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Synthesis and characterization of a new type of levan-graft-polystyrene copolymer

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ABSTRACT

Novel macromolecular graft copolymers were synthesized by reaction of the hydroxyl groups of the microbial polysaccharide levan, produced using *Bacillus licheniformis*, with polystyrene (Lev-g-PS). Synthesis was performed by the free radical reaction using potassium persulfate (PPS) as initiator. The prepared copolymer was characterized by FTIR, SEM, TG/DTA, XRD and ^{13}C NMR. The influence of the different conditions (reaction temperature, air or nitrogen atmosphere, reaction time, type of amines and ascorbic acid (AA) concentration) on the grafting reaction was investigated. Results showed that maximum percentage of grafting (58.1%) was achieved at a reaction temperature 70 °C, in a nitrogen atmosphere and using dimethylethanolamine (DMEA) as the amine activator. On the basis of the obtained results, the likely reaction mechanism was proposed. Synthesized copolymers have better thermal stability in comparison with their initial components. Copolymers such as Lev-g-PS could potentially have many applications, such as compatibilizers and material for membranes.

1. Introduction

In recent years, there has been a great interest in modifying synthetic polymers in order to increase their biodegradability. Polystyrene (PS) is one of the most dominant materials for plastic production due to its suitable mechanical properties and affordable production (Heartwin, Pushpadass, Weber, Dumais, & Hanna, 2010; Lambert & Wagner, 2016). However, PS is highly chemically inert and hydrophobic, and therefore, incorporating natural polymers to adjust these properties is a desirable approach to addressing these deficiencies. Modifications of polystyrene with lignin (Hilburg et al., 2014), chitin and chitosan (Francis, Baby, & Gnanou, 2015; Naim, Umar, Sanagi, & Basaruddin, 2013), dextran (Houga, Meins, Borsali, Taton, & Gnanou, 2007), cellulose (Espino-Pérez et al., 2016; Hajduová et al., 2014), and starch.

(Nikolic, Velickovic, & Popovic, 2014a,b; Sheikh, Akhavan, & Ataeivarjovi, 2013) have been reported. These resultant copolymers could be used as compatibilizers, (Sekharan, Abraham, & Thachil, 2012), and as conducting polymers, in separation technologies as material for membranes (Bhattacharya & Misra, 2004), or in the textile industry (Mechram, Patil, Mhaske, & Thorat, 2009).

Levan is a fructose-based polysaccharide polymer which can be excreted by diverse microorganisms as exopolysaccharide or produced in plants for storage. It comprises β-(2 → 6) linked β-D-fructofuranose units with occasional β-(2 → 1) branching and can vary in molecular weight and degree of branching in different organisms (Srikanth, Sundhar Reddy, Siddartha, Ramaiah, & Uppuluri, 2015). The biocompatibility, biodegradability, renewability and eco friendliness of levan make it a promising substrate

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for different applications in biomedicine, nanotechnology, and the food and cosmetic industries (Öner, Hernández, & Combie, 2016; Porras-Domínguez, Ávila-Fernández, Miranda-Molina, Rodríguez-Alegría, & López Munguía, 2015; Srikanth et al., 2015; Stefanović Kojić, Vrvić, Gojgić-Cvijović, Beškoski, & Jakovljević, 2016). Many studies on levan were focused on its prebiotic, antitumor, antioxidant, hypo-cholesterolemic, antidiabetic, anti-AIDS and cell-proliferating activities (Abdel-Fattah, Gamal-Eldeen, Helmy, & Esawy, 2012; Belghith, Dahech, Belghith, & Mejdoub, 2012). Thin plastic films, edible food coatings, encapsulating agents, carriers for flavor and fragrances derived from levan have also been reported (Demir Sezer, Kazak, Toksoy Öner, & Akbuğa, 2011; Esawy et al., 2011). However, no attempt has been made to use levan for a grafting reaction with synthetic polymers (polystyrene).

In the present study, levan-polystyrene graft copolymers were synthesized. Levan was obtained from *Bacillus licheniformis* strain cultivated on medium with ammonium chloride as a more convenient nitrogen source than regular organic sources. This study is the first to report the optimized grafting reaction between levan and polystyrene. The influences of different factors – temperature, atmosphere, reaction time, amines and ascorbic acid concentration – on the grafting reaction were investigated, and the mechanism of the grafting reaction between polystyrene and levan was suggested.

2. Materials and methods

2.1. Materials

Sucrose (edible; food grade, Serbia), potassium dihydrogen phosphate (Kemika, Croatia), magnesium sulfate heptahydrate (Alkaloid, Macedonia), ferrous sulfate heptahydrate, sodium chloride (all MERCK, Germany), ethanol, ammonium chloride, ascorbic acid, dipotassium hydrogen phosphate trihydrate (all Zorka Pharma, Serbia), sodium dodecyl sulfate, styrene, potassium persulphate and amines: propylamine, *iso*-butylamine, pentylamine, hexylamine, diethanolamine, dibutylamine, 4-(2-hydroxyethyl) morpholine, *N,N*-dimethylethanolamine, *N,N*-diethylethanolamine (all Fluka, Switzerland) were used as obtained.

2.2. Levan production

Levan was produced from *Bacillus licheniformis* NS032 strain (Genbank accession number JF826527). *B. licheniformis* was cultivated on medium which was described by Liu et al. (2010) with some previously reported modifications by our group (Kekez et al., 2015): 196.75 g sucrose, 2.44 g ammonium chloride, 3 g $K_2HPO_4 \times 3H_2O$, 3 g KH_2PO_4, 1 g NaCl, 0.2 g $MgSO_4 \times 7H_2O$, 0.001 g $FeSO_4 \times 7H_2O$ in 1 L of deionized water at pH 6.97. Medium (4 L) was prepared in eight 1L Erlenmeyer flasks and each one was inoculated with 3 mL of overnight culture suspension containing 10^6 CFU/mL. Cultivation was performed at 37 °C for 5 days without aeration.

Levan was isolated after biomass separation, ethanol precipitation, dialysis and finally lyophilization, then, its structure was confirmed by elemental analysis and FTIR, as described previously (Kekez et al., 2015).

2.3. Preparation of the Lev-g-PS copolymer

Levan dissolved in distilled water (20 wt%) was added to three-necked round-bottomed flasks equipped with reflux condenser and thermometer. After the polysaccharide solubilized, emulsifier (sodium dodecyl sulfate; SDS) and styrene were added. The levan-styrene w/w ratio was 1:1 in all syntheses. The reaction mixture was stirred for 30 min until it reached the desired temperature (between 35 and 70 °C, in 5 °C intervals) followed by addition of initiator PPS (1.5×10^{-4} mol). The grafting reaction was then continued for another 15–210 min. In some reactions, amines (0.02 mol), ascorbic acid (0.75×10^{-4}–2.25×10^{-4} mol) or nitrogen atmosphere were used (see below).

The mixture obtained after the grafting reaction was transferred into a vessel with methanol and left to stand 18 h to remove unpolymerized styrene monomer, which solubilized in the methanol. Extraction of styrene homopolymer was conducted by shaking with chloroform, which was repeated as long as PS homopolymer was precipitated by methanol. Consequently, levan-polystyrene graft copolymer was obtained. The ratio of polystyrene in Lev-g-PS was determined by hydrolysis in 1 M HCl. Hydrolysis lasted between 1 and 1.5 h depending on grafting percentage (G (%)) at 200 °C. The remaining unhydrolyzed polystyrene was collected by filtration and dried at 50 °C to constant weight (Nikolic et al., 2014a,b).

FTIR spectroscopy was used for confirmation that the product of hydrolysis was polystyrene. The syntheses were carried out in triplicate and the results obtained are shown as mean values.

2.4. Characterization of Lev-g-PS

The percentage of grafting, G (%), in the newly obtained graft copolymers was calculated using following equation:

$$G(\%) = (m_1/m_2) * 100$$

Where m_1 is the weight of unhydrolysed polystyrene and m_2 is the weight of Lev-g-PS.

FTIR spectra of levan, Lev-g-PS and polystyrene were recorded using a Thermo Nicolet 6700 Spectrophotometer and the samples were pressed with KBr in the form of discs.

Scanning Electron Microscopy (SEM) was used to investigate surface morphology of levan and Lev-g-PS. Samples of Lev-g-PS were mounted on copper and metalized with gold–palladium using a Polaron SC 502 sputter coater and recorded by SEM (Jeol JSM 6610 LV) with filament operating at 20 keV. Microstructure analysis of levan was carried out using a SEM (Jeol JSM 6390 LV) and the samples were coated with gold on BALTEC SCD 005 sputter coater.

Thermal properties of samples was analyzed on SDT Q600 (TA Instruments) for simultaneous thermogravimetry/differential thermal analysis (TG/DTA). Samples were analyzed in platinum plates and dynamic nitrogen atmosphere with 100 $cm^3 min^{-1}$ flow. The temperature interval was from 25 to 600 °C and heating rate was 20 °C min^{-1}.

X-ray diffraction (XRD) patterns of levan, polystyrene and Lev-g-PS were obtained on a Philips PW-1710 automated diffractometer using a Cu tube operated at 40 kV and 30 mA. The diffraction data were collected in the 2θ Bragg angle range from 3 to 65°, counting for 1 s Å at every 0.02° step. The XRD measurements were performed at room temperature in a stationary sample holder.

^{13}C NMR spectra were measured on a Bruker AVANCE III 500 spectrometer (125.80 MHz) using 5 mm broad-band probe head. Spectrum was obtained at 298 K in DMF for Lev-g-PS copolymer.

3. Results and discussion

3.1. Characterization of polysaccharide

The polysaccharide obtained from *B. licheniformis* strain was a white powder composed only of C, H and O. FTIR spectrum showed the characteristic pattern for levan type structures which was in accordance with previously reported data (Kekez et al., 2015).