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Development of Cross-Linked Gelatin Hydrogel Films Using Tannic Acid as Anti-Aging Active with Skin Care Potential

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Hydrogel films based on gelatin, glutaraldehyde and glycerol are widely reported in many studies with applications focused on food packaging, microencapsulation and release of active drugs. Thus, this study used this composition to produce hydrogel films and brought as a novelty the incorporation of tannic acid (TA) as anti-aging active, due to its great antioxidant capacity. The presence of TA in the hydrogel films was verified by the C–O stretch absorption band at 1020 cm^{-1}, as well as by its thermal degradation between 200 and 300 ºC. Furthermore, the presence of this active compound, which influenced the physical, morphological and mechanical properties of the film, was also confirmed by qualitative release tests. Additionally, the mechanical properties of the HT2 hydrogel film showed acceptable values of elongation at break (28.9 ± 3.1%) and Young modulus (6.4 ± 1.1 MPa), suggesting that this film has the potential to be applied in skin care routines.

Keywords: tannic acid, crosslinkers, mechanical properties, hydrogel films

Introduction

According to a research by the World Health Organization (WHO),[1] it is expected that, by the year 2050, the elderly population of the world (people over 60 years old) will surpass the number of adolescents and youths (people from 10 to 24 years old). This will lead to a general aging of the world population, which is a source of concern from governmental entities worldwide.

Aging is considered a natural process that, due to the decrease in fibroblastic cells over the years, reduces collagen production,[2,3] which can be accelerated by prolonged sun exposure.[2] This results in skin flaccidity, an increase in the amount of wrinkles, and expression lines.[3] Despite being a natural phenomenon, these signs of aging may have a negative impact on the self-esteem of the people, increasing the search for suitable skin care formulations to delay these aesthetic concerns. In this scenario, the market of skin care formulations in the form of films with anti-aging actives has been a growing category among dermatological products.[4] Additionally, anti-aging films based on hydrogel formulations are an advantageous alternative to injectable methods due to their easy application on the skin, non-invasive characteristics, and lower costs. According to the literature,[4] most films applicable on top of the skin contain preservatives, emulsifiers, active ingredients, and other additives, such as gelatin, which is considered an example of an emulsifying and gelling agent with a positive impact on skin health.[5,6] However, hydrogel films containing only gelatin exhibit poor mechanical properties and no antimicrobial activity, which limits their application.[7] Thus, crosslinking with glutaraldehyde has been an efficient approach to improve the mechanical, thermal, and antimicrobial activity of gelatin films, minimizing the perceived disadvantages.[8,9] Furthermore, it is possible to add active principles such as hyaluronic acid,[10] gallic acid,[11] and tannic acid,[12] which

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have been investigated in recent years, to these matrices, improving their anti-aging functional characteristics.

As previously stated, one of the active ingredients used in this process is tannic acid (TA), which is an affordable and non-toxic polyphenol highly available in a variety of plant sources. Besides being inexpensive and non-toxic, this active ingredient also shows astringent, antimicrobial, antitumor, antimutagenic characteristics and strong antioxidant activity.[13-15] Additionally, regarding the application of this active on skin, Daré *et al*.[16] report that non-cytotoxic doses of TA are able to prevent oxidative stress induced by UVB radiation in fibroblasts cells (L929), acting to prevent photoaging.

Although there are many studies related to the preparation and characterization of films based on gelatin (or collagen), glutaraldehyde and/or glycerol,[9,17] the investigation of the thermal, morphological and mechanical properties of these materials containing TA as an anti-aging agent has not yet been systematically investigated. Most of the research conducted involving this compound thus far has focused on its application as a polymeric crosslinker.[18-20] In this context, the study developed here focuses on the preparation of anti-aging hydrogel films based on glutaraldehyde, glycerol, gelatin with and without TA. The incorporation of this active compound aims at obtaining a new, differentiated, accessible material, with improved thermal, morphological, mechanical and anti-aging properties, with potential applications in the dermatological market.

Experimental

Hydrogel films preparation and characterization

Hydrogel films based on gelatin (Êxodo Científica, P.A., Brazil), glutaraldehyde (Dinâmica, P.A., Brazil), and glycerol (used as a plasticizing agent, Synth, P.A., Brazil), with and without tannic acid (Êxodo Científica, P.A., Brazil) were prepared according to a modified version of the methodology proposed by Kavoosi *et al*.[17] These alterations allowed the investigation of different proportions of gelatin (10 or 15% m/v), glycerol (25 or 30% m/m based on the weight of the gelatin powder) and glutaraldehyde (2% m/m based on the weight of the gelatin powder). Thus, after the addition of glutaraldehyde, each solution was carefully placed in Teflon™ dishes (diameter of 5 cm), filling the entire dish volume, which corresponded to 17 g of solution, and dried at ca. 37 ºC in an oven with forced air circulation (Model SP-100/21, SPLABOR, Brazil), in order to obtain thinner films with approximately 0.2 mm (Pachymeter model Vernier Caliper 150 × 0.05 mm, Kingtools, China). The hydrogel films in the absence of TA were named as H1, H2 and H3, as shown in Table 1.

Table 1. Compositions of gelatin, glycerol and glutaraldehyde used for the preparation of hydrogel films

Hydrogel film	Gelatin / %	Glycerol / %	Glutaraldehyde / %
H1	10	25	2
H2	15	25	2
H3	15	30	2

The hydrogel films containing TA (1% m/m based on the weight of the gelatin powder) were designated as HT1, HT2, and HT3. A screening of the physical (appearance and color), morphological (uniform surface suggesting better adhesion to the skin) and mechanical characteristics (elongation at break, Young's modulus, and puncture resistance strength), as well as the thermal stability of the components used in the preparation of hydrogel films, were taken into account in order to aid the selection of a more suitable material. The thermal properties were analyzed by thermogravimetric analysis (TGA) and differential thermal analysis (DTA), using a STA 8000, PerkinElmer (USA). These measurements were carried out using 45 mg of material, alumina sample holder, with a heating rate of 10 °C min^{-1} and under 80 mL min^{-1} flow of air. A Fourier transform infrared spectrometer equipped with attenuated total reflectance (FTIR-ATR, mod. Spectrum 100S, PerkinElmer, USA) was used to obtain absorption spectra between 4000-400 cm^{-1} with a spectral resolution of 4 cm^{-1}. Tensile and perforation assays were obtained using a universal texturometer (TA-HD Plus, Stable Micro Systems, England). The samples were kept inside a humidity box prior to the experiments in order to avoid dehydration, and all procedures were performed in triplicate. Tensile tests were realized with hydrogel films of 50 mm × 15 mm fixed to the pneumatic claws (A/TG - Tensile Grips, Stable Micro Systems, England) at 100 mm of distance, using test speed of 2 mm s^{-1} and trigger force of 5.0 g. For the perforation assay, the films were fixed in a circular support of texturometer, and an acrylic conical probe at 45º (P/45C, Stable Micro Systens, England) was used, with a test speed of 0.5 mm s^{-1} and a trigger force of 0.02 kg. The elongation at break was calculated from the equation 1.

$$\text{Elongation at break (\%)} = \left(\frac{L - L_0}{L_0} \right) \times 100 \quad (1)$$

where, L_0 is the length initial of the sample in mm; L is the length final of the sample in mm. The physical and

morphological characteristics of the hydrogel films were also investigated using the method of scanning electron microscopy (SEM, Zeiss EVO-MA10).

Release test

The release of TA incorporated into the hydrogel films was performed qualitatively through the Folin-Ciocalteau assay, using an immersion method.[21] The tests were carried out in aqueous medium, employing enough solution to fully submerge the samples, which were standardized at a 1:5 ratio (m/m). The hydrogel films containing TA were kept submerged for 0, 15, and 30 min. After each time interval, 20 µL of this solution were mixed with 100 µL of Folin-Ciocalteau reagent (2 mol L^{-1}) and 1580 µL of distilled water. Afterwards, 300 µL of an aqueous Na_2CO_3 solution (0.1 g mL^{-1}) were added. The resulting compound was kept in a thermostatic bath at 40 °C for 30 min, making it possible to observe the change of the color to blue, indicating that the anti-aging active had been released from the hydrogel film. The control assay was performed using the H2 hydrogel film at the same experimental conditions and all procedures were performed in triplicate.

Results and Discussion

Hydrogel films characterization

The physical properties of all hydrogel films on 0 and 72 days after preparation are shown in Figure 1. It can be seen that none of the films prepared showed the presence of granules or fungal growth on its surface, probably due to the use of glutaraldehyde, which may contribute to guarantee sterility in up to 72 days. The advantageous sterile characteristic of these films indicates that subsequent sterilization techniques, such as the use of ethanol, UV radiation and ethylene oxide, may be avoided. The increase in gelatin content (up to 15%) resulted in a more intense yellow coloration of the H2 and H3 materials when compared to the H1 (with 10% gelatin) hydrogel film, due to the greater degree of crosslinking between the protein and the glutaraldehyde.[9] It was also observed that the color of the films changed after 72 h, which may be related to chemical and physical changes of organic compounds, which are induced by external factors, such as temperature, humidity, and UV radiation.[21-23] Films that have color and transparency simultaneously represent an advantageous choice over completely transparent films (with absorption between 300-400 nm),[24] as they are more efficient in protecting the skin against UV-Vis radiation. Additionally, films with a very yellowish color and little transparency may not be attractive to final consumers.[9]

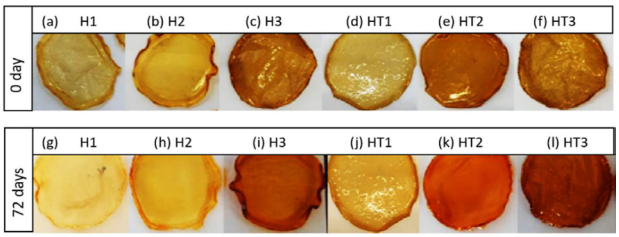

Figure 1. Hydrogel films with ca. 2 mm thickness without (a-c; g-i) and with (d-f; j-l) TA observed on 0 (a-f) and 72 (g-l) days after preparation.

The morphology of the hydrogel films that showed greater transparency (H1, HT1, H2 and HT2) were analyzed by SEM and are shown in Figure 2. From these micrographs, it was possible to verify that the surface of the H1 and H2 hydrogel films exhibited roughness, protrusions, and regions with cracks. On the other hand, the films that have TA in their composition exhibited granules (HT1) and a more leveled rough surface, as it is possible to observe in the HT2 sample, which may contribute to the adherence of the film to the skin.

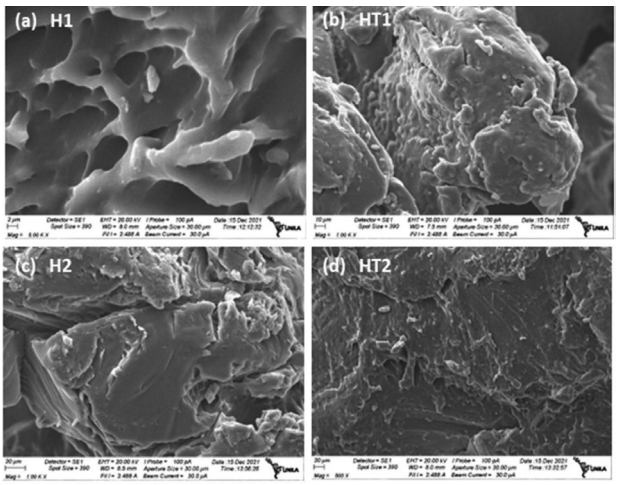

Figure 2. Scanning electron microscope (SEM) images depicting surface morphology of hydrogel films (a) H1 (mag. 5000×), (b) HT1 (mag. 1000×), (c) H2 (mag. 1000×), and (d) HT2 (mag. 500×), showing the influence of TA on film microstructure.

Figure 3a depicts TG/DTG curves of H1, HT1, H2 and HT2 hydrogel films, revealing that the thermal degradation of materials without TA (H1 and H2) occurs in three very similar steps. The first stage of weight loss happened from approximately 50 up to 120 ºC, due to the reduction of both free and bound water.[9] Consecutive weight decrease above 200 °C indicates evaporation of glycerol (which has a boiling point of 290 ºC), decomposition of amino acid residues and cleavage of the peptide bonds typically found in gelatin.[25,26] The same behavior was observed in

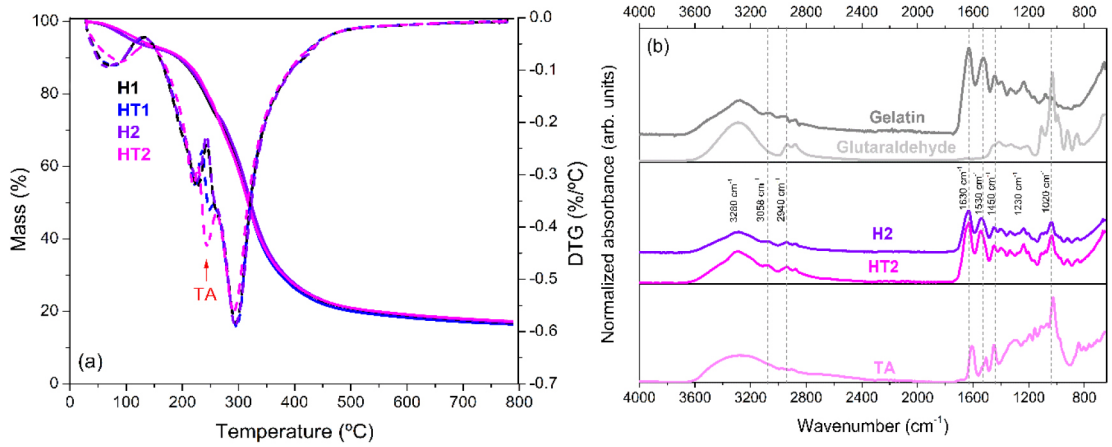

Figure 3. (a) TG/DTG obtained for H1, HT1, H2 and HT2 hydrogel films and (b) FTIR-ATR spectra acquired for H2, HT2, gelatin, glutaraldehyde and TA.

hydrogel films with TA (HT1 and HT2). However, it is possible to note that the thermal degradation of TA occurs between 200 and 300 ºC, indicating both the presence of the active and the thermal stability of the film formulation at temperatures below 50 ºC. This signals that the film is viable for storage and application on the skin at room temperature.[27]

ATR-FTIR spectrum of H2 and HT2 (Figure 3b) exhibits absorption bands that can be seen between 4000-600 cm^{-1}. A wide absorption band between 3600-3000 cm^{-1} can be assigned to –OH groups, which can also be seen in the spectra of gelatin, glutaraldehyde and TA. Furthermore, this region can also be attributed to amide-A and amide-B characteristic of gelatin.[28,29] Other absorption bands that appear at 1630 cm^{-1} (amide-I), 1570-1450 cm^{-1} (amide-II), 1240-1220 cm^{-1} (amide III), and 800-600 cm^{-1} correspond to amide IV, V and VI.[29] The absorption band at 1020 cm^{-1} is associated with the presence of C–O stretch, indicating the incorporation of glutaraldehyde and TA in the hydrogel films,[30] corroborating with the thermal analysis.

The hydrogel films H2 and HT2 presented better physical (color and aspect), structural (absorption bands characteristic of each component), thermal, and morphological characteristics; therefore, their mechanical properties were also investigated and are presented in Table 2. The mechanical tests allowed the verification of the manner in which the TA affected the characteristics of the hydrogel film, reducing the tensile strength, Young's modulus, and puncture resistance strength. Despite the reduction in these values, the HT2 hydrogel film exhibited acceptable elastic properties for skin applications, since the elongation at break (28.9 ± 3.1%) and Young's modulus (6.4 ± 1.1 MPa) are intermediate values to the ones obtained by Antosik *et al.*,[31] which indicates films with mechanical characteristics equivalent to those of human skin.

The Folin-Ciocalteau method, which is usually employed for the detection of the (poly)phenol compounds in foods and biological samples, was used for a preliminary evaluation of the ability of the HT2 hydrogel film to release TA. In this method, the reduction of Mo^{6+} to Mo^{5+} ions by phenolic compounds causes a color shift to blue.[32] According to the data shown in Figure 4a, it is possible to verify that the chromaticity of the blue coloration becomes more evident after 30 min, probably indicating a higher content of phenols released over time. Although Folin-Ciocalteau method is widely employed for the quantitative measurement of the total content of (poly)phenol compounds,[32] it can either be affected by non-phenolic reducing compounds present in the sample[33] or suffer from matrix effects.[34] Therefore, the TA release was also

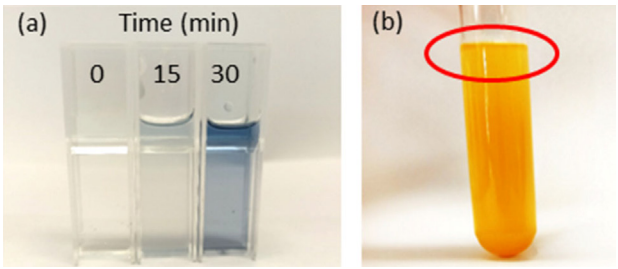

Figure 4. Qualitative assays of TA leaching by (a) Folin-Ciocalteau method and (b) ferric chloride test.

Table 2. Mechanical properties of H2 and HT2 hydrogel films

Hydrogel film	Average distance / mm	Tensile strength / N	Elongation at break / %	Young's modulus / MPa	Puncture strength / N
H2	14.4 ± 2.9	126.0 ± 1.6	28.7 ± 5.7	8.7 ± 1.4	24.0 ± 1.7
HT2	14.4 ± 2.4	92.0 ± 1.4	28.9 ± 3.1	6.4 ± 1.1	17.4 ± 1.8

detected by using the ferric chloride ($FeCl_3$) test, in which the presence of phenolic compounds in an aqueous solution is indicated by the formation of colored complex upon the addition of $FeCl_3$. Thus, the reddish ring formed when the assay was performed with the HT2 hydrogel film also indicated the presence of TA in the solution (Figure 4b).

Conclusions

Sterile hydrogel films based on gelatin, glutaraldehyde, glycerol and TA with a shelf life against fungi of up to 72 days were successfully prepared in this investigation. Absorption bands confirmed the presence of groups characteristic of each component used in the preparation of these materials, which exhibited a similar thermal degradation profile. However, in hydrogel films containing TA (HT1 and HT2), it was observed that the degradation of the active occurs between 200 and 300 ºC. In addition, the morphological and mechanical characteristics were also shaped with the addition of this active compound. Among the investigated properties, the H2 and HT2 hydrogel films showed the best results; thus, they were the only materials selected for the mechanical tests. Through these assessments, it was possible to verify that the 1% m/m of TA caused a reduction in the tensile strength, Young's modulus, and puncture strength. Despite this decrease, the values proved to be acceptable for dermatological use. Similarly, the release of TA from the films was confirmed through the Folin-Ciocalteu method and the ferric chloride test. As a result of this investigation, it was possible to verify that the release of the compound occurs slowly, being impacted by the passage of time, as it was expected. However, in order to determine precisely the quantity of TA released, more accurate analytical techniques, such as high-performance liquid chromatography (HPLC), should be employed. Due to its properties, the TA released may have a positive dermatological impact, diminishing oxidative stress on the skin and fostering anti-aging effects. Consequently, a comprehensive assessment of the controlled release is imperative to ascertain the optimal absorption of TA. Furthermore, additional procedures, such as cytotoxic assays, are required to validate the occurrence of non-toxic doses in the TA release.

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Author Contributions

All authors contributed to the study. G. B. Oliveira was responsible for formal analysis, investigation, methodology, software; I. S. Zamataro for formal analysis, investigation, methodology, software; M. S. Oliveira for formal analysis, investigation, methodology, software; A. S. Gomes for formal analysis, investigation, methodology; M. G. A. Barros for formal analysis, investigation, methodology, software; supervision; A. G. Oliveira for investigation, software, visualization, writing-original draft; K. Zanella for conceptualization, formal analysis, investigation, project administration, resources, supervision, writing-review an editing; C. C. S. Gonçalves for conceptualization, formal analysis, investigation, project administration, resources, supervision, writing-review and editing.

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