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Films based on neutralized chitosan citrate as innovative composition for cosmetic application

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ABSTRACT

In this work, citrate and acetate buffers, were investigated as neutralizers to chitosan salts in order to provide biocompatible and stable films. To choose the appropriate film composition for this study, neutralized chitosan citrate and acetate films, with and without the plasticizer glycerol, were prepared and characterized by thickness, moisture content, degree of swelling, total soluble matter in acid medium, simultaneous thermal analysis and differential scanning calorimetry. Chitosan films neutralized in citrate buffer showed greater physical integrity resulted from greater thicknesses, lower moisture absorbance, lower tendency to solubility in the acid medium, and better swelling capacities. According to thermal analyses, these films had higher interaction with water which is considered an important feature for cosmetic application. Since the composition prepared in citrate buffer without glycerol was considered to present better physical integrity, it was applied to investigate hyaluronic acid release in a skin model. Skins treated with those films, with or without hyaluronic acid, show stratum corneum desquamation and hydration within 10 min. The results suggest that the neutralized chitosan citrate film prepared without glycerol promotes a cosmetic effect for skin exfoliation in the presence or absence of hyaluronic acid.

1. Introduction

Nowadays, there is a high level of concern in the general population regarding aesthetic skin care. The search for an ideal standard of beauty – *i.e.* a healthy appearance of the skin (a hydrated skin without wrinkles and blemishes) – has been widespread [1,2].

Facial aging results from interactions between intrinsic and extrinsic factors that occur during our lifetime [3]. Facial aging is a consequence of a continuous process in which environmental damage in exposed areas overlaps with natural aging to determine the skin's overall appearance [4,5]. In the photoaging process, free radicals cause depolymerization reactions in essential components of the skin, such as collagen, elastin and glycosaminoglycans [6]. Photoaging reduces the amount of these components, which leads to a decrease in the natural elasticity of the skin. This results in the skin becoming more rigid and less hydrated, giving it a dry appearance (xerosis), which can give rise to fine wrinkles or hyperpigmentation [7].

Thus, topical use of facial permeation containing glycosaminoglycans such as hyaluronic acid (HA) appear as a way to minimize the effects of aging. HA is a non-sulphated glycosaminoglycan consisting of repeating units of *D*-glucuronic acid and *N*-acetyl glucosamine linked through β (1 → 3) and β (1 → 4) linkages [8]. >50% of the total HA found in the human body is located in skin tissue [9–11]. Despite the high molecular weight and hydrophilicity of HA, which would be expected to limit it's permeability, HA is known to be delivered through the skin in human tissue [12,13]. Brown et al. [12] presented the possibility of penetration and/or permeation of HA when applied in to the skin in gel form, finding it in the basal layer of the epidermis, dermis and lymphatic endothelium after topical application. The penetration and/or permeation of HA into the skin may be explained by the existence of hydrophobic patch domains in chain HA or by the presence of HA receptors distributed throughout skin tissue. These factors can enhance the permeability of HA across the stratum corneum and facilitate its localization in the skin tissue, respectively [14,15]. In this context, Pavicic et al. [16] demonstrated that the low molecular weight HA (M_w = 50,000 g·mol^{-1}) can penetrate into the stratum corneum, which provides moisturizing properties by clustering water in the stratum corneum leading to a reduction in the depth of wrinkles. This can be explained by the fact that HA is very hygroscopic, which enables the stratum corneum hydration.

Currently, the pharmaceutical industry is interested in the role of the excipients in these formulations. Thus, formulations containing an excipient capable of promoting a cosmetic effect and improving the effects of actives that are currently under development [17]. The use of films composed of natural polymers, which serve as excipients for such purposes, is a promising possibility. One possible natural polymer that could be used for this purpose is chitosan (CH). CH is a polysaccharide

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composed of *D*-glucosamine and *N*-acetyl glucosamine units linked through β (1 → 4) linkages. In aqueous acid solutions, CH forms hydrogels, which can be processed as beads, fibres or films [18–21]. An interesting property of CH in the water-soluble form is bioadhesion [22], owing to the cationic network that interacts with negatively charged residues at the skin surface. Due to this property, CH can be used in cosmetics and toiletries as a skin moisturizer and tone regulator [23], in pharmaceutical applications, including prolonged or controlled release drug delivery systems [24], in wound healing [25], and in tissue-regenerated materials [25,26].

However, the problem of poor stability of CH–based systems restricts its practical applicability [27], and maintaining the integrity of the physical structure of CH films during their preparation is a significant challenge. In diluted inorganic or organic acids, CH is positively charged as a result of protonation of the amino groups. This leads to film dissolution when these are immersed in water or acidic medium. Therefore, a neutralization step is required to improve the stability of these films.

After neutralization, CH films are stiff, an undesirable feature since the application of films on the skin requires easy handling of the material. The addition of glycerol, a substance with a plasticizer property, reduces the stiffness and improves handling of the films. In the pharmaceutical field, glycerol is considered a moist since it is a polar molecule with hygroscopic properties and it is used for treatment of dry skin conditions because of its ability to make the stratum corneum softer and more pliable [28].

According to Ali et al. [29], the pH of the medium affects integrity, cohesion and stratum corneum desquamation. Skin pH is normally acid (pH between 4.5 and 6.5) being a key factor in barrier homeostasis, stratum corneum integrity and antimicrobial defense. To preserve these features, the use of formulations with a pH similar to physiologic pH of the skin is recommended in products for skin care. Alternatively, buffer solutions that act as neutralizing agents and bring the pH close to that of the skin, can also be used.

The aim of this study is to prepare chitosan films neutralized in acetate (CHFA) and citrate (CHFC) buffers in the absence of the plasticizer glycerol, and in acetate (CHFPA) and citrate (CHFPC) buffers in the presence of glycerol (with neutralization of films to pH 5.0) in order to determine the film composition that presents the best physical integrity and biocompatibility with the skin. This film will then be tested in a hyaluronic acid release assay using a pig skin model, to determination of hydration measures.

2. Materials and methods

2.1. Materials

The following reagents were used in the preparation of chitosan films: chitosan ($M_w \approx 340{,}000\ g \cdot mol^{-1}$ and degree of acetylation ≈25%) purchased from Sigma-Aldrich (USA); acetic acid and citric acid provided by Nuclear and Farmaquimica (Brazil), respectively; sodium acetate and sodium citrate obtained from Fmaia (Brazil); and glycerol from Synth (Brazil). The following were used for the skin release experiments: the ears of freshly slaughtered pigs (species: *Sus domesticus*, sex: female, age: 5 months) obtained from the Frigorifico Borrussia (Brazil); sodium hyaluronate derived from *Streptococcus equi* ($M \approx 15{,}000–30{,}000\ g \cdot mol^{-1}$) obtained from Sigma-Aldrich (USA); and Carbopol® gel with hyaluronic acid (1.67% wt) provided by Farmatec (Brazil). The following were used for microscopy characterization: acetone obtained from Quimica Moderna (Brazil); and glutaraldehyde (GA, 25% wt/v aqueous solution) obtained from Merck (Germany). Monobasic sodium phosphate monohydrate and dibasic sodium phosphate dodecahydrate were purchased from Nuclear (Brazil) and used for both skin release experiments and microscopy characterization. All materials were utilized as received. Deionized water was employed for all preparation, characterization and washing procedures.

2.2. Preparation of neutralized films

Chitosan solutions (1.1% wt/v), with and without glycerol (33% wt), were prepared in an acetic acid solution (0.09 M) and stirred for 48 h. Aliquots of 20 mL of each solution were poured into Petri dishes (85 mm diameter) and dried in an oven for 3 h at 50 °C to obtain non-neutralized films. After, these films were neutralized in buffer solution acetic acid/sodium acetate (0.2 M) pH 5.0 for 24 h, washed in deionized water and dried in a desiccator at room temperature.

Additionally, neutralized films were prepared as described above, but employing a citric acid solution (0.04 M) as the dispersing medium, buffer solution citric acid/sodium citrate as a neutralizing agent and glycerol as a plasticizer. A total of six samples ($n = 6$) were used for each characterization.

2.3. Preparation of skin segments from pig ears

After the acquisition of the pig ears, they were washed in water, dried and dissected with a scalpel to extract the epidermis. Square segments of skin, with dimensions of 3.5 × 3.5 cm, were then cut. This measure was chosen to ensure a contact area with the receptor solution, in subsequent experiments, of 4.9 cm^2. Hairs present in the skin segments were extracted using scissors. The skin segments were wrapped in aluminium foil and frozen at −20 °C until the moment of the experiment. On the eve of the experiments, the frozen skins were transferred to the refrigerator for gradual thawing. The preparation of skin segments for release experiments was conducted in the Laboratory of Pharmacotechnique and Cosmetology of Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) after approval of the project *In vitro Evaluation of Hyaluronic Acid Release from Chitosan Films* by the Ethics Committee on Animal Research of UFCSPA, Opinion No. 196/2013.

2.3.1. Skin release experiments

In order to study the *in vitro* release of hyaluronic acid in pig ear skin, we used a diffusion system (adapted model, Fig. Fig. 1) which consisted of a receptor compartment filled with a phosphate buffer solution (0.2 M, pH 7.4) with a volume of 17 mL. This system was coupled to a

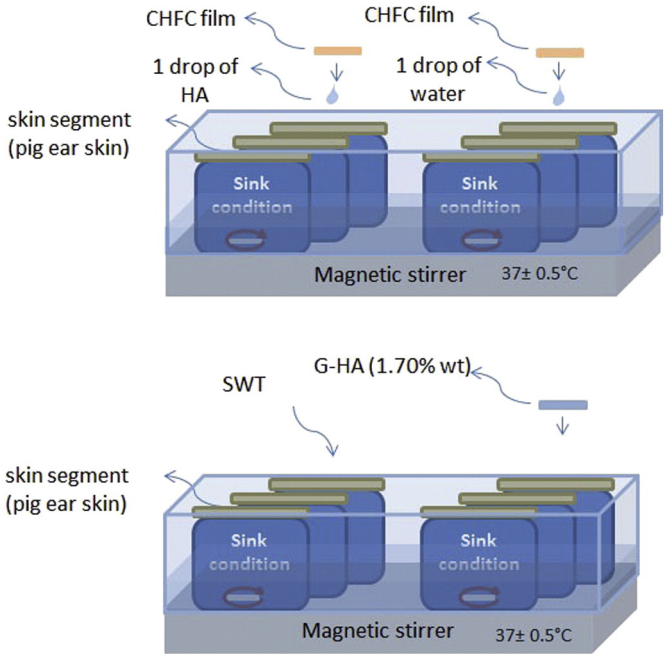

Fig. 1. Schematic illustration of skin release experiments: CHFC: chitosan film neutralized in citrate buffer without plasticizer, HA: hyaluronic acid, SWT: skin without treatment and G-HA: Carbopol® gel with hyaluronic acid.