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Mucoadhesive Properties of Thiolated Pectin-Based Pellets Prepared by Extrusion-Spheronization Technique

André Luiz Lopes Martins [1], Aline Carlos de Oliveira [1], Carolina Machado Ozório Lopes do Nascimento [1], Luís Antônio Dantas Silva [1], Marilisa Pedroso Nogueira Gaeti [1], Eliana Martins Lima [1], Stephânia Fleury Taveira [1], Kátia Flávia Fernandes [2], Ricardo Neves Marreto [1,*]

[1] School of Pharmacy, Federal University of Goiás, Goiânia, Brazil
[2] Laboratory of Polymer Chemistry, Department of Biochemistry and Molecular Biology, Institute of Biological Sciences - ICB II, Federal University of Goiás, Goiânia, Brazil

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ABSTRACT

The aim of this study was to develop mucoadhesive pellets on a thiolated pectin base using the extrusion-spheronization technique. Thiolation of pectin was performed by esterification with thioglycolic acid. The molecular weight and thiol group content of the pectins were determined. Pellets containing pectin, microcrystalline cellulose, and ketoprofen were prepared and their mucoadhesive properties were evaluated through a wash-off test using porcine intestinal mucosa. The *in vitro* ketoprofen release was also evaluated. Thiolated pectin presented a thiol group content of 0.69 mmol/g. Thiolation caused a 13% increase in polymer molecular weight. Pellets containing thiolated pectin were still adhering to the intestinal mucosa after 480 min and showed a more gradual release of ketoprofen. Conversely, pellets prepared with nonthiolated pectin showed rapid disintegration and detached after only 15 min. It can be concluded that thiolated pectin–based pellets can be considered a potential platform for the development of mucoadhesive drug delivery systems for the oral route.

Introduction

Mucoadhesion is defined as 2 materials, of which at least one is either mucus or a mucous membrane, holding together for a prolonged period of time.[1-3] The use of mucoadhesive polymers in drug delivery systems could increase their retention at defined sites, and thus increase the systemic bioavailability of the active pharmaceutical ingredient, and promote topical treatment.[4-6]

Mucoadhesive polymers should be nontoxic, nonirritating, stable low-cost materials. In addition, they should form a strong quick interaction with the biological constituent.[7-9] Some natural polymers have been used in medical, food, and pharmaceutical fields because of their mucoadhesive and film-forming properties. In addition, they are usually considered biodegradable and biocompatible.[10,11]

Pectin is a heterogeneous biopolymer present in most superior plants. In the pharmaceutical field, it is used as a binding and thickening agent, and for developing controlled drug delivery systems.[12-15] In addition, different types of pectin have presented mucoadhesive properties in the gastrointestinal tract, and it has been shown that pectin mucoadhesion is largely affected by the degree of esterification and molecular weight.[16,17] Pectin mucoadhesive strength may not be sufficient to guarantee the fixation of the dosage form at the site in the gastrointestinal tract.[18] In order to circumvent such problems, the pectin was thiolated. Thiolated polymers, also called thiomers, can interact covalently with the cysteine-rich subdomains of mucus glycoproteins and thereby form a disulfide bond, which could greatly contribute to its fixation in the gastrointestinal tract.[18,19]

Mucoadhesive polymers can be incorporated in many kinds of drug delivery systems, from tablets to nanoparticles.[20] Multiparticulate systems, like pellets, can be advantageous to oral delivery due to their spherical shape, small average diameter, and narrow size distribution. Pellets are rapidly emptied from the stomach irrespective of the feeding state. In addition, a uniform dispersion of this dosage form on gastrointestinal tract is expected, which thereby reduces the risk of high local active pharmaceutical

* *Correspondence to*: Ricardo Neves Marreto (Telephone: +55 62 3209 6037; Fax: + 55 62 3209 6044).
E-mail address: ricardomarreto@ufg.br (R.N. Marreto).

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Table 1
Formulation and Operational Parameters for the Preparation of Pectin Pellets via Extrusion-Spheronization

Formulation	Granulation			Spheronization
	Granulation Liquid	Ethanol[a] (%,v/v)	Granulation Liquid:Solid Mass Rate	Speed (rpm)
P1	CAS	20	1:1	1500
P2	CAS	23	1:1	1500
P3	CAS	26	1:1	1500
P4	CAS	23	1:1	3000
P5	CAS + PVP K30	23	1:1	3000
TP1	CAS + PVP K30	23	1:1	3000
TP2	CAS + PVP K30	23	0.85:1	3000
TP3	CAS + PVP K30	23	0.75:1	3000

P, pectin-containing pellets; TP, thiolated pectin-containing pellets; MCC, microcrystalline cellulose; CAS, 10% (w/v) citric acid solution; PVP K30, 5% (w/v) polyvinylpyrrolidone K30.
[a] Ethanol content (%) in CAS.

ingredient concentration and, consequently, decreases the irritation potential of the system.[21-23]

Several techniques are used for pellet preparation. Of these, the extrusion-spheronization technique is very popular, because it allows for the production of pellets with high drug loading and high productivity.[24] However, it is difficult to prepare pectin-based pellets with this technique, as pectin swells considerably and makes the wet mass difficult to process.[25] Developing thiolated pectin-containing pellets by extrusion-spheronization could result in a novel mucoadhesive solid oral platform for the treatment of several pathological conditions, using an industrially feasible production method.

With that in mind, the aim of this study was to prepare and optimize, for the first time, pellets containing thiolated pectin via extrusion-spheronization and evaluate their mucoadhesive potential in freshly excised porcine intestinal mucosa. Pellets with both thiolated and nonthiolated pectin were compared in terms of their mucoadhesion strength and ketoprofen (a model drug) release.

Experiment

Materials

Citrus pectin (GENU® pectin type USP/100, batch SK01589. DE ~68% and molar mass between 110 and 130 kg/mol) was purchased from C.P. Kelco Brazil (São Paulo, Brazil). Microcrystalline cellulose pH 101 was obtained from Mingtai Chemicals Company, Ltd. (Taoyuan Hsien, Taiwan), ketoprofen from Hubei Xunda Pharmaceutical (Hubei, China), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), thioglycolic acid, citric acid, L-cysteine, and toluene from Sigma-Aldrich Brazil (São Paulo, Brazil). Polyvinylpyrrolidone K30 was acquired from Basf Brazil (São Paulo, Brazil); hydrochloric acid, ethanol, and methanol from Hexis Científica (São Paulo, Brazil), and freshly excised porcine intestine mucosa from a local slaughterhouse (Frigorífico Caçula, Goiânia, Brazil).

Synthesis of Thiolated Pectin

Thiolated pectin was synthesized by esterification of pectin with thioglycolic acid in an acidic environment, according to a modified version of the Sharma and Ahuja[26] method. Sixteen grams of pectin (82.42 mmol) was fully dispersed in 200 mL of deionized water at 80°C. Then, 3.8 g of an 80% thioglycolic acid solution (41.25 mmol) and 2 mL of a 7M hydrochloric acid (14 mmol) were added to the pectin dispersion. The reaction medium was maintained at 80°C for 150 min. The mixture was then poured into 500 mL of methanol and the white precipitates formed were dried at room temperature and ground in a blender (Arno PerfomaMagic, São Paulo, Brazil). After that, the thiolated pectin was completely redispersed in 200 mL of deionized water. Successive washings (500 mL) with methanol were performed to eliminate the unreacted thioglycolic acid. The average yield was calculated as the ratio between the final mass of the thiolated pectin and the mass of citric pectin added at the beginning.

Characterization of Thiolated Pectin

Determination of Immobilized Thiol Group Content

The amount of immobilized thiol groups in the polymeric backbone was determined spectrophotometrically (Varian Cary® 50 UV-Vis; Agilent Technologies) after reaction with Ellman's reagent.[27] To do so, 2.5 mL of the thiolated pectin dispersion (0.2%, w/v) was diluted with 2.5 mL of 0.5 M phosphate buffer (pH 8.0) and allowed to react with 5 mL of Ellman's reagent (0.03% w/v DTNB in 0.5 M phosphate buffer, pH 8.0) in the dark for 120 min. Absorbance of the reaction mixture was measured at 450 nm. The immobilized thiol group content was calculated using an analytical curve obtained from the reaction of the pectin dispersion and varying amounts of L-cysteine with Ellman's reagent. The results were expressed as millimoles of immobilized thiol group/gram of pectin.

Determination of Average Molecular Weight of the Thiolated Pectin

The average molecular weight of both thiolated and nonthiolated pectin was determined by static light scattering, using Zetasizer Nano S equipment (Malvern Instruments, Malvern, UK). One hundred milligrams of pectin was dispersed in 20 mL of acidified water (pH 1.5). The polymeric dispersion was allowed to stand for approximately 12 h and was then sonicated for 2 h in an ultrasonic bath (Haver USC; Haver & Boecker, Oelde, Germany). After that, the dispersion was ultracentrifuged (Sorvall Discovery M150 SE; Thermo Scientific, Waltham, MA) at 140,000 rpm for 30 min (25°C) to remove gel fraction. The supernatant was collected and analyzed. Precipitate was also collected and dried at 60°C for 12 h. Its weight was determined and used to accurately calculate the pectin concentration in the supernatant. The intensity of the light scattered from samples of different concentrations was measured and the average molecular weight of pectin was calculated. Toluene was used as a standard in the light scattering experiments.

Pellet Preparation

Ketoprofen-containing pellets (ketoprofen is a poorly water-soluble model drug with anti-inflammatory activity) were prepared using the extrusion-spheronization technique (Table 1). Ketoprofen, microcrystalline cellulose, and pectin (2:2:1, w/w/w) were manually mixed and wetted with the granulation liquid. The wet mass was then fed into the extruder (Caleva Extruder 20; Caleva, Dorset, UK), set at 30 rpm, and forced through a plate containing 1-mm-diameter holes. What was extruded was then spheronized in a CALEVA MBS spheronizer (Caleva) equipped with