Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9291
ISSN on-line: 1807-8648
Doi: 10.4025/actascihealthsci.v34i2.11729

Levan in the developing of new colon-specific polymer material: evaluation of the permeability, moisture and thermal analyses in free films of Eudragit® FS 30 D

Leandro Freire dos Santos[1*], Edgardo Alfonso Gómez Pineda[2], Fernando César Bazani Cabral de Melo[1], Maria Antonia Pedrine Colabone Celligoi[1] and Osvaldo Albuquerque Cavalcanti[3]

*[1]Centro de Ciências Exatas, Departamento de Bioquímica e Biotecnologia, Universidade Estadual de Londrina, 86051-990, Londrina, Paraná, Brazil. [2]Centro de Ciências Exatas, Departamento de Química, Univesidade Estadual de Maringá, Maringá, Paraná, Brazil. [3]Centro de Ciências da Saúde, Departamento de Farmácia e Farmacologia, Univesidade Estadual de Maringá, Maringá, Paraná, Brazil. *Author for correspondence. E-mail: leandrofreire@onda.com.br*

ABSTRACT. Levan was used as agent in the synthesis of new colon-specific polymeric matrix together with Eudragit® FS 30 D. Eudragit® FS 30 D films incorporated with levan were made by casting process and characterized to: water vapour transmission, sweeling index, differential scanning calorimetry and thermogravimetric. The levan increased the films permeability ($p < 0.001$) however did not influenced in the sweeling index of the formulations ($p > 0.05$). The thermal analyses of the films indicated a glass transition temperature approximate at 47°C and thermal decomposition at 400°C. The results indicated that there is potential for using such site-specificity blend as pharmaceutical coating material.

Keywords: coating material, colon-specific release, physicochemical characterization, thermal analysis.

Levana no desenvolvimento de novo material polimérico cólon-específico: avaliação da permeabilidade, intumescimento e análises térmicas de filmes isolados de Eudragit® FS 30 D

RESUMO. Levana foi utilizada na síntese de novo material polimérico cólon-específico conjuntamente com o Eudragit® FS 30 D. Filmes de Eudragit® FS 30 D aditivados de levana foram feitos pelo método de "casting process" e caracterizados quanto à transmissão de vapor de água, índice de intumescimento, calorimetria diferencial de varredura e termogravimetria. A levana aumentou a permeabilidade dos filmes ($p < 0,001$), entretanto não influenciou no índice de intumescimento das formulações ($p > 0,05$). As análises térmicas dos filmes indicaram uma temperatura de transição vítrea aproximada de 47°C e temperatura de decomposição de 400°C. Os resultados indicaram que há potencial de uso desta nova blenda sítio-específica como material de revestimento farmacêutico.

Palavras-chave: material de revestimento, liberação cólon-específica, caracterização físico-química, análises térmicas.

Introduction

In the last decade many pathologies derived from the pattern of modern life, especially those from industrialized food, have gained interest in the medical and pharmaceutical research. These diseases have greatly affected the distal segment of the gastrointestinal tract, the colon. Among them, with the growing concern of medical authorities, highlights: irritable bowel syndrome, colorectal cancer, Crohn's disease and ulcerative colitis. These disorders impair the colon with chronic inflammations (swelling, fistulae and ulcers) and are often accompanied by abdominal cramps and diarrhea (JAIN et al., 2007).

In this perspective, polymers have been developed for the coating of solid oral dosage forms aiming to release the drug specifically in the place of action, the colon. Among the polymers developed in the last decade, deserves to highlight the pH dependent polymethacrylate Eudragit® FS 30 D. Anionic copolymer composed of methylacrylate, methyl-methacrylate and methacrylic acid have dissolution properties in slightly neutral environment (pH = 6.8). Such physiological conditions of pH, in healthy people, are mainly found in the distal portion of small intestine and proximal portion of large intestine (BECKERT, 2000).

A pH-dependent polymer, such as Eudragit® FS 30 D, has been used for pharmaceutical coating systems for oral administration and constitutes an important strategy for colon-specific release, based on changes in pH over the gastrointestinal tract (BECKERT, 2000; GUPTA et al., 2001).

However, systems based solely on strategies of pH-dependent release can easily fail in pathological

conditions such as ulcerative colitis that can become the colon significantly acidic (NUGENT et al., 2001).

Combining additional mechanisms, besides the pH-dependence, can decrease the chances of failure. To add the enzyme-dependent mechanism can be considered spectacular alternative to the formation of colon-specific films. The use of this mechanism is possible due to the enzymes presents in the human colon. The human colon microbiota, unique and complex, consisting of more than 500 bacterial species, that offers immeasurable possibilits, in a single system, of enzymatic and pH-dependent mechanisms (AKHGARI et al., 2006; QUINTANILHA et al., 2007).

The main representatives of the colonic flora are lactobacillus and bifidobacteria, producing numerous glycosidases (VARDAKOU et al., 2007). The notable growth of anaerobic microorganisms in this region, conducive to production of hydrolytic enzymes, ensures the enzymatic susceptibility to certain substrates.

Among the substrates with high potential for enzyme-specificity are the microbial extracellular polysaccharides (EPS). These EPS are potential alternatives to polysaccharide of plants traditionally used as additives in colon-specific films, due to the high yields and easy bioseparation (OLIVEIRA et al., 2007).

These exopolysaccharides (EPS) are polymers widely produced by different microorganisms and are known for their prebiotic effect and consequent susceptibility to enzymatic degradate by enzymes produced exclusively by colonic microflora. The prebiotic effect is characterized by the growth stimulation and increase of the metabolic activity of colonic microflora (RASTALL; MAITIN, 2002).

The levan, an exopolysaccharide composed predominantly of residues of D-fructose joined by glycosidic bonds β- (2→6), can be used as prebiotic agent in colon-specific films (PAULA et al., 2008). Nevertheless, little has been studied for the potential application of EPS, especially levan, on the development of new polymeric materials for the manufacture of coated oral systems focused on the treatment of colonic diseases.

The controlled release of drugs to the colon, through the convergence of additional mechanisms such as enzymatic specificity, offer the prospect of effective treatment by the increase the therapeutic response of active pharmaceuticals, as well as reducing their adverse effects.

Many substances, especially that are vulnerable to hostile conditions of the gastrointestinal tract, together with those already used in the treatment of colonic diseases, will be largely benefited by the colon-specific delivery systems. Among these substances can be mentioned growth hormone, insulin, endorphins and calcitonin. These substances do not support the inhospitable environment of the proximal gastrointestinal tract, stomach and small intestine (KATSUMA et al., 2006; OLIVEIRA; CAVALCANTI, 2007).

The levan, which shows susceptibility to the enzymes of the colonic flora, is presented as a candidate in the development of new polymer materials joined with Eudragit® FS 30 D, aiming to develop a system equipped with enzymatic and pH dependence.

Material and methods

Eudragit® FS 30 D (anionic copolymer composed by methylacrylate, methylmethacrilate and methacrylic acid) Evonik Industries® (Germany). Levan was obtained from *Bacillus subtilis* isolated from the Japanese food Natto (Department of Biochemistry and Biotechnology, State University of Londrina, Brazil).

Production of levan

The fermentations, to obtain levan from *B. subtilis* NATTO, were carried out in 2 L Erlenmeyer flasks with 500 mL under the following conditions: 16h of culture time, 37°C and 150 rpm in rotary shaker. The medium composition in g L^{-1}: 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.

Preparation of the films

The films were obtained from polymeric dispersions prepared at four different concentrations by varying the concentration of pseudo-látex and/or levan. The total solids content of the dispersions were always 4% (w v^{-1}). The dispersant was the water (BUNHAK et al., 2007). The proportions tested were 100:0 (Eudragit® FS 30 D: Levana), 95:5, 90:10 and 80:20. Furthermore a sliding agent was added; glyceryl monostearate (5%) and Tween 80 (2%) (IBEKWE et al., 2006). Dispersions containing initially Eudragit® FS 30 D plus the sliding agent were agitated during 30 minutes at room temperature (RT = 25°C ± 2.0). After complete homogenization, following the proportions listed above, adding the amounts of levan (5, 10 and 20% respectively), the dispersion was agitated for another 60 min. at room temperature. Was used vacuum pump during the entire homogenization process in order to avoid incorporation of air and the formation of undesired bubbles in the polymer mixtures.

After complete homogenization, 10.0 mL of volume of the various associantions were sampled and poured onto a Teflon® plate, previously marked and fixed in a nylon plate with an area of 28.27 cm^2. The plate was properly taken to heating in an air oven at 50°C for 24h; time conditions and minimum temperature for the formation of films adopted in this investigation.

After 24h of incubation the films were carefully removed from the substrate. Subsequently, the macroscopic analyses were realized to select samples with no cracks, bubbles or any imperfections that might interfere in the proposed tests.

Concomitantly, the thickness was determined (five samples for each formulation) using a micrometer (Mitutoyo®) with a sensitivity of 0.01 mm (CAVALCANTI et al., 2002).

Evaluation of permeability (Water Vapor Transmission - WVT)

The WVT study was carried out in accordance with the "B" method of the ASTM (American Society for Testing and Materials), guidelines E96-66. Initially, 10.0 mL of distilled water were added within each permeability cups (Payne permeability cup model, Belgium). Afterward, the candidates films for research (9.62 cm^2) were put (each one) into one of the cups, and the films were subsequently attached to the device. The cups with the films were weighed at time zero and stored in desiccator containing silica gel.

The cups were reweighed at 24, 48, 72, 96 and 120h in order to determine the permeated amount of water (mass loss %). The silica gel was replaced by another dried (50°C $24h^{-1}$) in each weighing. The different values of mass of the cups were recorded and fitted to the Equation (1) to calculate the rate of water vapor transmission through free films.

$$WVT = \frac{g \times 24}{t \times a} \quad (1)$$

where:

g represents mass loss, *t* time measured in hours and *a* the area of the film in m^2.

Determination of swelling index

The films of various associations were carefully cut with surgical scissors (Professional Model F/1) at approximately 1.0 cm^2, and then distributed onto Petri dishes properly identified. Subsequently, the Petri dishes were placed in an air oven at 50°C for approximately 24h, reaching constant weight.

After this period, the Petri dishes were removed and kept in desiccators during the experiment. The samples from dried films representatives of different compositions were weighed on an analytical balance and immediately immersed in a container containing Simulated Gastric Fluid (SGF) prepared according to 23th ed. Pharmacopoeia of the United States of America, without digestive enzymes, during different time at 37°C.

After predetermined intervals, the samples were removed from the media with tweezers and carefully wiped between two sheets of filter paper, to remove excess of surface water and it was reweighted. Samples were taken at time period of 1, 10, 30, 60, 75, 90, 105 and 120 min. of immersion. To calculate the swelling index (I_{eq}%) it was used the Equation (2) (BLANCHON et al., 1991):

$$I_{eq}\% = \frac{Ms - Md \times 100}{Md} \quad (2)$$

where:

Ws is the mass of the swelling film, at the correspondent time, and *Md* is the mass of the dried film.

Differential Scanning Calorimetry (DSC)

The differential scanning calorimetry (DSC) was performed utilizing a Netzch DSC-204 calorimeter apparatus with 6 mg of different films associations with nitrogen gas flow of 10 mL $min.^{-1}$. The samples were trapped in the aluminum crucible with pierced lid and undergone a program of controlled heating (0-600°C) at a heating rate of 10°C $minute^{-1}$. Before that, a 100°C isotherm for 15 minutes aiming at a total loss of moisture was applied.

The difference in the heat flow of sample and the standard was controlled by verifying the loss or gain of energy by the enthalpy variation outlined in the endothermic and exothermic peaks (CAVALCANTI et al., 2004).

Thermogravimetric analysis (TGA)

The thermogravimetric analyses were performed using an apparatus Netzsch TGA-204 with10 mg of sample (various films association) in a nitrogen gas flow corresponding to 10 mL $min.^{-1}$ and a temperature range of 0-600°C at a heating rate of 10°C $minute^{-1}$ (CAVALCANTI et al., 2004).

Statistical analyses

Statistical analyses were performed to verify the level of significance among the values obtained in all trials involving the films. These evaluations were determined using the software GraphPad Prism® (version 2.01, 1996). The different results regarding

the coefficients l_i% and WVT obtained in SGF were initially evaluated using variance analysis (ANOVA) ($p < 0.05$). To significant parameters, the Tukey's multiple comparison tests were applied, comparing the role of different polymer compositions (CAVALCANTI et al., 2002).

Results and discussion

Macroscopic morphological characterization and determination of the thickness of the films

The macroscopic morphological characterization and the determination of the thickness are critical steps to ensure the reproducibility of the proposed tests in the free films (OLIVEIRA; CAVALCANTI, 2007). Attention should be paid to the presence of cracks, air bubbles and other imperfections. The films obtained by evaporation (casting process) on Teflon® plates from water-based polymer dispersions demonstrated no cracks or air bubbles, and no evidence of incompatibility among the synthetic polymer Eudragit® FS 30 D and levan.

The compatibility of certain polysaccharides and polymethacrylate polymers are widely reported in the scientific literature. Oliveira and Cavalcanti (2007) found no incompatibilities among the α-glyco-oligosaccharide and polymer polymethylacrylate. Cavalcanti et al. (2002) evaluated the permeability and hydration of polymethacrylate films containing oligosaccharide inulin. They found no incompatibility among the polymethacrylate polymers and inulin, chemically very similar to the levan.

This reviewed studies could to suggest that the levan probably will not show incompatibility with the polymer polymethacrylate Eudragit® FS 30 D reinforcing its potential applicability in the formation of free films. The films obtained with the addition of the levan demonstrated homogeneity in the thickness among the formulations tested ($p = 0.114$).

However, the increase of the concentration of the levan decreased the transparency and flexibility of the free films. Bunhak et al. (2007) also showed loss of flexibility and transparency when increased the chondroitin sulfate in their polymethacrylate films although the flexibility and transparency still remained adequate.

Water Vapor Transmission

The water vapor transmission (WVT) evaluates the permeability of the polymeric matrix, as well as provides an index in which it is possible to relate the mobility of the matrix regarding the molecules of water vapor (MONDAL; YONG, 2006).

The results show that the levan alter the permeability of the films. The WVT about the Eudragit® FS 30 D (EF) films and levan (L) are shown in the Figure 1. The levan increased the permeability of the films ($p < 0.001$), although no statistically significant result was observed among the formulations EFL 95:5 and EFL 90:10 ($p = 0.069$), just a slightly increasing trend in the WVT of EFL 90:10. The WVT rate was increased considerably in the formulation EFL 80:20 ($p < 0.001$).

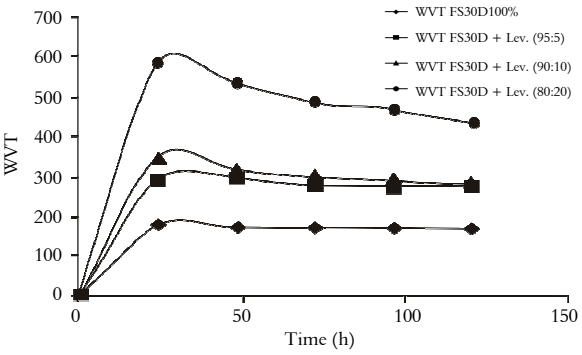

Figure 1. Water vapour transmission of films in various associations - Eudragit® FS 30 D and levan (n = 3) from *B. subtilis* NATTO.

These results are similar to the Bunhak et al. (2007) who analyzed the WVT of polymethacrylate free films containing the chondroitin sulfate polysaccharide. The authors found that the increase of the polysaccharide contents generated a proportional increase in the rate of WVT. This phenomenon can be explained by the relaxation of the polymeric matrix given by levan addition. The WVT indices were proportional to the concentration of levan in the free films. Excessive mobility of the matrix afforded by the formulation 80:20 should be avoid to prevent the premature release of drugs in the proximal portions of the gastrointestinal tract. Moderated mobility afforded by the formulation 95:5 and 90:10 assists the enzymatic attack of levan by colonic bacteria.

Determination of swelling index (I_{eq}%)

The swelling allows evaluating the hydrophilicity of the polymers by the hydration behavior demonstrated. The results show that levan did not affect the swelling index, $p > 0.05$ (date not shown). These results show similarities to those obtained by the Akhgari et al. (2006). These researchers demonstrated that the inulin concentrations 10-20-30% did not influenced the swelling of free films containing Eudragit® FS 30 D, the same synthetic polymer used in this study. The maintenance of

hydration behavior of Eudragit, despite the addition of levan, it is desirable to obtain a material with enzyme-dependent properties and minimal changes in the drug release. The Eudragit® FS 30 D due to its pH-dependent behavior was not immersed in simulated intestinal fluid (BECKERT, 2000).

Thermal Analyses (DSC e TGA)

The differential scanning calorimetry allows the analysis of the energy profile of the samples in a specific range of temperature. Studies have shown endothermic events attributed to the water loss (35 - 160°C), as well as events related to its merger; endothermic and exothermic events may also correspond to the thermal decomposition of products (CAVALCANTI et al., 2004).

The DSC curves for the films based on the Eudragit® FS 30 D are presented in the Figure 2. The results of the DSC curves for EFL indicated one glass transition temperature (Tg ≈ 47°C). This result can express the good compatibility among the materials as well as appropriate miscibility of the polymer blend in all formulations. This glass transition temperature is consistent with the manufacturer's specifications of the synthetic polymer applied (Evonik Industries). The specifications indicated that the Eudragit® FS 30 D has a glass transition temperature approximately to 48°C. There was also no significant differences among the control formulation and other formulations containing the levan (SD = 1.51); the exopolysaccharide not influence the glass transition.

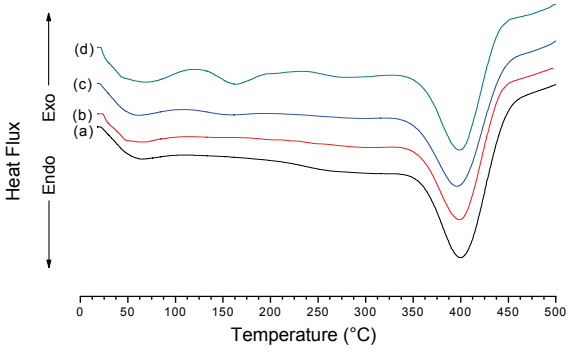

Figure 2. Differential scanning calorimetry in various associations of Eudragit® FS 30 D: Levan a) EF100%, b) EFL 95:5, c) EFL 90:10 and d) EFL 80:20.

However the EFL 80:20 formulations demonstrated an exothermic peak related to a relaxation (td = 120.6°C). This peak can be seen after the glass transition. According to the Sakamoto et al. (2003) the relaxation peak is due to the movement of the polymer chains causing an asymmetry in the electronic charge distribution around the molecules. This movement of the polymer chains is related to the transition temperature (SAKAMOTO et al., 2003).

The endothermic peak of decomposition was not influenced by the levan, on the EFL formulations, and it was around at 400°C. These results are consistent with the studies made by Rabito et al. (2012). In this paper is shown that films EF 100%, as well as in all arabinoxylans formulations used, the peak of decomposition was also observed around at 400°C; the plasticizer and concentration used were the same.

The thermogravimetric profiles of the EFL formulations are shown in the Figure 3.

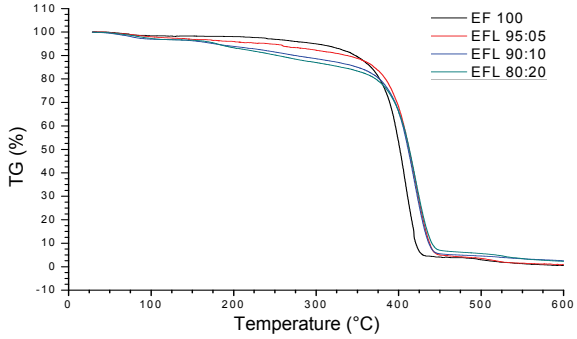

Figure 3. Thermogravimetric curves for free films Eudragit® FS 30 D added with levan from *B. subtilis* NATTO.

The derivative thermogravimetric curves (DTG) are shown in Figure 4. Thermogravimetric analysis assesses the thermal stability of the compounds while the derivative TGA curves facilitate the observation of different stages of degradation (CAVALCANTI et al., 2004). The TGA and DTG curves, for EFL formulations, indicated mass losses among 405.9 and 419.6°C, confirming high temperature degradation. On average, 90.8% of the mass was lost between 300 and 450°C. There were no significant differences in the weight loss among the different formulations containing Eudragit® FS 30 D at temperatures up to 100°C (EF 99.43 ± 0.62; EFL95: 05 99.33 ± 0.68; EFL90: October, 1998.77 ± 1.07; EFL80: 20 99.06 ± 0.88).

The levan increased proportionally the weight loss between 100 and 350°C. Formulations with higher concentrations of levan showed lower mass loss during the test; 97.52 and 97.89% for EFL 90:10 and 80:20 respectively, 99.40 and 99.15% for EF and EFL 95:5.

These results were considered positive in view of the new polymer formed containing levan. This new polymer coating showed degradation in high temperatures; as well as the levan did not affect the thermal stability of the material until 100°C.

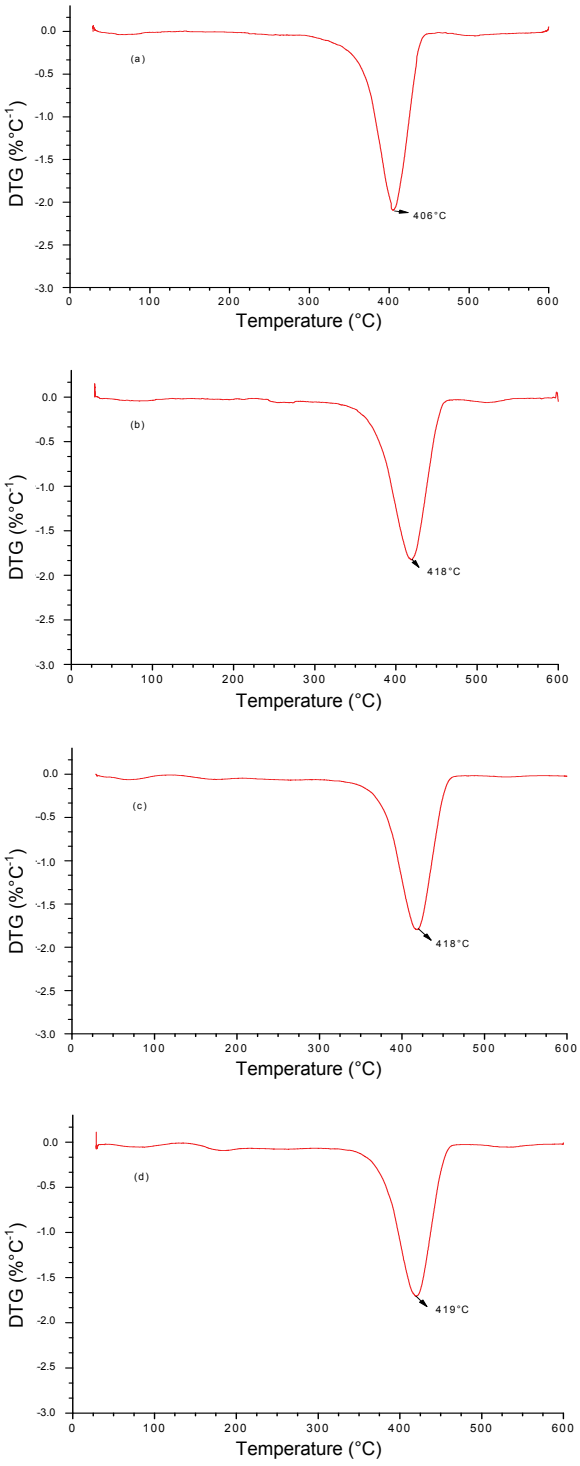

Figure 4. Thermogravimetric curves derived from various combinations of films Eudragit® FS 30 D: Levan: a) EF 100, b) EFL 95:05, c) EFL 90:10 and d) EFL 80:20.

Conclusion

The use of oligo and polysaccharides, mainly those produced by microorganisms, is still a major challenge in the area of pharmaceutical excipients. The results of this study indicated that the exopolysaccharide levan produced by *B. subtilis* NATTO, when incorporated into the Eudragit® FS 30 D on the free films formation, provided a promising initial information regarding the prospect of application of this new material in the development of coating process for oral solid-reservoir systems. Additional studies *in vitro* and *in vivo* are needed to achieve the implementation of this new material as a pharmaceutical excipient with colon-specific skills.

Acknowledgements

The authors thank Almapal (São Paulo State) for Eudragit® FS 30 D (Evonik Industries®) applied in this study, CAPES-Brazil for the financial support and Christian Lachenmeier for review.

References

AKHGARI, A.; FARAHMAND, F.; GAREKANI, H. A.; SADEGHI, F.; VANDAMME, T. F. Permeability and swelbling studies on free films containing inulin in combination with different polymethacrylates aimed for colonic drug delivery. **European Journal of Pharmaceutical Sciences**, v. 28, n. 4, p. 307-314, 2006.

BECKERT, T. Eudragit® Makes it possible. **Pharma Polymers**, v .7, n. 1, p. 1-3, 2000.

BLANCHON, S.; COUARRAZE, G.; RIEG-FALSEN, F.; COHEN, G.; PUISIEUX, F. Permeability of progesterone and synthetic progestin through methacrylic films. **International Journal of Pharmaceutics**, v. 72, n. 1, p. 1-10, 1991.

BUNHAK, E. J.; MENDES, E. S.; PEREIRA, N. C.; CAVALCANTI, O. A. Influência do Sulfato de Condroitina na Formação de Filmes Isolados de Polimetacrilato. Avaliação do índice de intumescimento e permeabilidade ao vapor d'água. **Química nova**, v. 30, n. 2, p. 312-317, 2007.

CAVALCANTI, O. A.; MOOTER, G. V. D.; SOARES, I. C.; KINGET, R. Polysaccharides as excipients for colon-specific coatings. permeability and swelling properties of casted films. **Drug Development and Industrial Pharmacy**, v. 28, n. 2, p. 157-164, 2002.

CAVALCANTI, O. A.; PETENUCI, B.; BEDIN, A. C.; PINEDA, E. A. G.; HECHENLEITNER, A. A. W. Characterisation of ethylcellulose films containing natural polysaccharides by thermal analysis and FTIR spectroscopy. **Acta Farmacéutica Bonaerense**, v. 23, n. 1, p. 53-57, 2004.

GUPTA, V. K.; BECKERT, T. E.; PRICE, J. C. A novel pH- and time-based multi-unit potential colonic drug delivery system. I. **International Journal of Pharmaceutics**, v. 213, n. 1, p. 83-91, 2001.

IBEKWE, V. C.; FADDA, H. M.; PARSONS, G. E.; BASIT, A. W. A comparative in vitro assessment of the drug release performance of pH-responsive polymers for

ileo-colonic delivery. **International Journal of Pharmaceutics**, v. 308, n. 1, p. 52-60, 2006.

JAIN, A.; GUPTA, Y.; JAIN, S. K. Perspectives of biodegradable natural polysaccharides for site-specific drug delivery to the colon. **Journal of Pharmacy and Pharmaceutical Sciences**, v. 10, n. 1, p. 86-128, 2007.

KATSUMA, M.; WATANABE, S.; KAWAI, H.; TAKEMURA, S.; SAKO, K. Effects of absorption promoters on insulin absorption through colon-targeted delivery**. International Journal of Pharmaceutics**, v. 307, n. 2, p. 156-162, 2006.

MONDAL, S.; YONG, J. L. H. Z. Free volume and water vapor permeability of dense segmented polyurethane membrane. **Journal of Membrane Science**, v. 208, n. 1, p.427-432, 2006.

NUGENT, S. G.; KUMAR, D.; RAMPTON, D. S.; EVANS, D. F. Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosalicylates and other drugs. **Gut**, v. 48, n. 4, p. 571-577, 2001.

OLIVEIRA, M. R.; SILVA, R. S. S. F.; BUZATO, J. B.; CELLIGOI, M. A. P. C. Study of levan production by *Zymomonas mobilis* using regional low-cost carbohydrate sources. **Biochemical Engineering Journal**, v. 37, n. 2, p. 177-183, 2007.

OLIVEIRA, F. M.; CAVALCANTI, O. A. Pré-bióticos na formação de filmes isolados de Eudragit® RS 30 D. Avaliação das propriedades de intumescimento e permeabilidade. **Latin American Journal of Pharmacy**, v. 26, n. 3, p. 325-331, 2007.

PAULA, V. C.; PINHEIRO, I. O.; LOPES, C. E.; CALAZANS, G. M. T. Microwave-assisted hydrolysis of *Zymomonas mobilis* levan envisaging oligofructan production. **Bioresource Technology**, v. 99, n. 7, p. 2466-2470, 2008.

QUINTANILHA, A. G.; ZILBERSTEIN, B.; SANTOS, M. A. A.; PAJECKI, D.; MOURA, A. G. H.; ALVES, P. R. A.; MALUF-FILHO, F.; CECCONELLO, I. A novel sampling method for the investigation of gut microbiota. **World Journal of Gastroenterology**, v. 13, n. 29, p.3990-3995, 2007.

RABITO, M. F.; REIS, A. V.; FREITAS, A. R.; TAMBOURGI, E. B.; CAVALCANTI, O. A. A pH/enzyme-responsive polymer film consisting of Eudragit FS® 30 D and arabinoxylane as a potential material formulation for colon-specific drug delivery system. **Pharmaceutical Development and Technology**, v. 17, n. 4, p. 429-436, 2012.

RASTALL, R. A.; MAITIN, V. Prebiotics and synbiotics: towards the next generation. **Current Opinion in Biotechnology**, v. 13, n. 5, p.490-496, 2002.

SAKAMOTO, W. K.; KANDA, D. H. F.; ANDRADE, F. A. Dielectric relaxation of vegetable based polyurethane. **Journal of Materials Science**, v. 38, n. 7, p. 1465-1470, 2003.

VARDAKOU, M.; PALOP, C. N.; GASSON, M.; NARBAD, A.; CHRISTAKOPOULOS, P. *In vitro* three-stage continuous fermentation of wheat arabinoxylan fractions and induction of hydrolase activity by the gut microflora. **International Journal of Biological Macromolecules**, v. 41, n. 5, p. 584-589, 2007.

Received on November 18, 2010.
Accepted on March 21, 2011.