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Crosslinked soy protein films and their application as ophthalmic drug delivery system

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ABSTRACT

In this research, the potential of soy protein (SPI) based-films as drug delivery devices for ocular therapy was developed. Hence, crosslinked films with a natural and non-cytotoxic crosslinking agent, genipin (Gen), coated with poly(lactic acid) (PLA), were prepared. Filmogenic solutions were loaded with timolol maleate (TM) as a model drug, to be used as drug delivery devices, a novel application for this material. The mechanical properties of the films were studied, observing that with the presence of PLA coating, more rigid materials with improved properties were obtained. Furthermore, the release behavior of TM was evaluated in aqueous medium, it being influenced by the degree of film crosslinking. Furthermore, it was determined that PLA coating decreased TM release rate compared to that of uncoated films. Similarly, this behavior was observed via indirect estimation of the release by assessing the hypotensive effectiveness of the films by in-vivo assays. Through intraocular pressure (IOP) determination tests in rabbits, it was demonstrated that, through the use of high crosslinked and coated films, a significant decrease in IOP could be achieved for prolonged time periods. These results suggest that the use of soy protein-based films as drug delivery systems is highly suitable.

1. Introduction

Biodegradable films derived from natural products have received considerable research interest during the last years due to ecological concerns aroused by the use of traditional petroleum-based materials. This has led to the use of several renewable agro-products as promising biodegradable materials [1]. Main products studied include polysaccharides and proteins [2]. However, protein-based films showed some advantages due to their excellent film-forming abilities, low cost and barrier properties against oxygen and lipid under low-to-intermediate humidity conditions [3,4]. In particular, soy proteins produce smoother, clearer and more flexible films as compared to those arising from other sources [5]. Novel soy protein based-materials are currently developed in order to be applied to several fields such as industrial coatings, food packaging, agriculture and medicine.

A particular interest in medicine is currently focused in the development of novel drug delivery systems that could improve the disadvantages arising from traditional dosage systems. In ocular therapy, for example, conventional dosage systems are generally based on topical application of eye drops. Most of these pharmaceutical formulations are administered for two main purposes: a) outside eyeball structure treatments, including conjunctivitis, blepharitis and dry keratitis; b) treatment of intraocular disorders such as glaucoma, uveitis and endophthalmitis. In the latter, the bioavailability of topically administered drugs shows significant limitations brought about by the rapid and extensive loss of formulation from the precorneal area due to drainage and lacrimal replacement [6]. In addition, the significant decrease in drug penetration by this route usually derives from the highly efficient barrier properties of the cornea.

With the administration of eye drops, less than 5% of the drug passes through the cornea and reaches intraocular tissues, while most is absorbed systemically via conjunctiva and naso-lacrimal ducts.

Further disadvantages of these dosage systems include discomfort experienced by the patient due to the high-frequency administration of small volumes of medicine (every 3 to 4 h). Hence, efforts are currently addressed to design more efficient drug delivery systems with sustained- or controlled-release properties in order to increase drug

Abbreviations: SPI, soy protein; Gen, genipin; PLA, poly(lactic acid); Gly, glycerol; TM, timolol maleate; IOP, intraocular pressure; RH, relative humidity; FTIR-ATR, Fourier Transform Infrared Spectroscopy in Attenuated Total Reflectance mode; TS, tensile strength; E, elongation at break; ANOVA, analysis of variance.

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concentration in the eye structure for an extended period of time. In addition, efforts are focused on the development of a more convenient and practical delivery system for patient's administration.

Some of the systems under study are related to the use of liposomes [7,8], nano- and microparticles [9–12], in situ gel formation [13–15], and more recently, to the novel use of non-cytotoxic films.

In particular, a method of increasing the residence time of the formulations during application is the use of bioadhesive systems capable of releasing controlled amounts of the desired drug formulation [16].

By their hydrophilic nature, films formed from natural and biodegradable materials generally have the ability of containing hydrophilic drugs (soy proteins contain 58% polar amino acids that cause hydrophilicity [4]). However, when they are immersed in aqueous environments, rapid drug diffusion is observed through the matrix, generating a fast release. An efficient release results from the presence of effective drug concentration in the environment for a preset time. By using non-sustained systems, several doses have to be applied to produce the same effect, as compared to the use of sustained systems. It can also be noted that, sometimes, by using conventional systems, the concentration yielded does not reach minimum levels to be effective, while in other cases, concentration reaches toxic values for the organism or tissues. In contrast, a sustained release system maintains concentration levels within the therapeutic window for a prolonged period of time.

Films formed by three-dimensional networks of crosslinked materials have the ability to swell in aqueous solvents in a controlled manner (according to crosslinking degree). When the swelling is generated in a drug-containing matrix, this can be dissolved and released to the medium by diffusion. As swelling properties, drug release can be controlled adjusting the crosslinking degree [17]. Therefore, films with correct structural relationship between hydrophilicity and crosslinking degree can release drugs with a sustained behavior [18–21].

Glaucoma is the main cause of blindness worldwide, particularly among elderly people. This eye disease is usually characterized by the pathological increase in intraocular pressure (IOP) due to lack of drainage of aqueous humor, showing a common optic neuropathy characterized by progressive loss of nerve fibers in the optic nerve. This asymptomatic disease causes progressive loss of visual function, accompanied by ocular hypertension. One of the drugs conventionally used for this condition is timolol maleate (TM). This is a non-selective blocking agent of the ß-adrenergic receptor. Due to its high stability and water solubility, this drug is perfectly suitable to be used as a model drug for the possible development of drug delivery systems.

This research work aims at employing a natural polymer such as soy protein, to develop materials to be used in the field of medicine and ophthalmology. For this, soy protein (SPI) films crosslinked with different amounts of genipin (Gen) previously obtained and characterized in our research group [22] were applied as TM delivery devices in ocular therapy.

Genipin is a novel and biocompatible cross-linking agent, about 10,000 times less cytotoxic than glutaraldehyde [23,24]. The colony-forming assay also showed that the proliferative capacity of cells after being exposed to Gen was approximately 5000 times greater than that of cells exposed to glutaraldehyde [23,25]. Genipin-crosslinked soy protein (SPI-Gen) films have the required characteristics as drug delivery devices for ocular therapy due to their hydrophilic and crosslinked structure, the biocompatible properties of their components, their low solubility in water and good mechanical properties and intense color, which would greatly help patients during their correct application. Presumably, due to small size, thickness and absence of sharp edges, these films do not cause inconveniences or discomfort in the eye.

The modification of mechanical properties produced by the addition of the drug was assayed, as well as the in-vitro release behavior in aqueous systems and the effect of the release on the ocular hypotensive effectiveness in in-vivo systems.

2. Experimental

2.1. Materials

The following chemicals were used: isolated soy protein SPI SUPRO E with 90% protein on fat-free, dry-weight basis (donated by The Solae Company, Argentina), glycerol (Gly) (Taurus, Argentina), genipin (Gen) (Wako, Japan), timolol maleate (TM) 99% (Parafarm, Argentina), NaCl (Cicarelli, Argentina), KCl (Cicarelli, Argentina) and $CaCl_2$ (Cicarelli, Argentina).

2.2. Animals

Twelve New Zealand white rabbits weighing 2–2.5 kg were used. These rabbits were provided with food and water ad libitum in a temperature-controlled room (21 ± 5 °C) and exposed to 12 h light:12 h dark cycles. Animal management procedures complied with ARVO (Association for Research in Vision and Ophthalmology) resolution on the use of animals in research from the European Communities Council Directive (86/609/EEC). The Institutional Animal Care and Use Committee of the School of Chemistry from the National University of Córdoba, Córdoba, Argentina reviewed and approved the protocols. After a week of adaptation in the facilities, animals were admitted to the experimental sessions.

2.3. Preparation of TM-containing SPI-Gen films

TM-containing films were prepared by casting method as described in a previous work [22] with the addition of TM in the last step of the preparation. In brief, SPI powder was dispersed under constant stirring in distilled water (8.33 g/100 mL) and Gly was added at 50% (w/w) of SPI while pH was adjusted to 9 with 0.5 M NaOH. The dispersions were stirred for 30 min at room temperature. Different volumes of 0.4% w/v Gen solution were then added to SPI dispersions to obtain the final SPI-Gen mixture with 0; 0.1; 1; 2.5; 5; 7.5 and 10% (w/w of SPI) of Gen. The dispersions were heated at 70 °C for 2 h. Once room temperature was reached, 1.5 mg of TM (equivalent to 6 normal doses) was added to the dispersions and stirred for 2 h. All dispersions were poured into plastic plates (polypropylene) and dried in an oven with air circulation at 40 °C for 12 h. Subsequently, films were removed and conditioned for 48 h at 25 °C and 50% relative humidity (RH) before use. The films were named SPI; SPI-Gen 0.1%; SPI-Gen 1%; SPI-Gen 2.5%; SPI-Gen 5%; SPI-Gen 7.5% and SPI-Gen 10%. The different films were cut in small 4 mm-diameter circles. In addition, PLA covered SPI-Gen-TM films were prepared. For this, the different disks were submerged for 3 min in a solution of PLA in chloroform (0.53% w/v) and dried at room temperature in a vacuum chamber until complete chloroform elimination.

2.4. FTIR-ATR analysis

In order to confirm the presence of PLA covering, disk surfaces were analyzed by Fourier Transform Infrared Spectroscopy in Attenuated Total Reflectance mode (FTIR-ATR) using a ZnSe crystal with an incidence angle of 45 grades. Different clean areas of five samples were analyzed to confirm the homogeneity of each surface. All spectra represent the average of 42 scans recorded at 4 cm^{-1} resolution in a 4000 to 400 cm^{-1} range, using air as background.

2.5. Mechanical properties

Stress–strain curves for each film (25 × 100 mm) were recorded; tensile strength (TS) and elongation at break (E) were determined according to ASTM methodology [26]. An Instron Universal Testing Machine (model 3342, Norwood, MA, USA) equipped with a 500 N capacity cell was used with an initial grip separation of 100 mm and