



Comprehensive characterization of chitosan/PEO/levan ternary blend films

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ABSTRACT

Ternary blend films of chitosan, PEO (300,000) and levan were prepared by solution casting method and their phase behavior, miscibility, thermal and mechanical properties as well as their surface energy and morphology were characterized by different techniques. FT-IR analyses of blend films indicated intermolecular hydrogen bonding between blend components. Thermal and XRD analysis showed that chitosan and levan suppressed the crystallinity of PEO up to nearly 25% of PEO content in the blend, which resulted in more amorphous film structures at higher PEO/(chitosan + levan) ratios. At more than 30% of PEO concentration, contact angle (CA) measurements showed a surface enrichment of PEO whereas at lower PEO concentrations, chitosan and levan were enriched on the surfaces leading to more amorphous and homogenous surfaces. This result was further confirmed by atomic force microscopy (AFM) images. Cell proliferation and viability assay established the high biocompatibility of the blend films.

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1. Introduction

Chitosan is obtained from chitin by removing its acetamide groups in concentrated alkali solution. Due to strong hydrogen bondings chitosan is insoluble in water. Although chitosan has many noticeable advantages as biomaterial its insolubility in water and incompatibility with most of the biodegradable polymers restrict its area of use. This has been partly overcome by extensive efforts devoted to improve its water solubility and compatibility with other polymers (Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Zohuriaan-Mehr, 2005). Chitosan derivatives and blends can now find applications as promising materials in drug and gene delivery (Bostan et al., 2013; Lai & Lin, 2009), wound healing (Muzzarelli, 2009; Muzzarelli, Greco, Busilacchi, Sollazzo, & Gigante, 2012; Sezer et al., 2008), enzyme immobilization and purification (Li, Cai, Zhong, & Du, 2012). Although chitosan has good film forming capacity, the films from pure chitosan have relatively high tensile strength and low flexibility.

PEO is a biocompatible semi-crystalline polyether, which is highly soluble in water via hydrogen bonding. This bio-inert polymer has been the subject of various research groups for surface modification of biomaterials, preparation of biocompatible surfaces (Sugiura, Eda, Sumaru, & Kanamori, 2008), surface and bulk modification of hemodialysis membranes (Amiji, 1995). Despite these favorable properties, when it is blended with variety of rigid biocompatible polymers, the resultant materials can have profoundly improved flexibility and mechanical strength.

Among the natural polysaccharides, microbial levan is a water soluble and high molecular weight extracellular polyfructan, consisting of glycosidic β -(2,6) major and β -(1,2) minor linkages. Levan has been proposed as a promising material in medicine, textile, cosmetic, waste water treatment, food and pharmaceutical industries (Calazans, Lima, De França, & Lopes, 2000; Iyer, Mody, & Jha, 2006; Poli et al., 2009). Despite these favorable properties, levan has a moderately low mechanical resistivity and poor film forming capability as well. Thus, it would be highly insufficient to use it alone as a film material.

Although many articles, concerning binary blend films of levan, chitosan and PEO with various polymers are available in the literature (Caykara, Demirci, Eroglu, & Guven, 2005; Zivanovic, Li, Davidson, & Kit, 2007), to best of our knowledge, there is no report on the preparation and comprehensive characterization of their ternary blend films which are expected to have high potential to

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Table 1
Composition of blend films and their TG, DSC and stress–strain data.^a

Sample code	Chitosan/PEO/Levan (w/w/w)	Heat of fusion (J/g PEO)	T_m (°C)	X_c (%)	Elastic modulus ^b (N/mm ²)	Tensile stress (N/mm ²)	Elongation at break (%)	DTG peak temperature of blend components (°C)		
								Levan	Chitosan	PEO
CPL1	78.3/8.7/13	105.0	55.1	53.3	4115 ± 152	63.1 ± 4.4	1.60 ± 0.10	218	294	444
CPL2	69.6/17.4/13	118.7	61.9	60.3	4568 ± 147	57.4 ± 4.2	1.10 ± 0.05	180	280–307	430
CPL3	60.9/26.1/13	171.0	62.7	86.8	4965 ± 95	31.4 ± 1.4	0.62 ± 0.02	180	283–312	424
CPL4	52.2/34.8/13	173.7	61.4	88.2	6825 ± 287	6.6 ± 0.8	0.34 ± 0.01	180	278–309	422

^a DTG peak temperatures (T_d) of pure polymer films; T_d (Levan) = 240 °C, T_d (Chitosan) = 390 °C, T_d (PEO) = 417 °C. Theoretically obtained heat of fusion for 100% crystalline PEO is 197.0 J g⁻¹ (Jurkin & Pucic, 2012).

^b Sample thicknesses were in the range of 50 ± 5 μm. Each result was average of 6 test results and deviations were determined.

find uses as active food packaging, hemodialysis membrane and artificial skin materials. Moreover, such blends could be augmented with additional superior properties of levan, such as anticoagulation, skin irritation-alleviating effects as a blending component in cosmetics and excellent cell adhesion and proliferation. Considering all these potentials of such a promising blend, this study was focused on preparation of ternary blend films of chitosan, PEO and levan to evaluate their morphological, thermo-mechanical, surface and biological properties.

2. Materials and methods

2.1. Materials

Chitosan was obtained from chitin of shrimp shell in accordance with the method used in the literature (Senol, 2005). The degree of deacetylation was determined as 86% using NMR spectroscopy by comparing the integrations of the selected peaks (Bostan et al., 2013). Levan was produced by microbial fermentation of *Halomonas smyrnensis* AAD6^T batch cultures growing on pretreated sugar beet molasses according to the method used in the literature (Poli et al., 2009). Poly(ethylene oxide), PEO (300,000) was supplied from Sigma–Aldrich and used as received.

2.2. Preparation of the blend films

Four different blend film compositions were prepared from the stock solutions of chitosan, PEO and levan, i.e. each in 1% acetic acid in water (v v⁻¹). They were mixed at desired proportions and then stirred for 12 h at room temperature. After degassing, the clear polymer solutions were introduced into 60-mm-diameter polystyrene Petri dishes. Solvent was slowly evaporated at room temperature. Thereafter, the films were kept in a vacuum oven for 2 days at 40 °C to remove traces of water and acetic acid. Thickness of the dry films was found to be in the range of 50 ± 5 μm. The film compositions referred as CPL are given in Table 1. In all compositions, levan contents of the blend films were kept as 13% (w w⁻¹).

2.3. Molar mass determination

Molar mass values of chitosan and levan were determined using online GPC–LS system equipped with Perkin Elmer-series 200 GPC pump, injector, differential refractive index detector (Optilab rEX), multi-angle static light scattering (LS) detector (Wyatt Dawn Heleos) and a column system consisting of a serial connected Shodex KW-804 Column and Ultrahydrogel DNA Column. Analysis method was reported in our previous article (Bostan et al., 2013). The specific refractive index increment (dn/dc) values were determined to be 0.128 ml g⁻¹ and 0.138 ml g⁻¹ for chitosan and levan, respectively. Mn, Mw and HI values were determined as 1.28 × 10⁵ g mol⁻¹, 1.86 × 10⁵ g mol⁻¹ and 1.46, respectively, for chitosan and 5.3 × 10⁶ g mol⁻¹, 7.2 × 10⁶ g mol⁻¹ and 1.35,

respectively, for levan. The molar mass of levan was similar to that reported by Poli et al. (2009).

2.4. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of the blend films were recorded on a Thermo Nicolet 6700 FT-IR spectrophotometer equipped with a Smart Orbit high performance diamond attenuated total reflectance (ATR) accessories. Measurements were conducted between 400 and 4000 cm⁻¹ in transmission mode.

2.5. Thermogravimetric analysis (TGA)

Thermal gravimetric analysis (TGA) of the blend films (8–10 mg) were conducted using Perkin Elmer Thermal Analyzer System equipped with Pyris6 model thermogravimetry, data of which were evaluated using Pyris TA software. TGA measurements were performed at a 10 °C min⁻¹ heating rate under dynamic argon atmosphere (20 ml min⁻¹).

2.6. Differential scanning calorimetry (DSC)

DSC measurements were performed using Perkin Elmer Jade DSC and Pyris software at a heating rate of 10 °C min⁻¹ under dynamic argon atmosphere (20 ml min⁻¹).

2.7. Mechanical measurements

2.7.1. Dynamic mechanical analysis (DMA)

DMA measurements were conducted using Diamond model Dynamic Mechanical Analyzer (DMA) supplied by PerkinElmer–Seiko (Japan). The data were evaluated by Muse software. The standard tension deformation mode with sinusoidal oscillation was applied to the test samples rectangular in shape (40 mm × 10 mm × 0.05 mm). The variation of storage modulus (E') and loss tangent ($\tan \delta$) with temperature was simultaneously recorded in multi-frequency mode (1, 2, 5, 10, 20 Hz) under nitrogen atmosphere. Measurements were carried out in the temperature range –80 °C – +55 °C, with a heating rate of 2 °C min⁻¹.

2.7.2. Stress–strain tests

Measurements were performed using DMA analyzer at 25 °C in stress–strain mode at a test speed of 0.1 mm min⁻¹. The test samples were in dog-bone shape. Ultimate tensile stress and strain values were determined at the rupture moment of the test samples and expressed in MPa and percentage elongation, respectively.

2.8. XRD measurements

Film samples were analyzed by an X-ray diffractometer (RIGAKU® D/MAX2200/PC) to obtain X-ray diffraction patterns. Each film sample (12 mm × 12 mm) was placed on special glass

holder for the analysis. X-ray beam originating from a copper target ($\lambda = 1.54056 \text{ \AA}$) working under 40 kV and 40 mA was used to scan the samples between 5° and 40° . The analyses were performed with a scan speed of 2° min^{-1} and a sampling width of 0.02° . The selected divergent, divergent height, scattering, and receiving slits were 1° , 10 mm, 1° , and 0.3 mm, respectively. The obtained diffraction patterns were analyzed using the MDI-Jade diffraction analysis software.

2.9. Surface characterization

2.9.1. Contact angle (CA) measurements

Static CA measurements were recorded using Krüss GmbH DSA-100 model instrument equipped with a single direct dosing system consisting of a High Performance Frame Grabber Camera T1C. The measurements were controlled by a DS3210 software. Liquid probes, deionized water, formamide, diiodomethane and glycerol were measured. Drops of each probe ($5 \mu\text{L}$) were deposited on a dry and clean film surface at room temperature and CAs were measured. Total surface energy of the films and its components were calculated from CAs using van Oss-Good contact-angle evaluation methodology (Van Oss, Chaudhury, & Good, 1988).

2.9.2. Atomic force microscopy (AFM)

Atomic force microscope (Park systems XE70 SPM Controller LSF-100 HS) was used for recording the surface topography and phase separation morphology of the films at ambient temperature. The probes, applied in non-contact mode, were triangular Si_3N_4 cantilever with integrated tips (Olympus). The normal spring constant of the cantilever was 20 N m^{-1} and the force between tip and sample was 0.87 nN .

2.10. In vitro biocompatibility test

To assess the biocompatibility of the blend films, WST-1 cell proliferation and viability assay (Roche Applied Science, Germany) was performed with the mouse fibroblast cell line L929 (ATCC number CCL-1). Cells were seeded on the sterile blend films at a density of 1.0×10^5 cells per well in 1 ml Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin ($100 \text{ units ml}^{-1}$) and streptomycin ($100 \mu\text{g ml}^{-1}$) and incubated for 24 h in an atmosphere of 5% CO_2 at 37°C . After incubation for 24 h, WST-1 reagent ($100 \mu\text{l}$) was added directly to culture wells. After 2 h of incubation with WST-1 at 37°C , the WST-1 reaction products were transferred from 24-well plate to 96-well plates and absorbance was measured at 450 nm with a GloMax Multi+ Microplate Multimode Reader (Promega, USA). For reference purposes, cells were seeded on the tissue-culture polystyrene plate with culture medium without film (control) under the same seeding conditions. Control cells were considered 100% viable. Statistical analyses were performed by one-way ANOVA followed by the Tukey test for multiple comparisons using the Prism analysis program (Graphpad, V 5.0).

To investigate the cell–film interaction, scanning electron microscopy (SEM) images were taken after L929 cells were allowed to attach and/or proliferate for a certain time, stated above. Surface morphology was examined using SEM (Jeol JSM-5910 LV, Tokyo, Japan) after sputter coating in vacuum with a thin layer of gold.

3. Results and discussion

3.1. FTIR analysis

Depending on the magnitude of the hydrogen bonding, IR absorption frequencies of the neighboring bonds of donor and

acceptor sites shift to lower values. This phenomenon can be considered as an evidence of the energetic interactions and therefore miscibility of blend components.

FTIR spectra of chitosan indicated broad N–H and –OH stretching bands between 3500 and 3100 cm^{-1} (Fig. 1A). Characteristic bands at 1646 , 1588 and 1324 cm^{-1} are due to the C=O vibration of amide I, N–H bending, –NH₂ (amide II) and N–H bending (amide III), respectively. The peaks at 1076 , 1021 and 972 cm^{-1} are assigned to the C–O–C bending vibration of glucose rings and glycosidic linkages.

The major absorption peaks of PEO, appeared at 1098 and 843 cm^{-1} are due to the C–O–C symmetric bending vibration, and sharp absorption bands at 1340 and 1359 cm^{-1} are due to the wagging vibrations of CH_2 groups in crystalline phase (Fig. 1A) (Pucic & Jurkin, 2012).

In FTIR spectra of levan (Fig. 1A), a broad stretching band at 3400 cm^{-1} is due to the –OH stretching of CH_2 –OH groups and fructofuranose ring. Sharp peaks at 950 , 1000 and 1100 cm^{-1} are due to the C–O–C symmetric bending vibration of fructofuranose rings and glycosidic linkages (Poli et al., 2009). Hydrogen bonding between blend components was evidenced as the absorption frequency of –NH₂ groups of pure chitosan shifted to lower wavenumber in the blend indicating an intermolecular interaction of chitosan with PEO and levan (Fig. 1B). Such an interaction was observed at CPL1 and CPL2, and no further shift was observed with an increasing PEO content, which could be due to the sterically restricted mobility of –NH₂ groups of chitosan, preventing further hydrogen bonding. On the other hand, in a previous study a clear evidence for such an interaction could not be found in IR absorption bands of chitosan/PEO blend films (Zivanovic et al., 2007). Hydrogen bonding between chitosan and PEO also caused lower crystallinity in PEO. This phenomenon was observed with the changes in shape and intensity of the CH_2 wagging vibrations bands of crystalline PEO at 1340 and 1359 cm^{-1} . These characteristic bands were partially merged into a single absorption band at 1344 cm^{-1} , belonging to CH_2 wagging vibration of amorphous phase (Fig. 1A and B) (Pucic & Jurkin, 2012). Moreover, amide carbonyl groups of chitosan made strong hydrogen bonding with free CH_2 –OH groups of levan. This was indicated with the shift of C=O bands of chitosan from 1646 cm^{-1} to 1636 cm^{-1} .

3.2. Thermal analysis

Thermal analysis techniques (TGA and DSC) were employed to investigate the effects of hydrogen bonding on the morphology and thermal stability of the blend films.

TGA: As shown in Fig. 2A, weight-loss curves of the pure components indicated significantly different thermal characteristics. While levan and chitosan displayed decomposition curves between 227 and 300°C and 294 and 400°C , respectively, PEO had substantially higher decomposition temperature between 381 and 425°C . Characteristic weight-loss processes of the components were quantitatively observed in their TG curves (Fig. 2A) and clearly appeared as distinct derivative peaks in their corresponding derivative weight-loss (DTG) curves (Fig. 2B). The area under the DTG curves and their peak values are direct quantitative measure of the rate of weight-loss and thus thermal stability of the blend components. Comparison of the DTG peak temperatures allowed us to obtain information on how intermolecular interactions affected the thermal stability. Intermolecular interactions reduced thermal stability of chitosan and levan from 390°C to 294°C and from 240°C to 218°C , respectively, while providing PEO with considerable extra thermal stability (Table 1 and Fig. 2B). It is worth noting that, in CPL1 composition, maximum weight-loss temperature of PEO was observed at 444°C , which is considerably higher than that of pure PEO (Fig. 2B). This might be due to increased number of

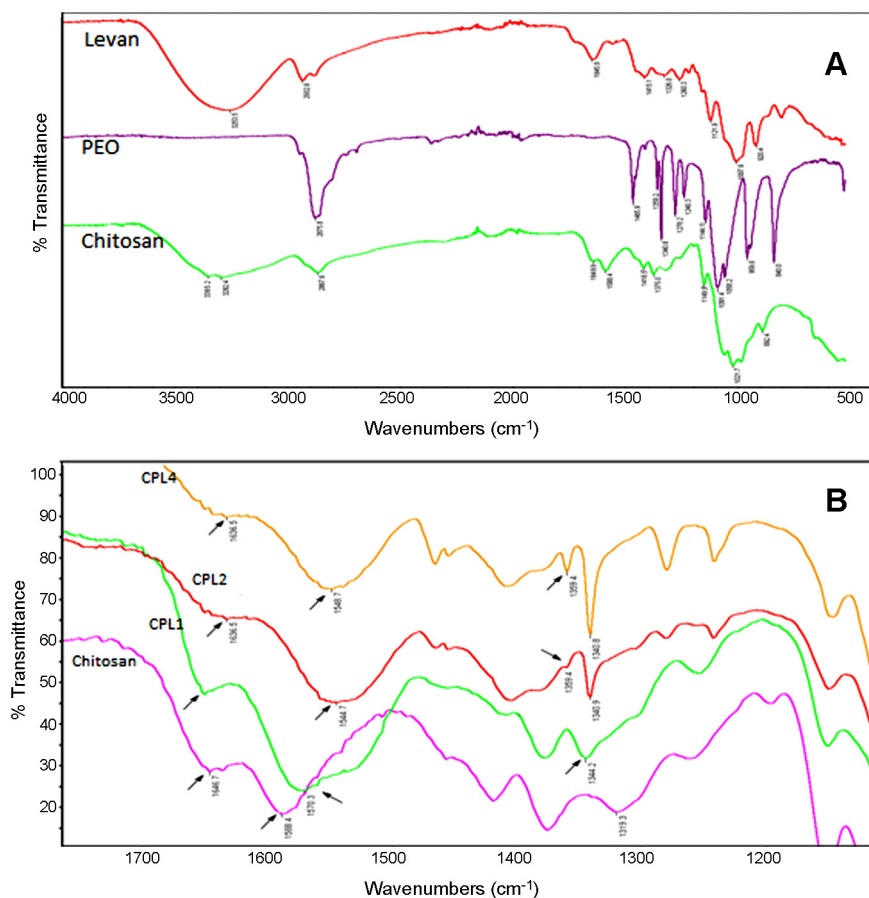


Fig. 1. FTIR spectra of (A) pure polymers and (B) blend films.

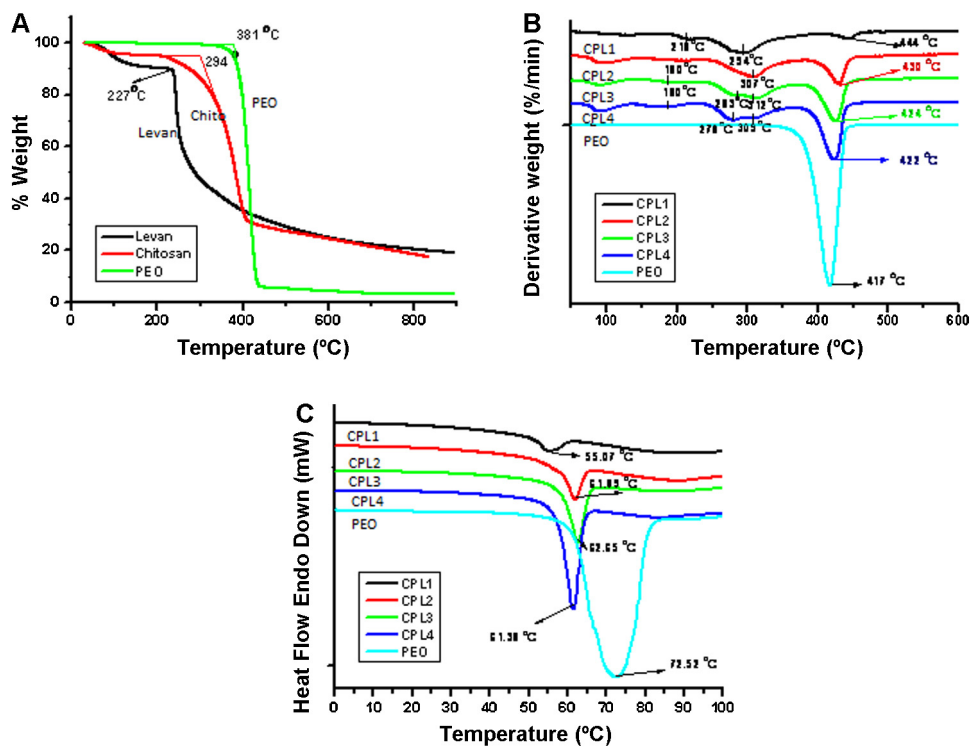


Fig. 2. (A) Weight-loss curves of pure components, (B) DTG curves of PEO and blend films and (C) DSC curves of PEO and blend films.

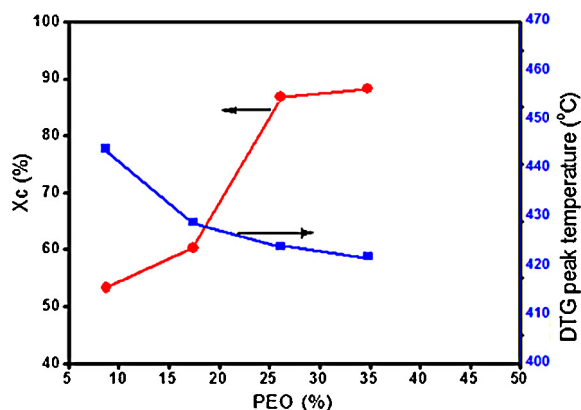


Fig. 3. The change of crystallinity and DTG peak temperature of PEO with composition.

intermolecular interactions for each PEO chain with decreasing PEO content (from CPL4 to CPL1). These results are additional convincing evidence for the intermolecular interactions as confirmed by FTIR results, which significantly reduced the thermal stability of chitosan.

DSC: As levan and chitosan do not display any melting behaviors in DSC operation range, heat of fusion values calculated from the melting peaks were considered for PEO portions. The crystallinity (X_c) of PEO in blend films was calculated from DSC data according to the following equation (Caykara et al., 2005; Jurkin & Pucic, 2012).

$$X_c = \frac{\Delta H}{f_w \Delta H_0}$$

Where, ΔH_0 is heat of fusion for per gram of 100% crystalline PEO. f_w is the weight fraction of PEO and ΔH is heat of fusion of blend sample. DSC melting behavior of the samples are shown in Fig. 2C and the data obtained from DSC and TGA are summarized in Table 1. The change of crystallinity and the DTG peak temperatures of PEO with its contents in blends are plotted in Fig. 3. Remarkable changes were noticed in the crystallinity and DTG peak temperatures of PEO in blends, indicating a decrease in number of hydrogen bonds for each PEO chain with increasing PEO content, which led to an increasing crystallinity in PEO. For the blends containing more than 25% PEO, no remarkable changes in both crystallinity and thermal stability were observed. This result implies that up to 25% of PEO content, morphological structure of PEO was considerably disrupted by chitosan and levan, leading to highly amorphous structure and lower melting temperature.

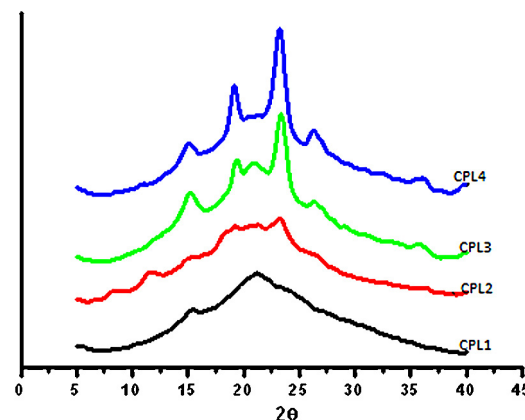


Fig. 5. XRD spectra of the blend films.

3.3. DMA analysis

DMA measurements, conducted at 10 Hz, indicated that, at all temperatures, the E' values of the blend films increased regularly with increasing PEO contents (Fig. 4A). Such a change is more clear with CPL1 and CPL2 compositions, above which only a small increase in E' values was observed. While at the concentration of CPL1 and CPL2, the blend films were more transparent, which is due to the more amorphous structure of PEO, above these concentrations, a phase separation was observed and the blend films became opaque. Loss tangent ($\tan \delta$, damping) curves of the samples were recorded between -80 and $+55$ °C (Fig. 4B). Glass transition temperatures (T_g) were obtained at the temperature values corresponding to maximum $\tan \delta$ values. Due to the high chain relaxation temperatures and rotation energy barriers of polysaccharides, T_g s of chitosan and levan (140 – 150 °C) are well above the melting point of PEO (72 °C) (Dong, Ruan, Wang, Zhao, & Bi, 2004). Thus, loss tangent curves of the blends exhibited only one T_g , belonging to the amorphous portion of PEO. The T_g s of the blend films decreased with increasing PEO contents approaching toward the T_g of PEO, which is about -20 °C (Fig. 4B). The T_g s of CPL1 and CPL3 were observed at about 1.7 and -5.6 °C, respectively. Closer T_g of CPL3 to pure PEO indicated more suppressed relaxation and rotational motion of PEO chains in amorphous phases of CPL1 and CPL2 than CPL3 by intermolecular hydrogen bonding. On the other hand, T_g of CPL4 could not be clearly observed from damping curve. This result is proof of the immiscibility of blend components at CPL4 composition.

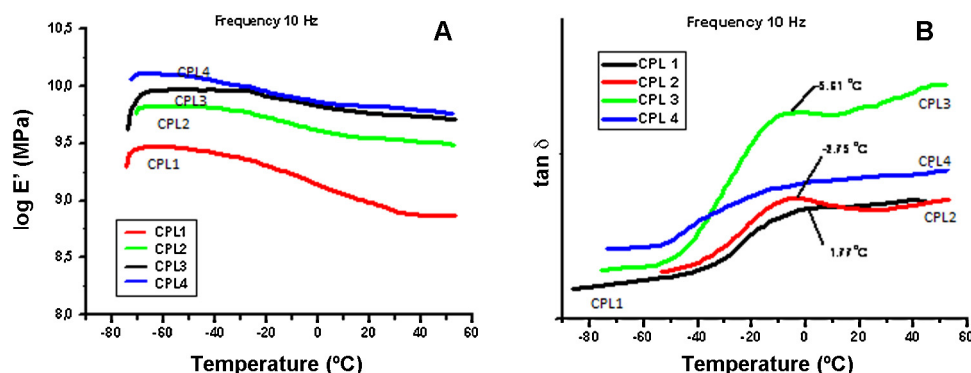


Fig. 4. (A) Storage modulus (E') and (B) loss tangent ($\tan \delta$) curves of the blend films.

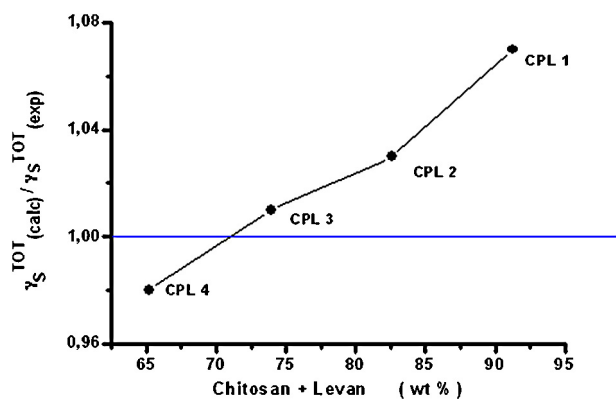


Fig. 6. Change of $\gamma_S^{\text{TOT}}(\text{calc})/\gamma_S^{\text{TOT}}(\text{exp})$ ratio with increasing chitosan + levan wt%.

3.4. Stress–strain analysis

As PEO is a highly hydroscopic and crystalline polymer, it has low capacity of forming films. In most cases, such attempts result in hard and brittle films, thus makes it hard to perform its stress–strain tests. However, PEO blends with chitosan and levan led to an increase in its amorphous portions via intermolecular hydrogen bondings. Then, the films became more flexible and transparent up to the CPL2 composition. Over this percent, the opacity of blend films (CPL3 and CPL4) observed and the films became more brittle, having less tensile stress and elongation values, and a phase separation was observed, which were confirmed by stress–strain measurements (Table 1). It was noticed that while elastic modulus increased, both tensile stress and elongation at break values of the blend films decreased with increasing PEO content.

3.5. X-ray diffraction

X-ray diffraction patterns of the blends were recorded to investigate the morphology of the films and determine the effect of compositions on crystalline tendency of PEO chains in CPL formulations (Fig. 5). Chitosan was reported to exhibit two broad and characteristic diffraction peaks at $2\theta = 11$ and 20° (Kweon, Um, & Park, 2001).

Moreover, the peaks observed at $2\theta = 15$, 19 and 23° were major crystallization diffraction peaks of PEO (Reddy & Chu, 2002). While the diffraction peak of chitosan appeared as a small and broad hump with CPL3 and CPL4 compositions, the characteristic diffraction

peak intensities of PEO increased with its increasing proportion in the blends and become more evident in CPL4. It needs to be pointed out that the characteristic peaks of PEO were not observed in diffraction patterns when PEO content was less than 26.1% (CPL3), which could be considered as a further proof of the suppression of PEO crystallinity via hydrogen bonding.

3.6. Surface characterization

3.6.1. CA measurements

Contact angle technique provides a quantitative evaluation of the surface dynamics and energetics of polymeric solids under their application conditions. Polymeric surfaces have dynamic nature. Thus, depending on the environmental conditions, surface compositions of polymers are considerably different from the bulk, and vary nanometers in-depth.

For the surface characterization, the total surface energy (γ_S^{TOT}), its apolar Lifshitz–van der Waals (γ_S^{LW}) and polar interactions, namely electron-donor (γ_S^-) and electron-acceptor (γ_S^+) components of the film samples were calculated by evaluating the one liquid CA data, applying the following equation:

$$\gamma_L^{\text{TOT}}(1 + \cos\theta) = 2(\gamma_S^{\text{LW}}\gamma_L^{\text{LW}})^{1/2} + 2(\gamma_S^+ \gamma_L^-)^{1/2} + 2(\gamma_L^+ \gamma_S^-)^{1/2} \quad (1)$$

where subscripts *L* and *S* refer to liquid and solid, respectively. θ is the contact angle of liquid drop on solid surface and γ_L^{TOT} is the total surface energy of liquid probe. γ_S^{LW} and γ_L^{LW} are apolar Lifshitz–van der Waals components of solid and liquid, respectively. γ_S^+ and γ_L^+ are the electron acceptor components (Lewis acid) of the surface free energy of solid and liquid, respectively. γ_S^- and γ_L^- are the electron donor components (Lewis base) of surface free energy of solid and liquid, respectively.

For an apolar liquid, $\gamma_L^+ = \gamma_L^- = 0$, which means $\gamma_L = \gamma_L^{\text{LW}}$. Hence, the Eq. (1) can be rewritten as follows:

$$\gamma_S^{\text{LW}} = \frac{\gamma_L(1 + \cos\theta)^2}{4} \quad (2)$$

Consequently, γ_S^{LW} of a solid surface can be calculated using CA value of an apolar liquid probe (diiodomethane). By using the known γ_S^{LW} value in Eq. (1), two unknown parameters of a solid surface, i.e. γ_S^+ and γ_S^- , were calculated for two liquid sets of water–formamide and water–ethylene glycol and the results were averaged. γ_S^{TOT} and its components were expressed as follows:

$$\gamma_S^{\text{TOT}} = \gamma_S^{\text{LW}} + \gamma_S^{\text{AB}} \quad (3)$$

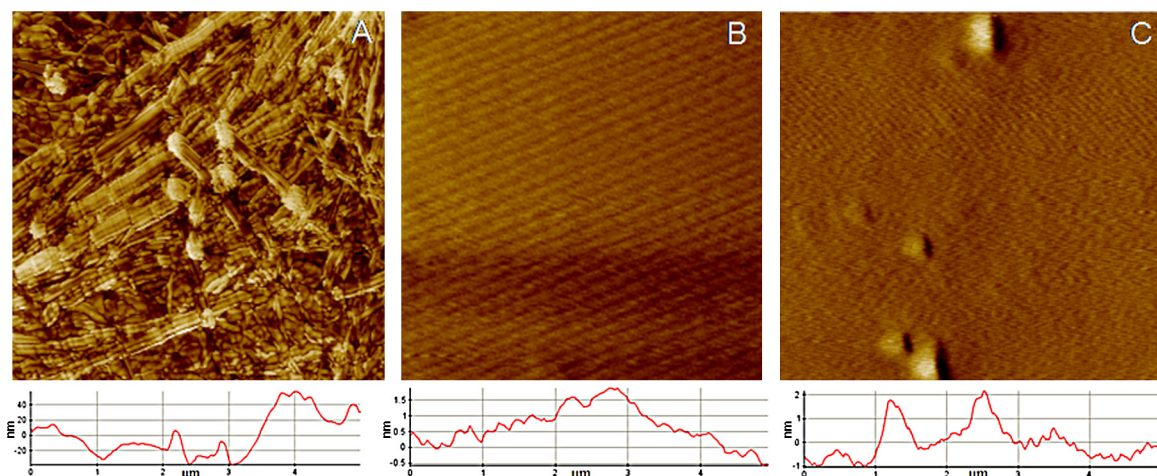


Fig. 7. AFM phase images ($5 \mu\text{m} \times 5 \mu\text{m}$) and surface cross-section analysis of (A) PEO (rms: 22.64 nm), (B) CPL3 (rms: 0.48 nm) and (C) CPL4 (rms: 0.58 nm).

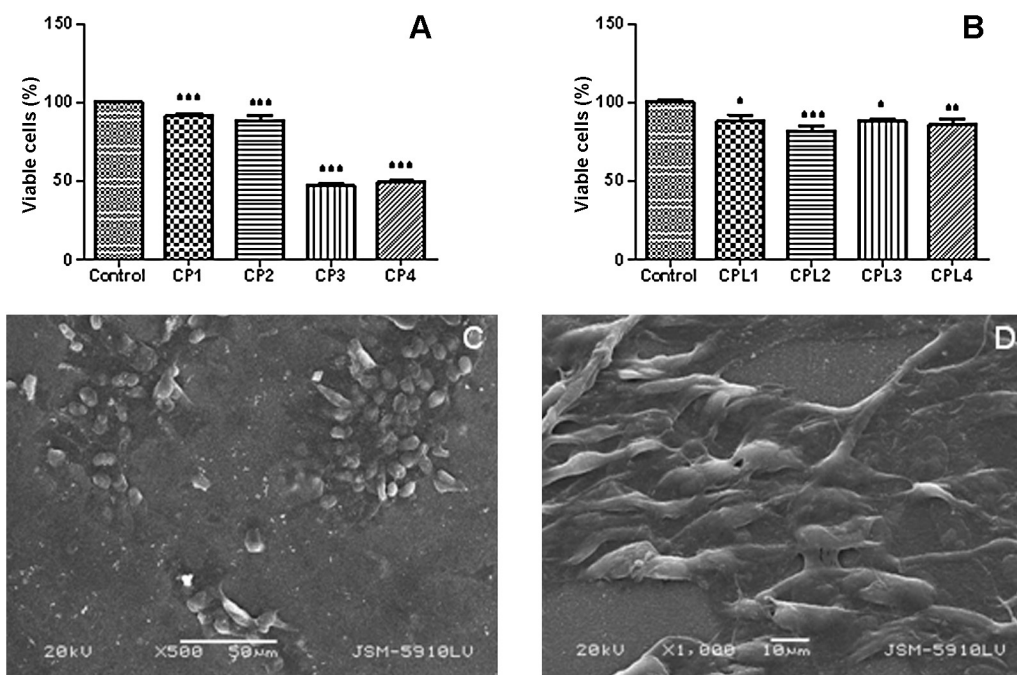


Fig. 8. Cell viability of L929 cells after being cultured with the blend films (CP1, CP2, CP3, CP4)⁺ (A) and (CPL1, CPL2, CPL3, CPL4)⁺ (B) for a period of 24 h. SEM images illustrating morphology of L929 cells after being cultured on CP1 (500 $\times$, scale bar = 50 μ m) (C) and CPL1 (1000 $\times$, scale bar = 10 μ m) (D). ⁺Composition of blend films with Chitosan/PEO ratio of CP1 (90/10), CP2 (80/20), CP3 (70/30), CP4 (60/40) and with Chitosan/PEO/Levan ratio of CPL1 (78.3/8.7/13), CPL2 (69.6/17.4/13), CPL3 (60.9/26.1/13), CPL4 (52.2/34.8/13).

$$\gamma_S^{AB} = 2(\gamma_S^+ + \gamma_S^-)^{1/2} \quad (4)$$

CA and surface energy values of the film samples were collected in supplementary file. γ_S^{TOT} of chitosan and levan were calculated to be 43.00 mJ/m² and 40.69 mJ/m², respectively. For chitosan, γ_S^{TOT} was found to be in close agreement with the literature data (Cunha et al., 2008), and for PEO, the literature data was employed in the calculations. PEO is strong monopolar basic polymer ($\gamma_S^- = 64.00$ mJ m⁻² and $\gamma_S^+ = 0$) and has different surface character than those of chitosan and levan, which are bipolar polymers and have slightly acidic and basic characters. A decrease in γ_S^{TOT} and γ_S^- values with decreasing PEO portions in the blend films was found to be an evidence for the surface enrichment of the lower surface energy components (i.e. chitosan and levan) on the film surfaces (from CPL4 to CPL1) (Erbil, Yasar, Suzer, & Baysal, 1997). Regarding the γ_S^- value of pure PEO, an increase in the surface basicity (γ_S^-) of the blend films was observed with an increasing PEO content. However, this was considerably small as compared to that of γ_S^- value of pure PEO. Surface basicity of the blend films went up from 0.02 mJ m⁻² to 1.14 mJ m⁻², whereas pure PEO had higher basic character ($\gamma_S^- = 64$ mJ m⁻²). This could be explained on the basis of the hydrogen bonding formed between chitosan and levan which led to a considerable attenuation in PEO basic character and therefore PEO depletion on the surface of CPL1 and CPL2. Regarding the dynamic nature of polymer surfaces, in order to estimate the surface configuration of the film samples, experimentally determined γ_S^{TOT} values of the blend films were compared with their calculated values obtained from the following equation. By considering the additivity rule,

$$\gamma_{(Calc)}^{TOT} = w_C \gamma_C^{TOT} + w_P \gamma_P^{TOT} + w_L \gamma_L^{TOT} \quad (5)$$

Where w_C , w_P and w_L are the weight percent of chitosan, PEO and levan in the blend compositions, respectively, and γ_C^{TOT} , γ_P^{TOT} and γ_L^{TOT} are their corresponding total surface energy. Fig. 6 depicts the ratios of the calculated total surface energies to the experimentally determined total surface energies ($\gamma_{S(calc)}^{TOT}/\gamma_{S(exp)}^{TOT}$) as a function

of chitosan + levan weight percent in the blend films. The ratio of $\gamma_{S(calc)}^{TOT}/\gamma_{S(exp)}^{TOT}$ increased with the increase of chitosan + levan contents. Interestingly, this ratio for the CPL4 composition was found to be less than 1.0, which indicated the enrichment of PEO on the surface. This ratio was higher than 1.0 for CPL3, CPL2 and CPL1, which could be due to the enrichment of low surface energy components (chitosan + levan) on the surface.

3.6.2. AFM

The AFM phase images and surface cross-section analyses of pure PEO, CPL3 and CPL4 films are shown in Fig. 7. The pure PEO film had relatively non-uniform surface morphology and lamellar crystal structures, formed within spherulites, having a root mean square (rms) roughness of 22.64 nm (Fig. 7A). On the other hand, the surface morphology of CPL3 and CPL4 blend films were predictably different, such that, while the amorphous CPL3 surface was homogeneous with a rms roughness of 0.48 nm (Fig. 7B), small crystalline islets were observed on the CPL4 film surface with relatively higher rms roughness of 0.58 nm (Fig. 7C). The difference in topography and surface roughness of CPL3 and CPL4 films might be attributed to the PEO crystallization on the CPL4 film surface (Beekmans, van der Meer, & Vancso, 2002). This result is in good agreement with CA observations where PEO enrichment on the CPL4 film surface was reported.

3.7. Biocompatibility

In order to understand the effect of levan on the cell viability, mouse fibroblast L929 cells were directly interacted with the CPL (with levan) and CP (without levan) blend films for 24 h and then subjected to WST-1 assay (Fig. 8). CP1, CP2 blend films showed no cytotoxicity to L929 cells. However, cell viability was decreased (46.74% and 49.56%) with increasing PEO content (CP3 and CP4, respectively), while the viability was significantly higher (87.64% and 85.78%) in CPL3 and CPL4, respectively. The results show that

the blend films containing levan (CPL blends) induced an increase in cell viability as compared with the simple CP blend films.

The morphology of L929 cells after they were cultured on blend films was examined by SEM (Fig. 8C and D). Clearly, blend films support the attachment and proliferation of L929 cells, which demonstrated their normal adherent morphology. These results pointed to the good biocompatibility of levan in the blend films.

4. Conclusions

FT-IR analysis revealed that blend components exhibited significant interactions with each other via hydrogen bonding when PEO blends contain less than 26.1%. It is notable that chitosan and levan suppressed the PEO crystallinity to some extent and therefore enhanced the mechanical and thermal properties of the blend samples. Hydrogen bonding between components hindered the relaxation and thus rotational motions of amorphous PEO chain segments. Thus, T_g s of the blend films increased with decreasing PEO content. CA and AFM analyses showed the PEO enrichment and crystallization on the surface at the weight ratio higher than 26.1% (CPL3 composition). Cell viability and proliferation studies confirmed the biocompatible behavior of the CPL blend films.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.carbpol.2013.09.096>.

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