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Development and characterization of crosslinked hyaluronic acid polymeric films for use in coating processes

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ABSTRACT

The aim of this work was to develop and characterize new hyaluronic acid-based responsive materials for film coating of solid dosage forms. Crosslinking of hyaluronic acid with trisodium trimetaphosphate was performed under controlled alkaline aqueous environment. The films were produced through casting process by mixing crosslinked or bare biopolymer in aqueous dispersion of ethylcellulose, at different proportions. Films were further characterized regarding morphology by scanning electron microscopy, robustness by permeation to water vapor transmission, and ability to hydrate in simulated gastric and intestinal physiological fluids. The safety and biocompatibility of films were assessed against Caco-2 and HT29-MTX intestinal cells. The permeation to water vapor transmission was favored by increasing hyaluronic acid content in the final formulation. When in simulated gastric fluid, films exhibited lower hydration ability compared to more extensive hydration in simulated intestinal fluids. Simultaneously, in simulated intestinal fluids, films partially lost weight, revealing ability for preventing drug release at gastric pH, but tailoring the release at higher intestinal pH. The physiochemical characterization suggests thermal stability of films and physical interaction between compounds of formulation. Lastly, cytotoxicity tests demonstrated that films and individual components of the formulations, when incubated for 4 h, were safe for intestinal cells Overall, these evidences suggest that hyaluronic acid-based responsive films, applied as coating material of oral solid dosage forms, can prevent the premature release of drugs in harsh stomach conditions, but control the release it in gastrointestinal tract distal portion, assuring safety to intestinal mucosa.

1. Introduction

The development of systems for modified release of drug, which provides delivering of active compounds to specific regions of the body, is still a major challenge for researchers both from academic community and pharmaceutical industrial. In order to direct the delivery of drug formulated into tablets, the use of polysaccharides for coating processes of solid oral dosage forms can contribute for a better tailoring of the release of active molecules for the intestinal sites of absorption. Such improvement has been particularly demonstrated when natural polysaccharides are used in association with well-established coating materials as ethylcellulose (EC) and its derivatives (Serverino et al., 2011).

Hyaluronic acid (HA) is a linear anionic polysaccharide formed by repeating disaccharide units of D-glucuronic acid and *N*-acetyl glucosamine. HA presents excellent biocompatibility, low toxicity and is fully biodegradable in vivo (El-Aassar et al., 2015; Lee et al., 2015; Oliveira et al., 2016). Moreover, HA is known for being easily modified with chemical functionalization of functional groups. It also possesses a natural affinity for specific receptors, such as CD44

Abbreviations: CHA, cross-linked hyaluronic acid; DSC, differential scanning calorimetry; EDS, energy dispersive spectrometry; EC, ethylcellulose; XRD, X-ray diffraction; SGF, fluid of gastric simulation; SIF, fluid of intestinal simulation; GIT, gastrointestinal tract; HA, hyaluronic acid; FTIR, infrared spectroscopy; SEM, scanning electron microscopy; SI%, swelling index; TGA, thermogravimetric analysis; STMP, trisodium trimetaphosphate; WVT, water vapor transmission.

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(Han et al., 2012; Mero and Campisi, 2014), which makes it a possible agent for target delivery. These receptors are overexpressed on various epithelial cells, as enterocytes (Misra et al., 2011; Saravanakumar et al., 2013).

Owing to the potential and versatility of this polymer, an increasing number of biomedical applications have been described. The HA have been widely used for viscosupplementation of synovial fluid in patients that suffer from arthritis (Lùrati et al., 2015), in intervertebral disc regeneration (Shen et al., 2010), for the treatment and diagnosis of atherosclerosis (Lee et al., 2015), for controlled drug release purposes (Kumar et al., 2015; Kwon et al., 2015; Zhong et al., 2015) and as component of a wide variety of cosmetics (Pan et al., 2013). The development of HA-based derivatives with enhanced or modulated properties has also been suggested (Schanté et al., 2011).

Still, HA is a highly hydrophilic polymer that can absorb a large amount of water, expanding up to 1000 times its solid volume, forming a loose network (Mero and Campisi, 2014). This feature results in inability to withstand the conditions founded in the upper gastrointestinal tract (GIT) when used alone in coating film forming process and may promote a premature release of drug trapped in the coated tablet. Trying to overcome this limitation, HA can be chemically modified by crosslinking agents such as trisodium trimetaphosphate (STMP), which is a low toxicity salt that does not cause adverse effects in humans and reacts with hydroxyl groups of the polysaccharide (Dulong et al., 2004; Prezotti et al., 2012; Souto-Maior et al., 2010).

Thus, the aim of this study was promote the crosslinking of HA with STMP, with subsequent production of new polymeric material in free film form. The characteristics of developed films for use in the coating process of solid oral dosage forms proposed for modified release of drug were also addressed. This crosslinked alternative intends to decrease hydrophilicity of bare HA, in combination with the mixture of insoluble EC, a classical film coating component. Also, it may be possibly to increase the intestinal targeting drug delivery due to the presence of HA (crosslinked or bare) due to its potential ability to bind to CD44 receptors (Vafaei et al., 2016).

2. Materials and methods

2.1. Materials

Hyaluronic acid 1.5 MDa (sodium salt, from *Streptococcus equi subsp. zooepidemicus*) was from Contipro (Czech Republic). Ethylcellulose (Surealease®) was a gift from Colorcon (Brazil) and trisodium trimetaphosphate from Sigma-Aldrich (Brazil). All other chemicals were of lab grade. Simulated gastric fluid (SGF, pH = 1.2) and simulated intestinal fluid (SIF, pH = 6.8) without addition of enzymes, were prepared according to United States Pharmacopoeia (USP).

2.2. Development of crosslinked hyaluronic acid

A solution of 1% HA (200 mL) was prepared in water (pH 12.0), which was stirred for 2 h to allow complete homogenization. At basic pH, a complex of di-polymer phosphate ester is formed from polysaccharide and STMP (Gliko-Kabir et al., 2000). Subsequently, 20 mL of 30% STMP solution in water were added at the previous HA solution and stirred for additional 2 h. After this process, the portions corresponding to the concentrations of 5 and 10% of crosslinked polysaccharide were added to the EC dispersion to obtain the free films (Bunhak et al., 2015).

2.3. Production of films

Conventional solvent evaporation method, also called casting process, was used for prepare films in seven different proportions (Rabito et al., 2012; Santos et al., 2013). Different formulations were obtained varying the concentration of the ethylcellulose and/or HA, maintaining the polymer mass constant at 4% (w/v). Polymeric solutions were prepared by magnetic stirring under vacuum conditions to avoid air incorporation and possible bubbles formation in the films, in a final volume of 10 mL. Then, solutions were poured onto Teflon® plates and oven-dry at 60 °C for 12 h (Rosiaux et al., 2013; Yang et al., 2010). Films were removed from the plates after drying and placed at desiccator at room temperature.

2.4. Macroscopic characteristics and free films thickness assessing

Films were analyzed by their macroscopic features as homogeneity, presence or absence of air bubbles and cracks. These characteristics may modify the films integrity and affect further analysis. Thickness of films were determined averaging five random points with a micrometer (Mitutoyo®) (Rabito et al., 2012; Santos et al., 2013). The films were kept at desiccator with silica gel until the tests accomplishment.

2.5. Water vapor transmission study

Initially, 10 mL of distilled water were added to permeability domes (Braive Instruments® type), where films samples were fixed. Then, each set (dome + distilled water + film) was weighed and stored in a desiccator containing silica gel at room temperature. The following measurements were made after 24, 48, 72, 96 and 120 h. Furthermore, silica was replaced after each weighing. The weight difference of each dome in the respective time intervals was recorded and Eq. (1) was used to calculate the vapor transmission rate of water transported through the films. The WVT was standardized for a period of 24 h (Oliveira et al., 2011; Santos et al., 2013).

$$WVT = \frac{g.24}{t.a} \quad (1)$$

where g is the weight loss, t is the time (h) during which the weight loss was accompanied and a is the film area (m^2). Measurements were performed in triplicate for each formulation.

2.6. Swelling index determination

The different hydration characteristics of the new polymeric material were determined by swelling assessment. Briefly, samples of films were cut into pieces of about 1 cm^2, accommodated in Petri dishes and left into an oven at 50 °C for 15 h, to achieve the complete loss of moisture. Afterward, films were stored in desiccators containing silica gel for conducting experiments. Samples of the different formulations were initially weighed on analytical balance to determine the dry mass and immediately immersed in containers containing SGF or SIF for predetermined periods of time (1–10, 30, 60, 90, 120 and 150 min) at constant temperature (37 °C). After, the films were removed from the media using tweezers, carefully dried between two sheets of filter paper to remove excess water, and then weighed again. This test was carried on in triplicate and Eq. (2) was used for SI% quantification (Oliveira et al., 2011; Santos et al., 2013).

$$\boldsymbol{SI}\% = \frac{\boldsymbol{weight\ of\ swollen\ film - weight\ of\ dry\ film}}{\boldsymbol{weight\ of\ dry\ film}} \times 100 \quad (2)$$