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Curcumin/xanthan–galactomannan hydrogels: Rheological analysis and biocompatibility

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ABSTRACT

Curcumin, a lipophilic compound found in the plant *Curcuma longa* L., exhibits a wide range of pharmacological activity; however, its therapeutic use has been limited because of its low bioavailability following oral administration. The aim of this study was to evaluate the viscoelastic characteristics and biocompatibility of a curcumin/xanthan:galactomannan hydrogel (X:G) system after topical application on chick embryo chorioallantoic membrane (CAM), a system established with a view toward curcumin nasal or topical pharmaceutical applications or possible administration in cosmetics or foods. A rheological analysis indicated that incorporation of curcumin did not alter the viscoelastic characteristics of the X:G hydrogel, suggesting that there was no change in the structure of the gel network. X:G hydrogels did not induce CAM tissue injury and the curcumin/X:G hydrogel system was also highly biocompatible. We conclude that the X:G hydrogel represents a potential matrix for curcumin formulations.

1. Introduction

Curcumin, a lipophilic compound found in the plant *Curcuma longa* L., has traditionally been used as a yellow pigment to color food and cosmetics (Jayaprakasha, Jagan Mohan Rao, & Sakariah, 2005). Curcumin possesses a wide range of pharmacological properties, including antioxidant (Miquel, Bernd, Sempere, Díaz-Alperi, & Ramírez, 2002), anti-inflammatory (Shakibaei, John, Schulze-Tanzil, Lehmann, & Mobasheri, 2007; Sharma, Gescher, & Steward, 2005; Xu, Deng, Chow, Zhao, & Hu, 2007), anti-angiogenic (Gururaj, Belakavadi, Venkatesh, Marmé, & Salimath, 2002), antitumor (Dorai & Aggarwal, 2004; Gafner et al., 2004; Kunnumakkara, Anand, & Aggarwal, 2008; Shishodia, Chaturvedi, & Aggarwal, 2007; Singh & Agarwal, 2003), and immunomodulatory (Srivastava, Singh, Dubey, Misra, & Khar, 2011) activities. Despite this large spectrum of biological activity, the therapeutic use of curcumin has been limited because of its low bioavailability when administered orally (Sharma et al., 2005). Thus, the development of alternative strategies for curcumin topical use could open up new possibilities for the development of medicinal applications.

Polymeric hydrogels are being increasingly studied as release matrices (Peppas, Bures, Leobandung, & Ichikawa, 2000). Polysaccharide hydrogels have been widely employed in various industrial and pharmaceutical applications. The polysaccharides xanthan (X) and galactomannan (G) alone are simple water-soluble thickening agents, but when they are mixed, an original gelation occur (Dea & Morrison, 1975). The galactomannan from the seeds of *Mimosa scabrella* Bentham used in xanthan:galactomannan (X:G) binary system has been tested as a hydrophilic matrix for the release of theophylline (Ughini, Andreazza, Ganter, & Bresolin, 2004) and sodium diclofenac (Vendruscolo, Andreazza, Ganter, Ferrero, & Bresolin, 2005) in the form of directly compressed tablets and capsules as well as hydrogel matrix to stabilize ascorbic acid (Koop, Praes, Reicher, Petkowicz, & Silveira, 2009). A system of xanthan and locust bean gum was studied as a hydrogel matrix to stabilize emulsions (Makri & Doxastakis, 2006). Considering the natural source of each of the individual components, a curcumin/X:G hydrogel system could have a variety of applications in food, cosmetic, and pharmaceutical industries. However, when a water-insoluble drug like curcumin is added to a hydrogel, it can only be dispersed, not dissolved; thus, a transparent aqueous gel cannot be obtained (Knighton, Ausprunk, Tapper, & Folkman, 1977; Peppas et al., 2000). Water-insoluble drugs are often dissolved in water miscible co-solvents, such as ethanol, before being added to the gel. Although the addition of ethanol to the gel could potentially

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precipitate polysaccharides, the OH group of the alcohol also helps to maintain a certain degree of hydrophilicity and thereby prevents precipitation (Montembault, Viton, & Domard, 2005). Another possible outcome of directly dissolving lipophilic drug molecules in a hydrogel mixture is that these drug molecules might be hosted within the three-dimensional gel network and lose their functionality. Accordingly, both rheological properties and biocompatibility of the curcumin/X:G hydrogel system must first be assessed.

The avian chorioallantoic membrane (CAM), the outermost extra-embryonic membrane lining the noncellular eggshell membrane, is one of the most commonly used models for studying angiogenic and anti-angiogenic effects (Eun & Koh, 2004; Hazel, 2003; Knighton et al., 1977; Nguyen, Shing, & Folkman, 1994). It has also been used as an *in vivo* model for evaluating tissue responses to biomaterials (Klueh, Dorsky, Moussy, & Kreutzer, 2003; Valdes, Kreutzer, & Moussy, 2002) and for assessing the biocompatibility of drug-delivery systems after topical administration on the CAM surface (Vargas, Zeisser-Labouèbe, Lange, Gurny, & Delie, 2007). The inflammatory response of the CAM to biomaterials is similar to that observed in mammalian models (Klueh et al., 2003; Valdes et al., 2002). This *in vivo* model enables constant observation of the site of the implant, providing rapid, simple, and low-cost monitoring.

The biocompatibility of the curcumin X:G hydrogel system has not yet been evaluated. Therefore, the aims of this study were to determine the rheological properties of curcumin/X:G hydrogels and verify their biocompatibility using the CAM model.

2. Materials and methods

2.1. Materials

Xanthan, curcumin, and galactomannan from locust bean gum (Man:Gal 3.5:1) were obtained from commercial suppliers (Sigma–Aldrich, Co, St Louis, MO, USA). The mannose:galactose (Man:Gal) ratio of galactomannan was determined by gas liquid chromatography (GLC) and ^{13}C NMR spectroscopy (Ganter & Reicher, 1999). PLLA (poly-L-lactide acid) resin was obtained from Birmingham Polymers (MW 13.7 kDa; Birmingham, AL, USA).

2.2. Preparation of the X:G hydrogel

Aqueous solutions of xanthan (X) and galactomannan (G) were prepared and then blended at 80 °C with mechanical stirring to obtain a X:G hydrogel (1:1) with a total polysaccharide concentration of 12.5 g/L. A 50-μL aliquot of a 10-mg/mL curcumin solution in ethanol was added to the xanthan solution prior to blending to obtain a X:G hydrogel containing 0.5 mg/mL of curcumin. X:G hydrogels were stored at 4 °C before analysis.

2.3. Preparation of PLLA films

A 1% solution (w/v) of PLLA was prepared by dissolving PLLA resin in chloroform overnight. Four milliliters of solution was used to cast a thin PLLA film on cleaned glass Petri dishes (55 × 20 mm). The solvent was allowed to evaporate slowly in air, ensuring the formation of a smooth, non-porous film (55 mm diameter, 0.02 mm thickness). The cast film was rinsed with deionized water, air-dried, and then kept under vacuum to remove any residual solvent (Nguyen et al., 2004). For sterilization, the PLLA-coated dish was exposed to UV irradiation for 45 min prior to the experiment.

2.4. Dynamic rheological analysis

Dynamic oscillatory measurements were performed using a dynamic stress rheometer (model Haake RS75) equipped with a PP35Ti sensor (plate, 35 mm in diameter). The temperature was controlled by a circulating water bath and maintained at 25 °C with a thermoelectric Peltier device. Small deformation oscillatory experiments were conducted in two stages: (1) deformation sweeps at constant frequency (1 Hz) to determine the maximum deformation attainable by the sample in the linear viscoelastic range and (2) frequency sweeps (0.01–10 Hz) at a constant deformation (1% strain) within the linear viscoelastic range. Mechanical spectra were obtained by recording the dynamic moduli (G' and G") and η^* as a function of frequency. G' is the dynamic elastic or storage modulus, which is related to the material response as a solid; G" is the dynamic viscous or loss modulus, which is related to the material response as a fluid; and η^* is the dynamic viscosity, which approaches zero-shear viscosity as both the shear rate and the frequency of deformation are reduced to zero. All tests were done at least three times, and mean and standard error values were calculated. The dynamic rheological studies were performed for X:G hydrogel samples (12.5 g/L total polysaccharide concentration) containing 0.10–0.50 mg/mL curcumin and without added curcumin.

2.5. Release experiments

Drug release was assessed in dissolution studies performed in triplicate using a USP rotating basket method. The hydrogel system (10 mL) containing 0.5 mg/mL of curcumin was placed into the basket. The dissolution media was 300 mL of isotonic phosphate buffer, pH 7.4, maintained at 37 °C and agitated at 50 rpm. At appropriate intervals, 1-mL aliquots of dissolution medium were withdrawn and assayed for drug release and replaced with 1 mL of fresh buffer. The aliquots were filtered through cellulose acetate membranes (0.45 μm) and evaporated under vacuum (Speed Vac) at 20 °C. Precipitates were dissolved in 0.5 mL ethanol, ultrasonicated for 10 min in ice-cold water, and centrifuged at 10,000 rpm for 20 min. Supernatant samples were assayed by fluorescence spectrophotometry (RF-5301 PC, Shimadzu) with excitation and emission wavelengths of 420 and 530 nm, respectively, and a 5-nm slit width. Dissolution medium without sample treated in the same manner as samples was used as a blank. A standard curve was generated by preparing serial dilutions of a stock solution of curcumin (5 mg/mL) in ethanol and then adding to dissolution medium to yield samples of curcumin in a concentration range of 2.5–500 ng/mL. Curcumin standards were assayed in triplicate in the same manner as samples. The emission intensity of samples at 530 nm was plotted against curcumin concentration. After linear regression analysis, the slopes, intercepts and the correlation coefficients (r) of curves were calculated using Excel 5.0 software.

2.6. CAM assay

Fertilized chicken eggs were obtained from a local hatchery. The eggs were cleaned with a 70% ethanol solution and incubated at 37 °C and 60% relative humidity. On day 5 of development, the surface of each egg was sterilized and part of the CAM containing the central vein was exposed by aseptically opening a circular window in the egg shell under a laminar flow hood. Test substances (50 μL X:G hydrogel with and without 50 μg curcumin) were applied from above onto the developing CAMs. A PLLA film (0.25 cm^2) was used as a positive control. The window was sealed with sterile plastic tape, and the eggs were returned to the incubator. On day 13 of incubation, the windows were extended to a diameter of about 3 cm, and the response to the different tested materials was analyzed by gross evaluation and histological analysis. Embryos that did not survive or in which disks could not be located were not included in the results.