

Extraction, Structural Modification and Characterization of Lotus Roots Polysaccharides (*Nelumbo nucifera* Gaertn). Excipient with Potential Application in Modified Drug Delivery Systems.

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SUMMARY. Due to its biodegradability, lastly polysaccharides have been largely studied for utilization in controlled drug release systems, since they can be specifically degraded by the intestinal bacterial flora. However, as a general rule polysaccharides are very hydrosoluble, causing a fast coating disintegration and precocious drug liberation. Nevertheless, these polysaccharides can be easily modified by chemical processes, like the cross-linking reaction. In this study, lotus (*Nelumbo nucifera*) root polysaccharides were cross-linked with trisodium trimetaphosphate (TSMP) through a phosphatation reaction involving the hydrophilic groups (hydroxyls) of the molecule, thus decreasing its hydrosolubility and, therefore, putting back the drug release until distal portions of the small and large bowel. Cross-linking reaction confirmation was based in Fourier Transformed Infrared Spectroscopy (FT-IR) analyses, where hydroxyls band showed alteration after chemical modification and a characteristic band of the P-O-C linkage emerged. Thermal analysis (Thermogravimetric Analysis, TGA and Differential Scanning Calorimetry, DSC) were also performed.

RESUMEN. "Extracción, Modificación estructural y Caracterización de los Polisacáridos de las Raíces de Lotus (*Nelumbo nucifera* Gaertn). Excipiente con Uso Potencial en Sistemas para Liberación modificada de Fármacos". Debido a su biodegradabilidad, recientemente los polisacáridos han sido bastante estudiados para su utilización en sistemas de liberación controlada de fármacos, ya que ellos pueden ser degradados específicamente por la flora bacteriana intestinal. Sin embargo, como regla general los polisacáridos son muy solubles en agua causando una rápida desintegración del revestimiento y liberación precoz del medicamento. Entretanto, estos polímeros pueden ser fácilmente modificados por el proceso químico de entrecruzamiento (reticulación). En este estudio, polisacáridos de raíz de lotus (*Nelumbo nucifera*) fueron reticulados con trimetafosfato de sodio (TMPS) por una reacción de fosfatación involucrando los grupos hidrofílicos (hidroxilos) de la molécula, disminuyendo de esta forma su solubilidad en agua y por lo tanto atrasando la liberación del medicamento para las porciones distales del intestino grueso y delgado. La reacción de reticulación fue confirmada con base en espectros de FTIR, donde la banda de hidroxilos mostró alteración después de la modificación química y también surgió una banda característica de enlace P-O-C. Los análisis térmicos realizados también son descritos: Análisis termogravimétrico (TGA) y Calorimetria Diferencial de Barrido (DSC).

INTRODUCTION

Delivery systems intended for specified regions of the digestive tract is a most challenging area in the field of development of the controlled drug delivery systems.

Colon-specific drug delivery has earned special attention in recent years due to several factors, like the high incidence of colon diseases, including irritable bowel syndrome, inflammatory bowel disease, Crohn's disease and ulcerative

colitis. Treatment for these pathologies needs drugs delivery directly in the colon whose uses lower drug dosages and intend to be more effective than systemic treatments using higher doses in traditional dosage forms ¹⁻³.

Several natural polymers have been applied like drug targeting, such as those found in the diet. These polymers present potential attractive over synthetic materials for colonic delivery because they are safer, more available and bio-

KEY WORDS: Modified drug release, Polysaccharides, Reticulation.

PALABRAS CLAVE: Liberación controlada de fármacos, Polisacáridos, Reticulación .

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degradable. The polysaccharides have recently been proposed as appropriate excipients for the development of colon-specific devices in oral administration based on their microbial specific biodegradability. A large number of these polysaccharides and oligosaccharides may form the basis for a suitable colonic biodegradable carrier⁴⁻¹².

These polysaccharides present film formation properties, nevertheless because of their high water solubility, they swell and become permeable in the presence of an aqueous environment, causing a premature liberation of the drug. On the other hand, some oligosaccharide has no film-forming properties by itself^{13,14}.

Natural polymers such as chitosan, chondroitin sulfate, dextran, pectin, guar gum and inulin have been chemically modified in an attempt to adjust them to the desired applications^{6,10,15,16}. These modifications can include chemically/physically crosslinking or synthetic polymer incorporation (used to formulate oral controlled release and sustained release delivery systems). The objective in both cases is to reduce the polysaccharide hydrosolubility and, thereby, prevent the premature drug liberation. The films produced from these crosslinked polysaccharides or from these polysaccharide/synthetic polymer blends for coating formulations, exhibited considerably changed properties, which provide possible means for delivering drugs in the colon^{6,10,15,16}. In this way, drug delivery happens into the large intestine due to polysaccharide specific degradation by the resident bacteria at this site. This process is pH independent, and is usually altered due to pathological process^{2,3}.

Galactomannans from different vegetable species have been employed as pharmaceutical excipients, especially for different types of dosage forms with modified release properties, including colon-specificity^{9,11}. Extraction, structural modification and characterization of Lotus roots (which contain appreciable quantities of non-starch polysaccharide arabinogalactan) was proposed in this study, in order to evaluate their application as a potential material destined to specific drug delivery carrier⁸.

MATERIAL AND METHODS

Lotus roots (*Nelumbo nucifera*) - San-Maru Ind. e Com. Ltda. (São Paulo). Eudragit® RS30D (ammonium metacrylate copolymer, type B, USP/NF/ Röhm Pharma, Darmstadt, Germany), triethyl citrate (Morflex, EUA). Another materials used in the assays were of analytic degree.

Polysaccharide Extraction

Methodology used in the polysaccharide extraction was adapted from the method proposed by Ramsden & Bic¹⁷, in which 100 g of dried roots were agitated with 1.4 L of distilled water for 6 hours at 10 °C. Low temperature is important to non-starch polysaccharides selective extraction. After shaking, extraction media was filtrated and centrifuged at 3.800 rpm for 5 minutes and the precipitate was rejected. Absolute ethanol was added to the filtrate in the rate of 4:1 to perform the polysaccharide precipitation. The obtained solid material was recovered by centrifugation and dried in a stove at 80 °C, weighed in analytic balance and stored in a drying keeper provided with silica gel until utilization.

Structural Modification

The chemical modification of the polysaccharide (Fig. 1) was carried out according to the experimental procedure proposed by Gliko-Kabir *et al.*¹⁶, starting from an aqueous dispersion at 1% of polysaccharide, previously prepared in alkaline water (NaOH 2M pH = 12.0) under agitation for 2 h, in order to attain its maximum swelling. To an aliquot of 100 mL of the dispersion formed, 10 mL of a sodium trimetaphosphate solution (STMP) at 30% were added, which was agitated for more than 2 hours, keeping pH value around 12.0. This dispersion was flowed into Petri's plate and dried in stove at 45 °C for 18 h.

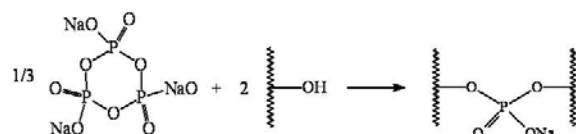


Figure 1. Cross-linking reaction.

Polysaccharide Characterization

Characterization of the polysaccharides, obtained by the employed extraction process, as well as of the product obtained through structural modification in presence of STMP at pH = 12.0 was accomplished by Fourier Transformed Infrared Spectroscopy (FTIR) and by thermal analysis (DSC and TGA).

Isolated Film Preparation

Isolated films were prepared through a method called *casting process*, starting from aqueous polymeric dispersions with only Eudragit® RS30D (control) and a mixture Eudragit® RS30D/polysaccharide in the proportion

of 90:10, being 4% (w/v) of final polymeric mass. The used Eudragit® RS30D was previously treated with triethyl citrate (a plasticizer agent) in the proportion of 20%, based on the polymetacrylate dry weight, shaking under vacuum for 1 h at room temperature.

After polysaccharide addition, the dispersion was stirred for 30 min and aliquots of 10 mL were flowed in Teflon-covered nylon plates and dried in stove at 45 °C for 24 h. Obtained films were carefully removed and analyzed for morphologic characteristics (air bubble presence, homogeneity, cracks and transparency) and stored in drying keeper provided of silica gel until time of utilization¹⁸.

Isolated Films Thickness Determination

The films thickness determination was accomplished with a micrometer (Mitutoyo®, Japan) in five different points chosen randomly in three films.

FTIR Spectroscopy Analysis

Infrared spectroscopy analysis was performed across KBr tablet from powdered samples (STMP, natural and modified polysaccharide) and by the photoacoustic method for films (Eudragit® RS30D 100% and Eudragit® RS30D/Polysaccharide 90:10) in a Bomen® MB-100 Michelson device, operating in a wave number range of 4000 to 400 cm⁻¹. Powdered samples were previously dried in a stove at 100 °C for 15 h and stored in drying keeper provided of silica gel until time of utilization, with the purpose of avoid air relative moisture interference in the analysis of the polysaccharide.

Thermal Analysis

Differential Scanning Calorimetry (DSC)

DSC was performed with samples of about 6 mg in a Shimadzu® DSC-50 calorimeter under nitrogen atmosphere at a flow rate of 10 mL/min. Temperature varied between room temperature (25 °C) to 1000 °C in a rate of 10 °C/min.

Thermogravimetric Analysis (TGA)

TGA was performed with samples of about 6 mg in nitrogen atmosphere flowing at 10 mL/min. Temperature varied between room temperature (25 °C) to 500 °C in a rate of 10 °C/min.

RESULTS AND DISCUSSION

The films prepared were evaluated for their

morphologic characteristics; they showed good homogeneity, translucency and absence of air bubbles or cracks, appropriate to exigency of the assays.

In the thickness study, films of Eudragit® RS30D 100% (standard) a medium thickness of 0.120 mm (± 0.019) was measured, while films formed by Eudragit® RS30D/Polysaccharide association in the proportion of 90:10, presented 0.160 mm (± 0.010), being these slightly thicker.

A number of features are apparent from the TGA and DSC analyses for the polysaccharide (Figs. 2 and 3, respectively). The thermal decomposition starts at 115 °C and about 14% of weight loss occurs in a first step, up to near 220 °C. Between 220 and 300 °C further 25% of mass are lost in a second step and from 300 °C on, a slightly continuous mass loss is observed until 1000 °C where about 30% of residual mass is still present.

The DSC thermogram (Fig. 3) shows an endothermic peak starting at approximately 100 °C and centered at 175 °C, which coincides with an initial mass loss at about 115 °C, observed in Fig. 2, indicating the occurrence of a degradation process in this temperature range. Other endothermic events are visible at about 200 °C and 240 °C. At higher temperatures exothermic peaks can be seen (280 °C, 350 °C, 420 °C and 490 °C).

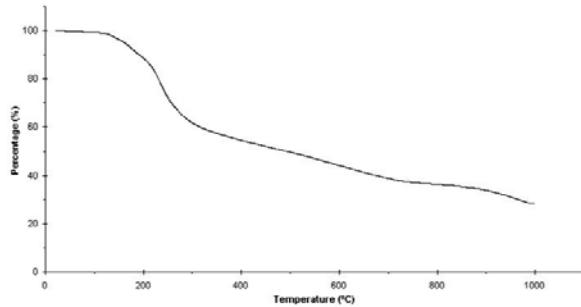


Figure 2. Natural Polysaccharide Thermogravimetric (TGA).

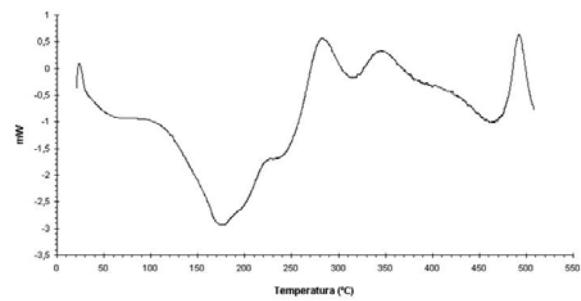


Figure 3. Natural Polysaccharide Differential Scanning Calorimetry (DSC).

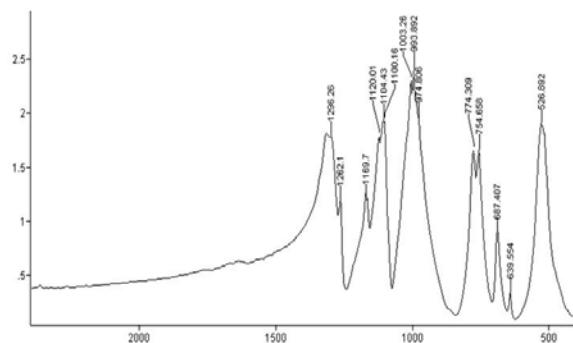


Figure 4. Sodium Trimetaphosphate Infrared Spectrum.

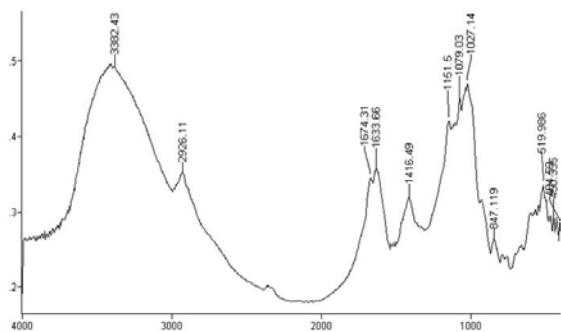


Figure 5. Natural Polysaccharide Infrared Spectrum.

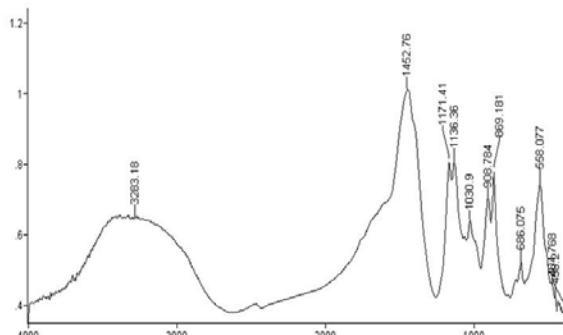


Figure 6. Modified Polysaccharide Infrared Spectrum.

Figure 4 represents the infrared spectrum of the STMP, where it is possible to observe characteristics peaks, like that located at 994 cm^{-1} related to P-O-P bonds and at 1296 cm^{-1} related to P=O bonds^{10,19,20}.

Figure 5 represents an infrared spectrum of the natural polysaccharide in which the bands in the regions of 3363 cm^{-1} referring to vibration of axial deformation of OH; 2928 cm^{-1} , to vibration of axial deformation of C-H; 1674 and 1635 cm^{-1} suggesting carbonyl of non-substituted amide and water; 1416 cm^{-1} , assigned to OH, C-H and C-N; and 1029 to 1153 cm^{-1} to C-O, C-O-C and C-C, so that these last peaks are characteristics of the polysaccharides.

However, in the modified polysaccharide spectrum (according to Fig. 6) it can be observed the apparition of a band in the region of 1030 cm^{-1} not so much visualized into previously discussed spectrum as pure STMP spectrum. This peak indicates the presence of the P-O-C group and proves the occurrence of the phosphatation reaction in the polysaccharide, with its consequent reticulation.

CONCLUSIONS

The results obtained in this study suggest that STMP can be used as a chemical agent employed in the reticulation reaction of the lotus roots polysaccharides, since the procedure guaranteed structural modification of the biopolymer. Besides, films prepared with the aqueous dispersions containing the studied polysaccharide in combination with Eudragit® RS30D revealed satisfactory for their morphologic characteristics. In this way, the studied association can be suggested as a suitable material to be applied in the coating of solid pharmaceutical forms. However, other studies are necessary to evaluate the hydrosolubility decrease in the modified product, as well as, the evaluation of its biodegradability, reminding that this property can indicate it as a potential candidate for drug carrier in the development of new systems for modified release, especially for colon-specific systems.

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