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Topical curcumin-loaded hydrogels obtained using galactomannan from *Schizolobium parahybae* and xanthan

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ABSTRACT

The curcumin (CUR)-loaded binary hydrogel was formulated using xanthan and galactomannan from *Schizolobium parahybae* (guapuruvu). The binary hydrogels presented gel characteristics, stable pH values and mechanical stress resistance even after 45 days of heat exposure (45 °C). The CUR-loaded hydrogel content was 98.6% for XGMC (xanthan and galactomannan with CUR-microemulsion) after the stability test. The *in vitro* cytotoxicity analysis suggested non-cutaneous membrane irritation, and the *in vitro* skin permeation analysis indicated 2.15 to 2.50 μg mL^{-1} CUR at the stratum corneum, epidermal and dermal levels. The XGEC (xanthan and galactomannan with CUR solubilized in ethanol) and XGMC hydrogels presented 76.8 and 63.2% inhibition of topical inflammation, respectively. Chemical stability and non-cytotoxicity analysis confirm the safety of prolonged exposure of the skin during the topical treatment, offering long-lasting XGEC and XGMC action.

1. Introduction

Mixed polysaccharide systems are used in a wide range of applications because of their ability to interact synergistically (Mannion et al., 1992) and to render materials with controlled properties. In particular, galactomannan blends are used by themselves or in combination with xanthan in a range of applications that include drug delivery (Ughini, Andreazza, Ganter, & Bresolin, 2004; Vendruscolo, Andreazza, Ganter, Ferrero, & Bresolin, 2005). The drug dissolution profile from matrices of galactomannan and xanthan revealed zero order model drug release, indicating their potential as sustained-release vehicles (Ughini et al., 2004). The general structure of seed galactomannans consists of polymeric main-chains of (1 → 4)-linked β-D-mannopyranosyl residues substituted at *O*-6 by single unit side-chains of α-D-galactopyranose. The galactomannan extracted from the seeds of a Brazilian native plant, *Schizolobium parahybae* (Guapuruvu), presented a mannose/galactose (M/G) ratio of ~2.5, are abundant throughout Brazilian forests and could be an additional source of galactomannan for biotechnological applications. This galactomannan is similar to a galactomannan widely used in the pharmaceutical industry, obtained from the seeds of the locust bean gum, which presents a M/G ratio of ~3.5 (Bresolin, Milas, Rinaudo, & Ganter, 1998; Ganter, Heyraud, Petkowicz, Rinaudo, & Reicher, 1995). The M/G ratio depends on the source and method of extraction (Bento et al., 2013; Salvallagio, Freitas, Franquetto, Koop, & Silveira, 2014).

Xanthan gum is an extracellular polysaccharide of *Xanthomonas campestris*. Its primary structure is a cellulose backbone of D-glucose linked β-1,4 and on every alternate glucose unit, there is a side chain consisting of β-D-mannose-(1,4)-β-D-glucuronic acid-(1,2)-α-D-mannose. The terminal mannose moiety may carry pyruvate residues linked to the 4- and 6-positions. The internal mannose unit is acetylated at *O*-6 (Jansson, Kenne, & Lindberg, 1975). Xanthan solutions exhibit weak gel-like properties at low shear rates, making it suitable as a suspending agent; it does not gel at any concentration or temperature (Dea et al., 1977; Milas, Rinaudo, & Tinland, 1985). Xanthan has the remarkable feature of forming physical hydrogels when mixed with galactomannans, *e.g.* locust bean gum in 1:1. The formation involves synergistic interactions between these polysaccharides (Richter, Brand, & Berger, 2005; Sandolo, Coviello, Matricardi, & Alhaique, 2007). Examples of current applications of xanthan and the locustbean gum are drug delivery tablet systems, commercially known as TIMERx®, developed by Penwest Pharmaceuticals Company (Baichwall & Neville,

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2002), which in the presence of dextrose (50%) form a strong binder gel in water. The function of xanthan in tablets is to erode or dissolve slowly and thereby yield a more delayed release of active ingredients, compared to formulations devoid of hydrocolloids (McCall & Baichwall, 1994).

Xanthan and galactomannan from the seeds of *Mimosa scabrella* form a hydrogel matrix, studied to improve the stability of ascorbic acid in pharmaceutical formulations (Koop, Praes, Reicher, Petkowicz, & Silveira, 2009). Recently, a hydrogel made from CUR/xanthan and galactomannan from the seeds of *Ceratonia siliqua* (locust bean gum) was investigated by the authors (Da-Lozzo et al., 2013; Koop et al., 2012). We determine its rheological properties as well as its biocompatibility using the chorioallantoic membrane from *Gallus domesticus*. This binary system could be an alternative for hydrophobic compounds, such as CUR, which showed therapeutic effects on wound healing and protection against the deleterious effects of UVA-induced skin cancers injury by attenuating oxidative stress and suppressing inflammation upon topical application (Heng, 2010; Huang et al., 1997; Santibáñez, Quintanilla, & Martinez, 2000).

CUR is a low molecular weight polyphenol extracted from the rhizomes of turmeric (*Curcuma longa* Linn.). It has the chemical structure [1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione] and is water-insoluble. CUR is widely used in traditional Chinese medicine and in its food industry (Ammon & Wahl, 1991). It has additionally demonstrated several types of biological and pharmacological activities, including anti-inflammatory and antioxidant properties. However, previous studies have shown that the retention time of CUR in the body is limited due to its rapid systemic elimination (Kurd et al., 2008; Yang, Huang, & Lin, 2007).

The percutaneous route may be suitable for the administration of CUR for both local and systemic therapeutic uses. To enhance its bioavailability, the technique of encapsulation or incorporation with different polymers have been employed, including chitosan (Sanoj Rejinold et al., 2011), cyclodextrin (Tomren, Másson, Loftsson, & Tønnesen, 2007), methoxy poly(ethylene glycol)-graft-chitosan film (Li, Nan, Li, Zhang, & Chen, 2012b), nanocomposite of *N*,*O*-carboxymethylchitosan and oxidized alginate (Li et al., 2012a), and poly(ethyleneglycol)-poly(ε-caprolactone)-poly(ethyleneglycol) hydrogel (Gong et al., 2013). Previously we develop a lecithin-based CUR-containing microemulsion. However, the CUR mobility was thereby facilitated through the most external skin layers, including the stratum corneum (Koop et al., 2012).

In the present work, we proposed a strategy for cutaneous wound healing, which combined a CUR-loaded microemulsion (MC) without ethanol with an *in situ* gel-forming hydrogel system of xanthan and galactomannan from *S. parahybae* (XGMC). Furthermore, the stability, rheological properties, *in vitro* drug release, skin permeation and *in vivo* anti-inflammatory effect were determined to investigate the topical effects on cutaneous wound models.

2. Materials and methods

2.1. Chemicals and reagents

CUR powder (89.5% purity), isopropyl myristate and egg L-α-phosphatidylcholine were purchased from Sigma-Aldrich Co. (Steinheim, GER). Benzylic alcohol and xanthan (X) were obtained from Merck (Darmstadt, GER). Polysorbate 80 (Tween 80), 1,2-propanediol and phosphoric acid were obtained from Synth (São Paulo, Brazil). Water was purified using Easy Pure equipment, with resistivity above 18 MΩ cm (Barnstead/Thermo scientific, Waltham, USA). The other chemicals are of HPLC or analytical grade.

Seeds from *S. parahybae* (guapuruvu) were acquired from *Viveiro Mata Atlântica*, São Paulo. The galactomannan was extracted from the isolated endosperm of guapuruvu seeds (G) using water at 25 °C for 1 h with mechanical stirring; the extract was centrifuged at 1,680 × *g* for 30 min at 10 °C (yield 42.1%). The supernatant was precipitated by adding ethanol and dried under vacuum (Bresolin et al., 1997). The M/G ratio of the galactomannan of G, determined by alditol acetate derivatization, was 2.5 (Wolfrom & Thompson, 1963a,b). Size exclusion chromatography (SEC) was carried out as described by Vianna-Filho, Petkowicz, and Silveira (2013); the average molar mass was 7.73×10^5 g mol^{-1} for the G, and the recovered mass from the system was 91.5% at 25 °C, suggesting good solubility in this aqueous solvent.

2.2. Preparation and characterization of the microemulsion

The MC was obtained by the method described previously (Lin, Lin, Chen, Yu, & Lee, 2009) with minor modifications. Oil (0.6 g), lecithin (0.765 g), Tween 80 (1.785 g), and CUR (0.09 g) were added in a test tube. The oil phase (isopropyl myristate) was kept at 50 °C and well mixed by a vortex. Water (15 g) was added with constant agitation, after sonication until a transparent mixture, an isotropic O/W MC, was obtained. The average hydrodynamic diameters (D_h) of the oil droplets were determined by dynamic light scattering in a BI 9000 multiangle laser light-scattering apparatus from Brookhaven Instruments (Holtsville, NY, USA). MC stability was assessed as a function of temperature. The samples were analyzed by DLS—Nano DLS Brookhaven Instruments (Holtsville, NY, USA) between 25 °C and 60 °C at a fixed angle of 90°. All measurements were performed at 25 °C in triplicate.

2.3. Preparation of hydrogels

Hydrogels were used as CUR vehicles in the *in-vivo* and *in-vitro* studies. The hydrogel formulations were prepared with X and G. In the first formulation (XG), X and G (50% of both) were dispersed in distilled water for 16 h at 1.25% total polysaccharide concentration in the presence of 1% benzyl alcohol (w/w) as a preservative and were used as a control without CUR. In the second formulation (XGMC), the MC was added to the XG hydrogel to compare the influence of MC on the hydrogel structure and to observe whether the MC preparation facilitates the "entrapment" of drug, such that the drug is not released. In the third formulation (XGEC), CUR was solubilized in ethanol (EC) and added 7.6 mL to 100 g of XG hydrogel, this amount was experimentally defined to not precipitate the polysaccharides. All samples were stirred for 10 min at room temperature, and all hydrogels were stored at 4 °C.

2.4. Stability and incorporation efficiency of curcumin-loaded hydrogel

The stability of the hydrogels was evaluated to verify that their chemical stability was maintained and that the following aspects of each active ingredient of the product was maintained: (a) the chemical integrity; (b) CUR concentration within the specified limits; (c) constant CUR concentration during storage and use; and (d) the properties and characteristics of the product (Isaac et al., 2008).

XG, XGEC and XGMC hydrogel samples were placed into a sealed glass bottle and in an oven at 45.0 ± 0.2 °C for 45 days. The samples were withdrawn 1, 15, 30 and 45 days after preparation. The stability was confirmed by centrifugation (Sigma 2K15, Osterode am Harz, Germany) at 1,107 × *g* at 25 °C for 30 min. The pH of the hydrogels was determined by using digital pH meter, directly in the sample, but promoting rupture of the gel structure by stirring, and measuring in different parts the samples. Additionally, we compare the pH of one gram of gel dissolved in 10 mL distilled water and stored for 30 min prior to measurement. The measurement of pH of each formulation was done in triplicate and average values are