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Papain wound dressings obtained from poly(vinyl alcohol)/calcium alginate blends as new pharmaceutical dosage form: Preparation and preliminary evaluation

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ABSTRACT

Transparent, soft, flexible, mechanically resistant films, which are ideal for use as wound dressings were prepared in the presence of 2% papain, a proteolytic enzyme that can play a role in the chemical debridement of the skin and can accelerate the healing process. The films, based on poly(vinyl alcohol):calcium alginate blends with increasing concentrations of polysaccharide (10, 20, and 30% v/v), were obtained by casting method. FTIR and DSC analyses were performed to assess the composition and miscibility of blends. Mechanical properties such as tensile strength, elasticity modulus, and elongation at breakpoint were evaluated. The influence of different concentrations of calcium alginate on physical attributes of films like wettability, swelling capacity and mechanical properties was determined. The stability of papain in the films was assessed indirectly by hemolytic activity assay employing direct contact method and confirmed by technique based on blood agar diffusion. Preliminary cytotoxicity was evaluated with the XTT method. The results showed that at the polymer concentrations tested, the blends were miscible. The increase in the content of the calcium alginate increased the wettability and swelling capacity of the films, which is desirable in wound dressings. On the other hand, mechanical resistance decreased without causing breakage of the films during the swelling tests. The hemolytic activity of the films was maintained during the studied period, suggesting the stability of papain in the proposed formulations. Cellular viability indicated that the films were non-toxic. The analysis of the results showed that it is possible to prepare interactive and bioactive wound dressing containing papain from blends of PVA and calcium alginate polymers.

1. Introduction

Wound dressings are intended to protect wounds from physical, thermal, chemical, biological, and other traumas. An enhanced, optional feature is the ability to promote healing. An ideal dressing should protect the application site, maintain a moist environment in the wound area, allow gas exchange with the environment, remove excess exudates, and act as a local physical barrier. They must be nontoxic, non-allergenic, and non-adherent, and contribute both to the healing and comfort of the patient. From a mechanical point of view, they must have a good level of resistance and flexibility to allow easy application and removal [1–3]. Traditional dressings, such as those based on gauze, are known as passive products. Other polymer-derived dressings are called interactive products and are intended to cover, preferably, low exudative wounds. These dressings are permeable to gases and impermeable to particles. Bioactive products also contribute to wound treatment. They are based on covering polymers and may contain substances with endogenous activity, such as proteins and drugs, which are released locally. Another classification system of dressings, which is based on their materials, divides them into non-resorbable, hydrophilic, occlusive, hydrogels, and interactive dressings [4–6].

The latex of papaya (*Carica papaya*) is traditionally employed in the treatment of different skin disorders, including in wound healing processes and for the treatment of burns. The latex contains a complex mixture of cysteine endopeptidases, among them, papain,

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which are implicated in the chemical debridement of tissues, therefore, accelerating tissue regeneration [7–9].

Natural and synthetic polymers, alone or in combination, are employed in the preparation of wound dressings. The aim is to satisfy as many requirements as possible to result in the ideal product. Natural and synthetic polymers blends are of high interest in the biomedical and pharmaceutical areas, because of superior properties to those of individual polymers. Among the advantages of these blends are better chemical and mechanical resistance, processability, permeability, biodegradability and biocompatibility. Many efforts have been undertaken to obtain and characterize such blends, especially as the discovery of new materials with superior properties is more time consuming and expensive [10–12].

Poly(vinyl alcohol) (PVA) is a linear polymer obtained by vinyl acetate polymerization, followed by partial (70%) or full (100%) hydrolysis. The molar mass of the different types of PVA varies from 10.000 g mol^{-1} to 190.000 g mol^{-1} and glass-transition temperatures (T_g) reported for the different types of PVA are between 70 and 85 °C. PVA properties such as tacticity, crystallinity, and solubility influence its other properties such as the Tg, melting temperature (Tm), viscosity, resistance to solvents, permeability, adhesiveness, and swelling capacity. PVA has good interface and mechanical characteristics and its presence in blends aims to change the permeability to gases, improve processability, increase mechanical and thermal resistance, promote controlled swelling, and enhance the stabilization of polymeric matrices [13]. PVA's chemical inertness, crystallinity, and stability towards biological fluids make it a biocompatible synthetic polymer, when used in appropriate conditions. The main characteristics exploited in the use of PVA as an excipient are its chemical compatibility, mechanical resistance, capacity to form films, swelling capacity, adhesiveness, and biocompatibility. PVA is a safe pharmaceutical excipient when administered orally. It is not absorbed and is rapidly eliminated from the body. Many studies have reported its use in experimental systems for the release of drugs administered by different routes such as the oral, buccal, topical, transdermal, ophthalmic, intramuscular, intravaginal, and pulmonary routes [14–16]. However, pure PVA hydrogels are rigid, have very little or no elastic behavior, and have a low swelling capacity; therefore, they are not good candidates for use as wound dressings.

Biomaterials based on alginate are of great interest in the medical, pharmaceutical, dental, and engineering areas. Alginates are polysaccharides obtained from renewable sources and widely used as pharmaceutical excipients. They display excellent biocompatibility and biodegradability properties, in addition to possessing a chelating capacity and a lack of immunogenic activity. Alginates are anionic, hydrophilic macromolecules, which contain numerous functional groups, notably carboxyl and hydroxyl groups. These groups can establish hydrogen bonds, undergo hydration and swelling when in aqueous solution, and adhere to mucosal layers in the body. Alginates are obtained from brown algae and bacteria; their basic structural units are uronic acid, α-L-glucuronic acid, and β-D-mannuronic acid. Although good candidates for the manufacture of wound dressings, pure alginate films have low mechanical resistance, a feature that can be manipulated through blend preparation [16–19]. Calcium alginate (AlgCa) wound dressings have a high swelling capacity when in contact with body fluids that are present in exudative wounds, in addition to maintaining hydration and local protection. Once in contact with the exudates, ion exchange occurs between the calcium ions from the film and the sodium ions of fluids. As sodium ion levels increase, the alginate fibers swell and dissolve, which increases gelation and keeps the environment moist for healing. Some authors suggest that the presence of the calcium ion promotes ionic exchange that assists in autolytic debridement and hemostasis, and produces a gel with a high absorptive capacity [4,20–22].

Papain-containing pharmaceutical formulations in the form of gels, hydrogels, creams, and ointments at concentrations ranging from 0.5 to 10% are already commercially available. Some of the many advantages of bioactive dressings include the ease of application and removal, which contributes towards greater patient comfort and treatment success. Furthermore, the presence of a drug in the dressing reduces the need to manipulate the wound. Sterile dressings can be manufactured by aseptic casting, since polymers can be sterilized. This provides justification for the proposition of a wound dressing as an alternative topical formulation.

In this context, the main objective of this work was to produce and characterize films containing papain, prepared from PVA: AlgCa blends, for use as wound dressings. The films were tested using differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) to assess the composition and miscibility of blends. The resistance of films was studied via mechanical tests. An investigation of the wettability of the films was carried out using goniometry, and the swelling capacity in water and phosphate buffered saline (PBS) was also determined. The hemolytic ability of papain was determined in order to study the maintenance of enzyme activity within the film.

2. Materials and methods

2.1. Materials

Alginic acid calcium salt from brown algae, papain from *C. papaya* (powder ⩾ 3 U/mg, EC 3.4.22.2), and PVA (molecular weight 85.000–124.000 g mol^{-1}, ⩾99% hydrolyzed) were purchased from Sigma-Aldrich, USA. Papain (pharmaceutical grade, EC 3.4.22.2), sodium chloride, and calcium carbonate were purchased from VETEC, Brazil. PBS (pH 7.2 ± 0.1, technical grade) was purchased from LaborClin, Brazil.

2.2. Preparation of the films

The PVA solution was prepared by dissolving 12.5 g of PVA in distilled water, whilst heating above 90 °C and stirring at 1500 rpm on a digital mechanical stirrer (FISATOM, model 713D, São Paulo, Brazil) until complete polymer dissolution was achieved. The final concentration of the obtained PVA solution was 5% *w/v*. Simultaneously, 10 g of AlgCa was dispersed in 250 mL of water containing 2.5 g of sodium carbonate at 70 °C, whilst being continuously stirred for 6 h, resulting in a 4% *w/v* colloidal solution.

For the preparation of the blends, aqueous solutions of the individual polymers were mixed on a magnetic stirrer (FISATOM, model 752A, São Paulo, Brazil) at 25 °C for 30 min. Papain was incorporated by direct solubilization in the blends, at a suitable concentration to result in 2% papain films, as reported by other authors [23,24]. Films were prepared from the blends using the casting technique: ten milliliters of each blend were deposited on standardized diameter plates (8 cm in diameter) and left exposed to air for 48 h, until completely dry. Table 1 presents the compositions and the thicknesses of the films.

2.3. pH measurement

The pH of the starting solutions and blends was determined using a pH meter (DIGIMED, model DH-22, Brazil) via the direct immersion of the electrode in the solution.