Levan as a new additive for colon-specific films: A new approach in the use of exopolysaccharides in time-dependent free films (Aminoalkyl Methacrylate Copolymer RS)

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Abstract: Time-dependent films, augmented with prebiotics, offer potential strategy for colon-specific controlled drug release. In this study, we produced films containing levan (L) and Aminoalkyl Methacrylate Copolymer RS (ER). Free films of ER combined with levan were produced by the casting process and characterized by the mobility of the polymeric matrix, hydration, differential scanning calorimetry (DSC) and thermogravimetry (TGA). The results of this study suggest that the exopolysaccharide levan can be used in combination with ER for colon specific materials. No evidence of incompatibilities between the levan and the synthetic polymer were detected, and levan improved the mobility of the polymeric matrix and the hydrophilicity of the system. Levan may have positively altered the density of the polymeric matrix, as visualized by thermal characterization. The endothermic decomposition peak was shifted with increasing amounts of levan. This new barrier polymer utilized a combination of time-dependent enzymatic mechanisms and can be considered promising for use in the coating of solid oral drugs for specific release.

Keywords: Swelling index, WVT, hydration, DSC, TGA.

INTRODUCTION

The development of drugs that target specific regions of the body and the release of drugs exactly where they should act to avoid systemic distribution, coupled with the extinction of long-held paradigms, is a major challenge for academic and industrial researchers (Akhgari *et al*., 2006).

Although the applicability of exopolysaccharides is well known, there has been little research on their direct insertion in time-dependent free films. Exopolysaccharides may contribute to enzymatic-specific mechanisms because of their vulnerability to colonic microflora. This action will allow the release of specific drugs in the colon (fig. 1).

This combination of strategies can be effective in the treatment of numerous pathologies, primarily those affecting the distal segment of the gastrointestinal tract (GIT) or those in which current drugs are vulnerable to proximal GI tract conditions (Akhgari *et al*., 2006; Tsuda and Miyamoto, 2010).

The pharmaceutical formulations currently available to treat these pathologies involve pH/time-dependent systems or enemas without enzyme-specific mechanisms. These traditional treatments are not site or target specific and often fail, primarily due to changes in physiological conditions and restricted access of the drug to the damaged region (Asghar and Chandran, 2006).

The exopolysaccharide levan is formed predominantly by residues of D-fructose and shows activity to specific enzymes in the colonic ecosystem; it can be used as an additive in time-dependent film coat, such as ER (Jain *et al*., 2007; de Paula *et al*., 2008).

This study added the exopolysaccharide levan, produced by *Bacillus subtilis* (Natto), to the manufacture of ER polymeric free films and evaluated the influence of levan on the mobility of the polymeric matrix, hydration and thermal properties of the films. This is the first time a frutan exopolysaccharide has been applied to time-dependent free films for use in colon pathologies.

MATERIALS AND METHODS

Materials

The materials used were Aminoalkyl Methacrylate Copolymer RS (Eudragit RS30D) and levan, which was obtained from *Bacillus subtilis* isolated from the Japanese fermented food Natto (Departamento de Bioquímica e Biotecnologia da Universidade Estadual de Londrina, Brazil) (Melo *et al*., 2010). All other materials used in the trials were of analytical grade.

Production of levan

The fermentation to obtain levan from *B. subtilis* Natto, were carried out in 2/L Erlenmeyer flasks with a working

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volume of 500/mL for 16 h at 37°C and 150 rpm with the solution (in g/L): sucrose 400.0, yeast extract 2.0; KH_2PO_4 1.0, (NH_4) $2SO_4$ 3.0; $MgSO_4.7H_2O$ 0.06, $MnSO_4$ 0.02 and distilled water (Euzenat *et al.*, 1996). Levan was characterized in prior studies (*in press*).

Preparation of films

The films were obtained as described by Bunhak *et al*. (2007) (fig. 1). Four different formulations were prepared by water-based polymer dispersions, and the concentration of pseudolatex and/or the levan polymer mass was constant at 4% (w/v). The proportions tested, expressed as the ratio of ER to levan, were 100:0, 95:5, 90:10 and 80:20. To obtain the films, the plasticizer agent triethyl citrate was used at a concentration of 20% (Abbaspour *et al*., 2008).

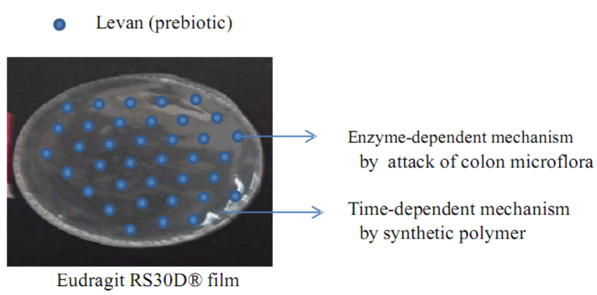

Fig. 1: Casting process of the ER and levan films.

ER dispersions plus the plasticizer agent were stirred for 30 min at room temperature (TA = 25°C ± 2.0). After complete homogenization, levan was added according to the proportions listed above and agitated for 60 min at room temperature. Throughout the homogenization process, a vacuum pump was used to prevent air bubble formation in the polymer blends.

The drying time was 24 h. Macroscopic analysis of the films was used to select samples of the material that lacked cracks, air bubbles or other defects that might interfere with the proposed tests. The thickness of the homogeneous mixture was evaluated using a micrometer. The selected membranes were placed in a desiccators (Cavalcanti *et al*., 2002).

Mobility of the polymeric matrix (water vapor transmission - WVT)

The WVT study was developed in accordance with method "B" of the ASTM (American Society for Testing and Materials), designated E96-66. Initially, 10.0 mL of distilled water was added to each permeability dome (Payne permeability cup model, Belgium). The films, with an area of 9.62 cm^2, were arranged individually. The set (desiccator + film + distilled water) was weighed at baseline and stored in a desiccator containing silica gel. The domes, which were made in triplicate for each film, were weighed at intervals of 0, 24, 48, 72, 96 and 120 h. The difference in the mass of the domes at each time interval was recorded and used in Equation (1) to calculate the water vapor transmission through the free films (Cavalcanti *et al*., 2002).

$$WVT = \frac{g \times 24}{t \times a} \tag{1}$$

Equation 1 – Water vapor transmission (WVT)

Where g is the mass loss (g), t is time (h) and a is area (m^2).

Swelling index determination

The formulation of the different films were carefully cut with surgical scissors (Professional model F/1) at approximately 1.0cm^2 and distributed in properly identified Petri dishes. The Petri dishes were then placed in an oven at 50°C for approximately 24h, where they reached a constant weight. The dishes were removed and stored in a desiccator during the experiment. The samples of dry films were weighed on an analytical balance and immediately immersed, for different time intervals, at room temperature and without the presence of enzymes, in containers of either the fluids of gastric simulation (FGS) or intestinal simulation (FIS), which were prepared according to 23rd edition of the United States of America Pharmacopoeia. After 1, 10, 30, 60, 75, 90, 105 and 120 min of immersion, the samples (in triplicate) were removed from the media with tweezers and carefully dried between two sheets of filter paper; the films were then weighed. To calculate the swelling index (Si%), Equation (2) was used as follows (Blanchon *et al*., 1991):

$$Si\% = \frac{sW - dW}{dW} \times 100 \tag{2}$$

Equation 2 – Swelling index (Si %)

Where sW is the weight of the swollen film at the corresponding time and dW is the weight of the dry film.

Differential scanning calorimetry

Differential scanning calorimetry was performed using 6 mg of each association in a Netzch calorimeter DSC-204 with an atmosphere of nitrogen gas at a flow rate of 10 mL/min. The samples were trapped in closed aluminum pans with pierced lids and subjected to a controlled heating program of 0-600°C at a heating rate of 10°C per min. The difference in the heat flow of the sample was controlled by verifying the loss or gain of energy by the variation of enthalpy from the samples, and endothermic and exothermic peaks were plotted (Cavalcanti *et al*., 2004).

Thermogravimetric analysis

Experiments were conducted to investigate thermal stability by thermogravimetry using a Netzsch TGA-204 apparatus. Approximately 10/mg of each sample was tested in an atmosphere of nitrogen gas at a flow rate of 10 mL/min. The temperature range was 0-600°C at a heating rate of 10°C per min (Cavalcanti *et al*., 2004).

STATISTICAL ANALYSIS

Statistical analyses were used to determine if there were significant differences between the values obtained with the various films. These tests were performed with the GraphPad Prism ® (version 2.01, 1996) software. The results for the coefficients Si% and WVT obtained in FGS and FIS were initially evaluated using an analysis of variance (ANOVA) ($p<0.05$). Tukey's multiple-omparison test was used for significant results ($p<0.05$) (Cavalcanti *et al*., 2002; Cavalcanti *et al*., 2004).

RESULTS

In this study we have studied levan as a new additive for colon-specific films. The compatibilities between levan and synthetic polymer and its contribution to the mobility of the polymeric matrix, hydrophilicity and thermal analyses were obtained using WVT method, swelling index determination, differential scanning calorimetry and thermogravimetric analysis. Our results are shown in table 1 and figs. 2, 3, 4 and 5.

Table 1: Swelling index in FGS and FIS.

Formulation	Swelling index, Si (%)	
	FSG PH 1,2	FIS PH 6,8
ER100%	21,63±7,85	34,13±10,70
ERL95:5	48,87±19,45	43,10±16,40
ERL90:10	60,05±26,70	67,85±14,24
ERL80:20	18,66±10,45	43,72±11,77

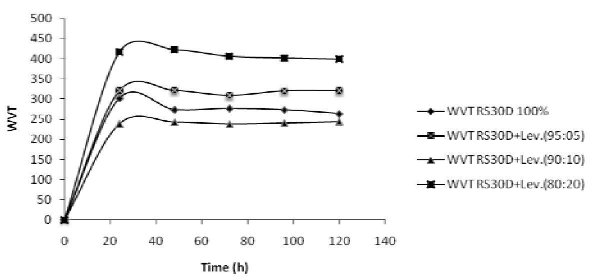

Fig. 2: Water vapor transmission- ER and levan from *B. subtilis* Natto (n=3)

The morphological macroscopic characterization and the thickness determination of the films are essential steps to guarantee the reproducibility of the assay (Cavalcanti *et al*., 2002). The films must be assessed for the presence of cracks, air bubbles, thickness homogeneity and any other imperfections. The films obtained by the casting process in polytetrafluorethylene plates did not show cracks or air bubbles, nor did they show evidence of incompatibilities between levan and the synthetic polymer. In our study, we observed that there was change compared with control formulation when levan was mixed. Levan had a positive influence on the formulation stability (figs. 3, 4 and 5) and did not alter the glass transition (fig. 3). Interestingly, hydrophilicity and mobility of the polymeric matrix were increased since a suitable concentration of levan be dully used (table 1 and fig. 2).

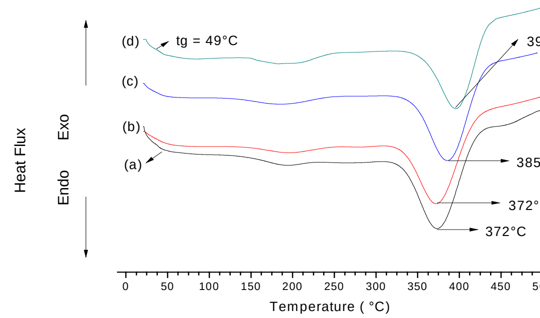

Fig. 3: DSC curves for the different formulations from ER and levan. a) ER 100%, b) ERL 95:5, c) ERL 90:10 and d) ERL 80:20

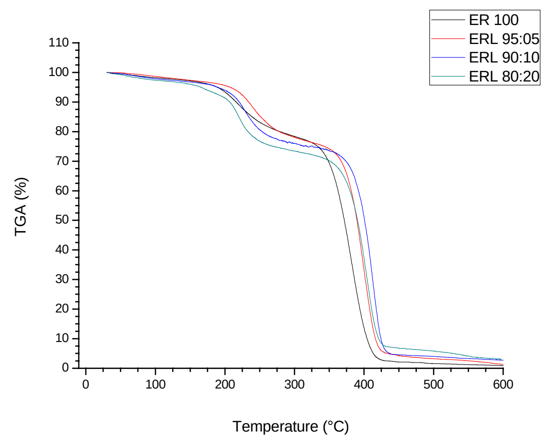

Fig. 4: Thermo gravimetric profiles from free films of ER and levan

DISCUSSION

Mobility of the polymeric matrix - water vapor transmission (WVT)

The WVT assay evaluates the hydrophilic nature of the polymers in the films and provides an index by which it is possible to demonstrate the mobility of the polymeric matrix using water vapor molecules (fig. 2) (Mondal *et al*., 2006).

The results showed that levan altered the WVT of the films ($p<0.05$). The 95:5 and 80:20 formulations increased the WVT. This increase was proportional to the amount of exopolysaccharide in the film ($p<0.001$). The highest value of the WVT was obtained in the 80:20 formulation, which contained the largest proportion of polysaccharide. Levan has a large number of hydroxyl groups (-OH), which may explain the increase in the hydrophilicity of the system and the increase in the WVT

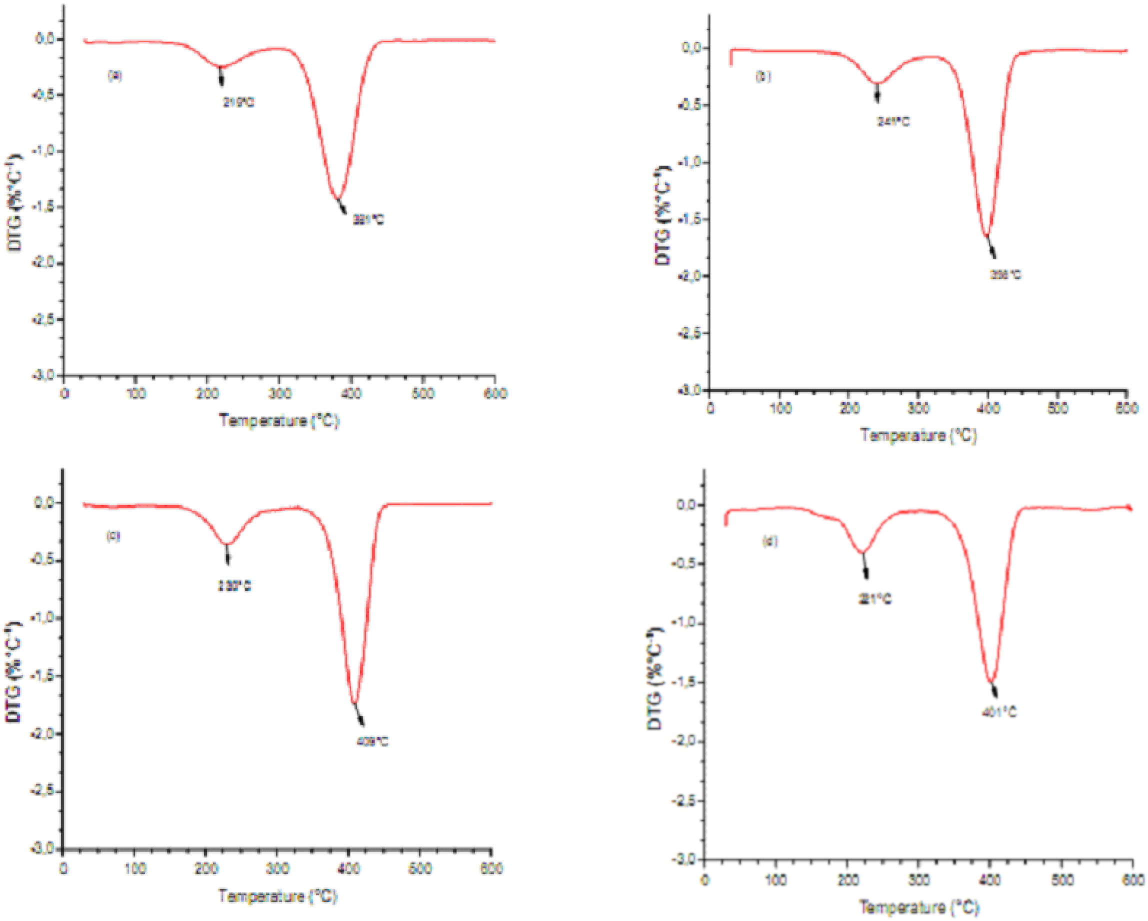

Fig. 5: Derived thermo gravimetric curves from free films of AMCRS and levan: a) ER 100%, b) ERL 95:5, c) ERL 90:10 and d) ERL 80:20.

(Bunhak *et al*., 2007). However, in the 90:10 formulation, the WVT decreased. This decrease may be because the increased density of the polymer restricted the diffusion of water vapor molecules (Mondal *et al*., 2006). The increased polymer density is supported by alterations in the interchain interactions seen in the thermal characterization. A moderate mobility of the polymeric matrix is appropriate for vulnerability to enzymatic attack by colonic microbiota.

Swelling index determination

The swelling index evaluated the hydrophilicity of the polymeric films through the hydration behavior. A moderate hydrophilicity in physiological fluids provides a strong indication of the integrity of the films during passage through the gastrointestinal tract and the vulnerability to enzymatic attack by colonic microbiota (table 1) (Cavalcanti *et al*., 2002).

Levan influenced the swelling index of ERL 95:5 and 90:10 formulations in FGS. The change in the swelling index was relatively proportional to the amount of the exopolysaccharide. However, the ERL 80:20 film did not show a statistically significant difference compared to the ER 100% control in FGS (p=0.074). We suggest that higher concentrations of levan could change the interchain interactions and, consequently, change the polymer mesh density. This would lead to a similar swelling index in the ERL 80:20 formulation as in the control. These density changes can also be seen in the permeability and thermal characterization tests. In FIS, only the ERL 90:10 formulation differed from the control. In ER 100% and ERL 80:20, the swelling index was higher in FIS than in FGS. These data may be explained by the presence of quaternary ammonium groups from ER in the form of chloride salt. The dissociation of these groups is responsible for the hydration of the polymer films. The FGS contains chloride anions that are less selective for ionic exchanges than the phosphate in FIS. Consequently, less hydration occurs in FGS compared in FIS (Akhgari *et al*., 2006).

Thermal analyses (DSC and TGA)

Differential scanning calorimetry enables analysis of the energy profile of the samples at a specific range of temperatures. Studies typically note that a wide range of endothermic events (35°C-160°C) lead to water loss, and endothermic events are assigned to the melting point; endo- and exothermic events can also correspond to the thermal decomposition of the products (figs. 3, 4 and 5) (Cavalcanti *et al.*, 2004).

We noted that the peak of endothermic decomposition shifted with increasing amounts of levan in the ERL 90:10 and 80:20 formulations. These changes in decomposition temperature may be due to changes in the polymer mesh density. Alterations in density occur when interchain interactions from a polymeric system are reorganized (Kanis *et al*., 2005). The glass transition temperature (tg) was 49°C. These results corroborate those obtained by Abbaspour *et al*. (2007) that demonstrated that the glass transition temperature is 50°C. In addition, according to Haines (1995), these variations in tg may be due to differences in equipment and analyses. Levan did not influence the tg; low tg values are ideal for film manufacturing (fig. 3) (Wei *et al*., 2009).

The thermogravimetric analysis evaluates the thermal stability of the compounds, while the derived thermogravimetric (DTG) analysis allows the observation of different stages of degradation. The TGA and DTG curves indicated mass loss between 218.9°C and 241.5°C and 381.1°C and 409.4°C, respectively (figs. 4 and 5). Based on these analyses, we concluded that this new polymeric material possesses high degradation temperatures; levan does not negatively influence the degradation temperature of the film.

CONCLUSIONS

This is the first time that a frutan exopolysaccharide has been used in time-dependent free films for colon pathologies. The results of this study suggest that the exopolysaccharide levan can be used as additive in ER for colon-specific materials. No evidence of incompatibilities between the levan and the synthetic polymer were detected, and the levan improved the mobility of the polymeric matrix and hydrophilicity of the system. Levan may have positively altered the density of the polymeric matrix, as shown by the thermal characterization. The peak of endothermic decomposition was shifted with increasing amounts of levan.

These results suggest that the free films produced in this study may be used as an oral solids coating for the treatment of colon pathologies after complementary studies.

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