Orally Disintegrating Films Containing Propolis: Properties and Release Profile

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ABSTRACT: The objective of this work was the production and characterization of orally disintegrating films of gelatin and hydrolyzed collagen containing the ethanol extract of propolis. The films were produced by casting with different concentrations of hydrolyzed collagen with and without the extract. The mechanical properties, mucoadhesive properties, swelling degree, *in vitro* release kinetics, stability of active compounds, Fourier transform infrared spectroscopy (FTIR), and antimicrobial activity of the films were evaluated. The films with the highest concentration of hydrolyzed collagen were less resistant and more elastic, and films containing the extract were more resistant than the control. In addition, the films with the extract showed higher mucoadhesion, which is important for ensuring the release of active compounds in the oral cavity. Generally, all formulations showed a high swelling capacity, which may have contributed to the quick release also demonstrated by the release kinetics model. Interactions between the extract compounds and the polymeric matrix were observed by FTIR spectroscopy, which may have contributed to an improvement in the mechanical properties. Films containing the extract had good stability and effective antimicrobial properties against *Staphylococcus aureus*, which shows that these films can potentially be used to release active compounds in the oral mucosa.
Keywords: oral drug delivery; stability; polymeric drug delivery systems; mathematical model; kinetics

INTRODUCTION

Currently, there is growing interest in developing delivery systems for active compounds in the oral cavity because this is an attractive region either for the local and systemic application.[1] Thus, a growing number of studies on the development of new mechanisms for oral release have been performed, including tablets,[2] gels,[3] sprays,[4] pastes,[5] patches,[6] wafers,[7,8] and films.[9,10]

Orally disintegrating films have the advantage of ease of handling and transportation because they are thin and flexible,[11] and they also have good acceptance by patients with difficulty in swallowing.[12] These films are mainly composed of polymers, plasticizers, and the active compound of interest, and other ingredients may be incorporated such as flavorings and sweeteners to increase patient acceptance of the films.[13]

In the production of orally disintegrating films, gelatin has great potential because of its film-forming ability;[11] furthermore, it is a natural polymer with good mucoadhesive properties.[14] Several studies have used gelatin-based films incorporated with active compounds for different applications.[15,16]

Natural products have been used for many years in popular medicine as teas and infusions and have gained more prominence because of consumer desire to replace synthetic products with natural ones.[17] Many plants, spices, and other natural sources are rich in active compounds such as phenolic compounds, which have interesting properties such as antimicrobial,[18,19] antioxidant,[20,21] anti-inflammatory,[22] and anticarcinogenic activities.[23]

Propolis is a natural substance composed mainly of organic acids, phenolic compounds, some enzymes, vitamins, and minerals.[24] Studies show that propolis has great potential for use against pathogenic bacteria and fungi[25–27] in addition to its anti-inflammatory properties,[19] making its incorporation into orally disintegrating films an interesting proposal for a local application.

The objective of this work was the production and characterization of orally disintegrating films from gelatin and hydrolyzed collagen containing the ethanol extract of propolis.

MATERIALS AND METHODS

Materials

Porcine gelatin type A (260 Bloom/40 MESH/Lot LFP7466 P 11) and hydrolyzed collagen (B50) were purchased from GELITA Brazil Ltd. (São Paulo, Brazil). The plasticizer used was sorbitol (Nuclear, São Paulo, Brazil), and 12-type resin (Star Rigel Raffard, São Paulo, Brazil) was used for production of the ethanolic extract of propolis.

Production and Characterization of the Ethanol Extract of Propolis

The ethanol extract of propolis was produced with a ratio of 30 g of resin per 100 mL of ethyl alcohol (80%) according to Nori et al.[28] The extraction was performed under agitation (500 rpm) for 30 min at 50°C. After this period, the solution was refrigerated for 24 h, and then the supernatant was filtered to obtain the ethanol extract of propolis.

The concentrations of phenolic compounds in the ethanol extract of propolis were characterized using the Folin–Ciocalteau method.[29] The extract was dissolved in absolute ethanol (1:1000), and an aliquot of 0.5 mL of this solution was placed in a tube containing 2.5 mL of Folin–Ciocalteu reagent (1:10).

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After 5 min, 2 mL sodium carbonate solution (4%) was added. The solution was left to stand for 2 h, and the reading was performed at 740 nm using a spectrophotometer (SP-22 UV; Biospectro, Curitiba, Brazil) and expressed in milligram of gallic acid per gram of extract.

Production of Orally Disintegrating Film

The orally disintegrating films were produced using the casting technique as described by Borges et al.[30] with a constant mass of gelatin and hydrolyzed collagen ($m_G + m_{HC} = 2\%$, w/v) and plasticizer concentration (0.6%, w/v). The mass of hydrolyzed collagen was varied (0%, 10%, 20%, and 30%, w/w) in relation to $m_G + m_{HC}$. The previously hydrated (30 min, room temperature) gelatin and hydrolyzed collagen were solubilized at 50°C (10 min), and sorbitol (solubilized in water) was incorporated into the solution under magnetic stirring. The film-forming solution was kept at 50°C for 10 min. The extract (4%, w/v) was added to the filmogenic solution under stirring using an ultraturrax (IKA T-25) at 6000 rpm (1 min). The filmogenic solution was then poured in a plate and dried in forced-circulation oven (Marconi AM-035) at 30°C for 24 h. The thickness was kept constant by controlling the mass–area ratio. Before analyses, films were conditioned in desiccators containing a saturated salt solution of NaBr (RH = 58%, 25 ± 2°C) for 5 days. For Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy, films were conditioned in desiccators containing silica.

Mechanical Properties

The mechanical properties were determined using the tensile test according to American Society for Testing and Materials (ASTM) standards[31] using the TA.XT Plus texture analyzer (Stable Microsystems SMD, England). Samples of film (100 × 25 mm^2) were fixed in the probe at an initial distance of 100 mm. The test speed was set at 50 mm/min until breakage. The tensile strength at break (MPa), elongation (%),elastic modulus (MPa/%), and the area under curve (AUC) were determined by Exponent software. The energy at break (normalized to the film's volume) was determined using the following equation:

$$EB = \frac{AUC}{V} \tag{1}$$

where EB is the energy at break (MJ/m^3), AUC is the area under the load versus displacement curve (MJ), and V is the volume of the film (m^3).

The analysis was performed for all formulation with 10 replicates each.

Mucoadhesive Properties *In Vitro*

The mucoadhesive properties were evaluated with a TA.XT Plus texture analyzer as described by Bruschi et al.[32] Chicken pouch was used to simulate the oral mucosa.[33] Samples of films were fixed on the equipment platform, and the chicken skin was placed in a cylindrical probe with a diameter of 20 mm. The sample was compressed by the probe covered with the skin at a constant force (0.1 N) for 30 s to ensure uniform contact between the skin and the film. The sample and the skin were completely separated at a constant speed (1 mm/s), and the maximum force required for this separation was determined as the mucoadhesive force. The analysis was performed for all formulation with 10 replicates each.

Swelling Degree

The swelling degree was determined gravimetrically using a phosphate buffer solution (pH 6.8) following the method described by Mohamed et al.[34] Film samples (2 cm diameter) were immersed in 30 mL of phosphate-buffered saline at 37 ± 1°C. The samples were collected with the help of a net support at intervals of 30 s and weighed. The analysis was performed for all formulation. The swelling degree was determined using the following equation:

$$SD = \frac{m_i - m_f}{m_i} 100 \tag{2}$$

where SD is the swelling degree (%, w/w), m_i is the initial mass of sample (g), and m_f is the wet mass of the sample at different times (g).

Release *In Vitro*

The *in vitro* release was measured according Perumal et al.[35] for formulations with ethanol extract of propolis. Samples of the orally disintegrating films (22 × 35 mm^2) were immersed in a phosphate buffer solution (pH 6.8) at 37 ± 1°C and stirred at 100 rpm. At different times (0, 2, 3, 4, 5, 10, and 15 min), aliquots (0.5 mL) of this solution were collected to determine the concentration of phenolic compounds by the Folin–Ciocalteu method,[29] and it was replaced with 0.5 mL of fresh dissolution media after every aliquot. The analysis was performed in triplicate.

Release Kinetics

The release kinetics for the phenolic compounds were evaluated using the following mathematical models: zero order,[36] Higuchi,[37] Korsmeyer and Peppas,[38] and Peppas and Sahlin,[39] as shown in Table 1, using the computer program Statistica (version 11).

Comparisons between the release profiles of the films were made by difference factor ($f1$)[40] and similarity factor ($f2$)[41] using the following equations:

$$f1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} 100 \tag{3}$$

Table 1. Release Models Used to Evaluate the Release Kinetics of Phenolic Compounds in Orally Disintegrating Films

Release Model	Equations
Zero order[32]	$\frac{M_t}{M_\infty} = K_0 t + b$
Higuchi[33]	$\frac{M_t}{M_\infty} = K_H \sqrt{t} + b$
Peppas and Sahlin[35]	$\frac{M_t}{M_\infty} = K_1 t^m + K_2 t^{2m}$
Korsmeyer and Peppas[34]	$\frac{M_t}{M_\infty} = K t^n + b$

M_t/M_∞, fraction of drug released over time (t); b, initial concentration of drug in the solution; K_0, K_H and K, kinetics constants; n, exponent of drug release; K_1, constant related to Fickian diffusion mechanism; K_2, constant related to erosion/relaxation mechanism (Case II); m, Fickian diffusion exponent.