\delta(\text{CH})$, $\delta(\text{OH})$
1370	$\delta(\text{CH})$, $\delta(\text{OH})$
1265	$\nu(\text{CH})$
1248	$\nu(\text{CH})$
1165	$\nu(\text{CO})$ linkage
1093	$\nu(\text{CO})$ linkage
945	$\nu(\text{CO})$ furanose ring
813	$\nu(\text{CO})$ furanose ring

Vibrations: ν = stretching, δ = bending.

fraction. The critical glycerol concentration also corresponded to the point at which films became visually clear and cohesive and were pliable enough to test in tension. 100 g samples were extruded, therefore at $c = 0.2$ weight fraction glycerol there were 0.22 moles glycerol and 4×10^{-5} moles of levan for a mole ratio of glycerol to levan of 5500. The molecular weight of the fructose monomer was 180 g/mol. Therefore, there were $2 \times 10^6 / 180 = 11111$ monomers. This means that at $c = 0.2$, about half of the monomers had a glycerol molecule associated with them, which appeared to be a critical point for plasticization.

A glycerol was associated with each monomer at 35 wt% glycerol as evidenced by a molar ratio of glycerol to levan of one. For cold-mixed and compression-molded samples, at

$c \geq 0.35$, the blend was too soft to test in tension because of a molar excess of glycerol. The peak at 1220 cm^{-1} originated in CH_2OH on glycerol. Monitoring this peak intensity can serve as an internal standard for the amount of glycerol in the blend. This peak steadily increased in intensity with glycerol concentration, with the value at 35 wt% glycerol falling on the trend line. Therefore, no glycerol was lost during extrusion. The 35 wt% glycerol blend that was extruded was cohesive enough to test in tension whereas the same blends cold-mixed and compression-molded were not. Therefore, extrusion may provide a much more thorough incorporation of glycerol into the levan structure so that there are more glycerol–levan interactions than glycerol–glycerol interactions. The glycerol–glycerol interactions made the blends more “liquid”-like than “solid”-like and were not strong enough to test. The fact that the $c = 0.35$ weight fraction value for the 3280 cm^{-1} peak in Fig. 8 lies slightly above the trend line may indicate that levan–glycerol hydrogen bonding interactions after extrusion are more pronounced than without extrusion, which was how the other points were obtained.

4. Conclusions

The results of this paper show that levan can be successfully thermally processed through traditional molding and

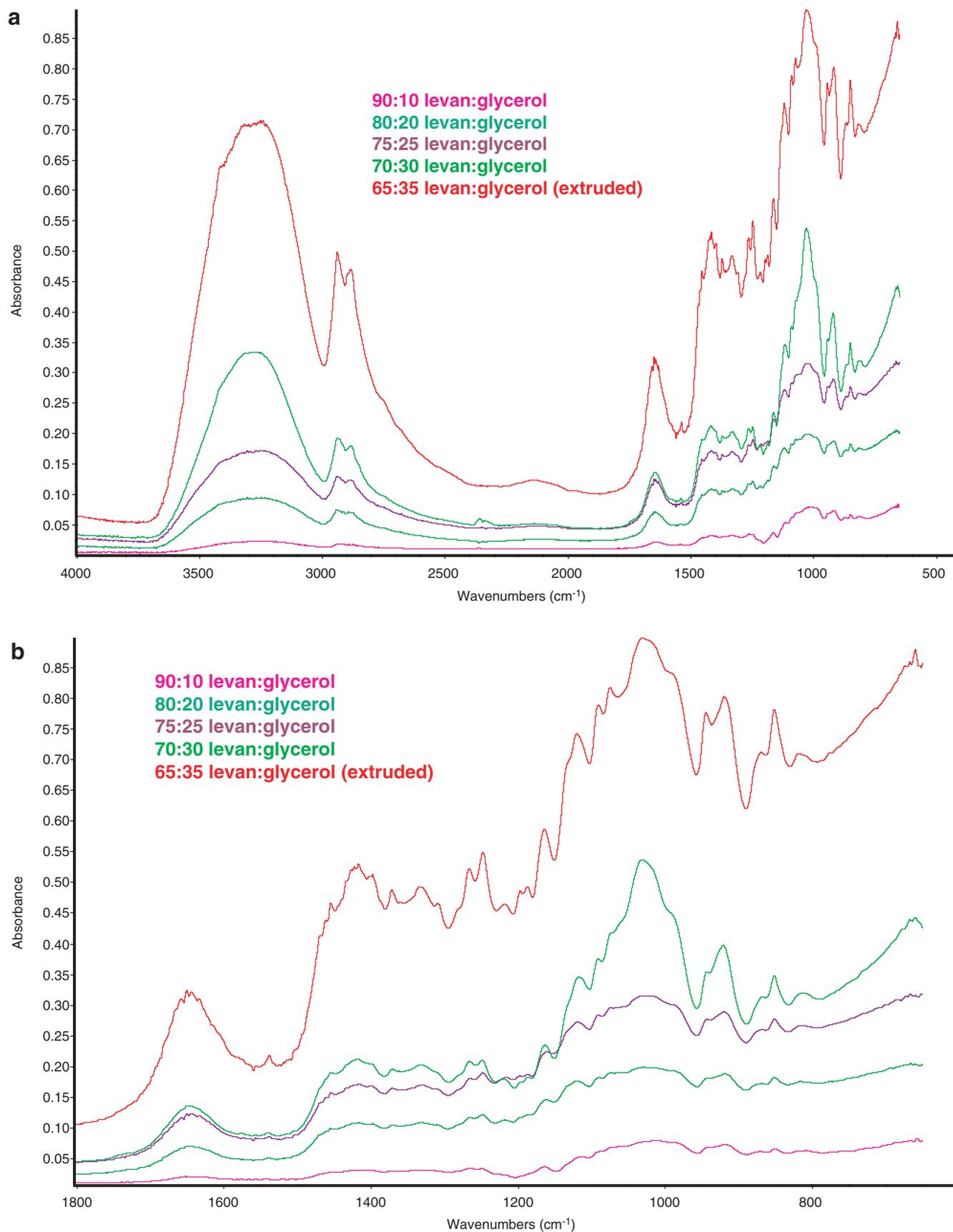


Fig. 7. FT-IR spectra (a) full IR range and (b) 600 cm^{-1} to 1800 cm^{-1} range for levan:glycerol blends. The 65:35 levan:glycerol blend was extruded at 110 $^{\circ}\text{C}$. The y-axis is a common scale.

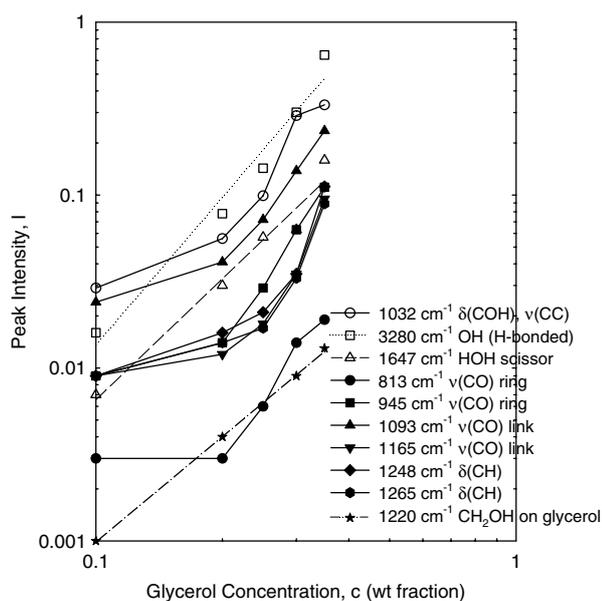


Fig. 8. Plot of FT-IR peak intensity, I , as a function of glycerol concentration (weight fraction), c .

extrusion techniques. Assuming levan could be economically produced through a microbial process, the molecule has potential to be formed into films and molded products for commodity polymer applications. Glycerol was an effective plasticizer in the solid state and glass transition reducer to allow for efficient processing at low temperatures without degradation. A critical concentration of 20 weight percent glycerol was necessary for effective plasticization of levan. Extrusion appeared to more thoroughly incorporate glycerol into the levan structure with increased hydrogen-bonding interactions observed over cold-mixed and compression-molded samples.

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