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Article

Locust bean gum hydrogels are bioadhesive and improve indole-3-carbinol cutaneous permeation: influence of the polysaccharide concentration

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The locust bean gum (LBG) is a polysaccharide with thickening, stabilizing and gelling properties and it has been used in the preparation of pharmaceutical formulations. Hydrogels (HGs) are obtained from natural or synthetic materials that present interesting properties for skin application. This study aimed to develop HGs from LBG using indole-3-carbinol (I3C) as an asset model for cutaneous application. HGs were prepared by dispersing LBG (2%, 3% and 4% w/v) directly in cold water. The formulations showed content close to 0.5 mg/g (HPLC) and pH ranging from 7.25 to 7.41 (potentiometry). The spreadability factor (parallel plate method) was inversely proportional to LBG concentration. The rheological evaluation (rotational viscometer) demonstrated a non-Newtonian pseudoplastic flow behavior (Ostwald De Weale model), which is interesting for cutaneous application. The HET-CAM evaluation showed the non-irritating characteristic of the formulations. The bioadhesive potential demonstrated bioadhesion in a concentration-dependent manner. Permeation in human skin using Franz cells showed that the highest LBG concentration improved the skin distribution profile with greater I3C amounts in the viable skin layers. The present study demonstrated the feasibility of preparing HGs with LBG and the formulation with the highest polymer concentration was the most promising to transport active ingredients through the skin.

Keywords: Locust bean gum. Topical application. Hydrogels. Indole-3-carbinol. Skin permeation.

INTRODUCTION

The potential characteristics presented by the use of natural polysaccharides have been the subject of research because of their low cost, ease of purchase, biocompatibility and patient acceptance. Natural gums are versatile and present different structures and unique rheological properties. They act as stabilizing, emulsifying and viscosity modifying agents besides being biodegradable and biocompatible polymers. Due to their great potential, the application of these natural polymers has been explored in biotechnology, food, textile and pharmaceutical industries (Ahmad *et al.*, 2019; Saidin, Anuar, Meor Mohd Affandi, 2018). Regarding pharmaceutical products, natural gums are used to produce gels, films and controlled-release devices, where these polymers can be used in an isolated way, in association with other gums or be chemically modified to meet the desired requirements for drug delivery systems (Ahmad *et al.*, 2019; Akkaya *et al.*, 2020; Jana *et al.*, 2015).

Considering polysaccharides, the locust bean gum (LBG) is a non-ionic polysaccharide composed of mannose and galactose (ratio 4:1). It is classified as a high molecular weight galactomannan obtained from the endosperm of locust bean seeds (*Ceratonia siliqua L.*). This vegetable

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gum is on the rise in the pharmaceutical area due to its thickening, gelling and stabilizing properties in a wide pH range and temperature (Verma *et al.*, 2019; Zhu *et al.*, 2019). In addition, LBG is being used in the preparation of pharmaceutical formulations because of its ability to modulate the active principle release, promoting a sustained release (Coviello *et al.*, 2015; Pettinelli *et al.*, 2020). Thus, the LBG use has already been evidenced in microspheres (Jana *et al.*, 2015), hydrogels (Coviello *et al.*, 2015), films (Akkaya *et al.*, 2020; Kaur *et al.*, 2019) and hard capsule wrappers (He *et al.*, 2017).

Regarding its cutaneous application, LBG has already been used in the development of films in association with carrageenan gum for the transdermal release of curcumin with adequate mechanical properties and controlled release (Kaur *et al.*, 2019). Zepon and collaborators (2019) developed LBG and k-carrageenan films containing cranberry extract with antimicrobial potential. Akkaya and colleagues (2020) developed LBG and agar films with interesting properties, such as cellular hyperproliferation and antimicrobial properties for the treatment of wounds.

Coviello and collaborators (2015) developed niosomes containing ammonium glycyrrhizate incorporated into hydrogels prepared with xanthan gum and LBG at 1%. The formulations were stable and able to promote a controlled release of the active substance. Pettinelli and collaborators (2020) developed LBG and K-carrageenan hydrogels containing microparticles of ketoprofen and mupirocin. The associated gums and microparticulate system combination showed a controlled release profile and biocompatibility. Although LBG stands out in the pharmaceutical field, it should be highlighted that most studies have used LBG in association with other gums (Akkaya *et al.*, 2020; Coviello *et al.*, 2015; Pettinelli *et al.*, 2020) with no studies reporting its isolated use to obtain cutaneous hydrogels.

Hydrogels are semi-solid formulations consisting of a three-dimensional polymeric network that absorbs and retains water in interstitial spaces. The bonds and intermolecular interactions responsible for the formation of the three-dimensional network classify the hydrogels as physical or chemical ones. In physical hydrogels, the molecular tangle is formed by non-covalent bonds, while in chemical hydrogels, covalent bonds form the polymeric network, normally by the addition of a crosslinking agent. A third type of hydrophilic systems includes viscous matrices that do not form "true" gels, that is, three-dimensional networks, but form dispersions that are very similar to conventional gels. These formulations are obtained from the use of polymers of natural or synthetic origin and they stand out for being hydrophilic bases of easy application and quick drying (Ahmad *et al.*, 2019; Ullah *et al.*, 2015). For this reason, hydrogels have been used to deliver active ingredients on the skin, facilitating the permeation and promoting controlled release (Ferreira *et al.*, 2020; Pettinelli *et al.*, 2020; Sari *et al.*, 2020).

Currently, there is a growing interest in active substances of natural origin to develop pharmaceutical products. In this context, indole-3-carbinol (I3C) is a compound obtained through glycosinolates hydrolysis, which are present in cruciferous vegetables of *Brassica* genus (Ahmad, Sakr, Wahidur Rahman, 2011). This bioactive has a log-P of 1.39 and a solubility of 3.75 mg/mL in water, being easily soluble in organic solvents. In addition, studies have demonstrated its anti-inflammatory, antioxidant and antitumor potential (Gehrcke *et al.*, 2017; Kim, Park, 2018). Therefore, it is believed that due to its ability to inhibit inflammatory processes, this natural compound may play an important role in treatments of skin disorders.

Despite I3C potential, it is known that it undergoes oligomerization reactions when in contact with the stomach acid, which results in its low bioavailability to oral administration (Banerjee *et al.*, 2011). Therefore, it is interesting to develop semi-solid formulations containing I3C to be applied on skin, improving its performance and bypassing the limitations associated to its oral administration. In addition, as far as we know, this is the first report on I3C skin permeation. This way, this study was designed to develop LBG-based hydrogels using I3C as a bioactive model to suggest a new alternative hydrophilic vehicle for cutaneous application.

MATERIAL AND METHODS

Material and solvents

I3C was obtained from Sigma Aldrich (São Paulo, Brazil). LBG was kindly donated by CP Kelco (Limeira,

Brazil). Propylene glycol was purchased from Impex (São Paulo, Brazil). All other solvents and reagents used had analytical grade.

Hydrogels preparation

Hydrogels were prepared using LBG at 2%, 3% and 4% (w/v). The gum was transferred to a glass mortar (0.2, 0.3 or 0.4 g for 2%, 3% or 4%, respectively) and dispersed in 400 µL of propylene glycol using a pestle. Then 9.5 mL of cold ultrapure water was slowly added to this solution. The prepared base remained resting for 24 h at room temperature (20 - 30 °C). In sequence, the I3C (5 mg) previously solubilized in 100 µL of propylene glycol was incorporated into the formulation. The theoretical final bioactive concentration in hydrogels was 0.5 mg/g and the formulations were named HG-LBG-2%, HG-LBG-3% and HG-LBG-4%.

Hydrogels characterization

pH determination

The hydrogels pH was assessed by diluting 1.0 g of formulation in 10.0 mL of ultrapure water (10% w/v). The dilution was submitted to magnetic stirring until its complete dispersion. Then, the pH was determined in a potentiometer (model pH 21, Hanna Instruments, Brazil) previously calibrated and at room temperature. The analyses of each hydrogel were performed in triplicate (n = 3).

Indole-3-carbinol content determination

The I3C content in the formulations was determined by diluting an amount (0.45 g) of hydrogel in methanol, which was kept under magnetic stirring for 20 min and then taken to the ultrasound bath for an equal period to ensure the complete bioactive extraction. The samples were filtered on a 0.45 µm membrane and analyzed by high performance liquid chromatography (HPLC), using the methodology previously validated by our research group (Gehrcke *et al.*, 2017). The content determination was assessed in triplicate for each hydrogel (n = 3).

It was used the LC 10A HPLC (Shimadzu, Japan) system equipped with an LC-20AT pump, a UV-VIS SPD-M20A detector, a CBM-20A system controller, a SIL-20A HT automatic injection valve system and a RP C_{18} LiChrospher Phenomenex column (250 mm x 4.00 mm, 5 µm; 100 Å) maintained at room temperature (24 °C). The mobile phase consisted of water and acetonitrile (70:30, v/v) with a flow of 1.0 mL/min, detection at a wavelength of 288 nm and an injection volume of 20 µL.

Spreadability factor

The parallel plate method was used to determine the spreadability (Rigo *et al.*, 2012). A plate containing a 10 cm scale was placed on an HP Officejet 4500 desktop scanner. On the top, it was placed a mold plate with a central hole (1 cm) where the sample was leveled, in sequence, the mold was removed, leaving only the hydrogel on the base glass. Subsequently, a glass plate with known weight was placed on the top of the sample and the spread sample image was captured after 45 s. This procedure was repeated with a total of 10 plates, one per minute. The scattered surface was calculated using the Image J 1.49q software. Spreadability analyses were performed in triplicate for each formulation in the same day at 25 - 30 °C. The spreadability factor was determined applying equation 1.

$$Spreadability = \frac{A}{W} \quad (1)$$

A corresponds to the dispersed area (mm^2) and W corresponds to the plates' accumulated weight (g).

Rheological behavior

The rheological evaluation was performed at 25 ± 1 °C temperature using a Brookfield rotational viscometer DV-I prime model, requiring approximately 30 g of each hydrogel (n = 3). *Spindles* RV03 (HG-LBG-2%), RV06 (HG-LBG-3%, HG-LBG-4%) and speeds in the range of 0.05 s^{-1} to 1.67 s^{-1} were selected by preliminary tests to maintain the ideal torque (10-90%), ensuring analyses reliability. To evaluate the rheological model that best

describes hydrogels behavior, Bingham equations (2), Casson (3) and Ostwald-de-Waele (4) were tested.

$$\sigma = \sigma_0 + \eta.\gamma \quad (2)$$

$$\sigma^{1/2} = \sigma^{1/2} + \eta^{1/2}.\gamma^{1/2} \quad (3)$$

$$\sigma = K.\gamma^{\eta} \quad (4)$$

η is viscosity, σ is shear stress, γ shear rate, *n* is plasticity index, *K* is consistency coefficient and σ_0 is yield stress.

Irritation potential assessment

The irritation potential of the formulations was determined by the chorioallantoic membrane assay (CAM), using embryonated chicken eggs on the 10th day of fertilization donated by Languiru Company (Teutônia-RS, Brazil). This test was carried out in accordance with the Interinstitutional Committee rules for the validation of alternative methods (ICCVAM) and it was approved by the Institutional Committee for Animal Care and Use of the Federal University of Santa Maria (protocol number 5428271020/2021). For this purpose, the fertilized chicken eggs had their shell and outer membrane removed with the aid of tweezers, being exposed the CAM, which was carefully washed with physiological solution (0.9% NaCl). Then, 0.3 g of each formulation ($n = 5$) was added to about 50% of CAM. The occurrence of irritating phenomena such as hemorrhage, lysis and coagulation were observed in the uncovered portion for a period of 300 s.

Aiming to observe the isolated I3C possible irritating effect, the isolated bioactive was evaluated in a solution at the same concentration present in the hydrogels (aqueous solution of I3C containing 10% propylene glycol at 0.5 mg/mL), as well as the vehicle used for solubilization. 300 µL of I3C solution and the vehicle were added to the entire CAM for 20 s. Then CAM was washed with 0.9% NaCl and analyzed to check if the phenomena would happen. Solutions of 0.9% NaCl and 0.1 M NaOH were used as negative and positive controls, respectively. All experiment phases were photographed. The irritation score (IS) was determined according to equation 5 and, from this, the formulations were classified as non-irritant (0 - 0.9); irritant (1 - 4.49); moderately irritating (5 - 8.9) and severely irritant (9 - 21).

$$IS = 5.\frac{301-h}{300} + 7.\frac{301-l}{300} + 9.\frac{301-c}{300} \quad (5)$$

h= bleeding time; *l*= lysis time; and *c*= coagulation time.

Bioadhesive potential

The bioadhesive strength was determined according to Osmari and collaborators (2020) methodology, with some modifications. For this test, discard healthy skins obtained from abdominoplasty surgery performed in female patients were used. This experiment was approved by the Research Ethics Committee of the Federal University of Santa Maria (CAAE: 27168719.4.0000.5346; number: 895.464). After obtaining the skin, the adipose tissue was removed, and the skin was kept under refrigeration until its use. To perform this experiment, the skin was cut and fixed on a glass plate. In an upper support 0.8 g of each formulation ($n = 3$) was added, which were kept in contact with the skin for 1 min, applying the force of 1 N. Subsequently, in a falcon tube positioned in the other end of the apparatus, water was steadily added until the separation between skin and formulation happened. The amount of water added was measured in a beaker to determine the volume required for the detachment. For comparative purposes, a 0.5% Carbopol® Ultrez hydrogel (HG-CARBOPOL) was also evaluated as a positive bioadhesion control since this substance is well recognized has bioadhesive polymer. The bioadhesive strength was determined using equation 6. The analyses were carried out at 25 ± 1 °C.

$$Bioadhesive\ strength = \frac{m.g}{A} \quad (6)$$

m corresponds to the necessary amount of water for the detachment, *g* gravity acceleration (980 cm s^2) and *A* is contact area (3.14 cm^2).

Skin permeation study

The permeation study was performed according to Rigon and collaborators (2019). To perform this experiment, Franz diffusion cells and circular cuts of human skin were used as membrane. The human skin was obtained from abdominoplasty surgeries and had the subcutaneous tissue removed with a scalpel and the skin thickness standardized with a digital caliper. Afterwards, the skin was stored at -20 °C until the time of the experiment (CAAE: 27168719.4.0000.5346; number: 895.464). The skin was positioned with the dermal layer facing the receptor medium, which consisted of pH 7.4 phosphate buffer (32 °C) and *stratum corneum* facing the donor compartment, where 0.5 g of each formulation was added ($n = 4$). After 8 h of incubation, the skin was removed, the excess of formulation was removed, and the recipient medium was collected and filtered for further HPLC analysis.

To quantify the active in different skin layers, the *stratum corneum* was removed by tape stripping technique, using 18 tapes (Qualitape, Adelbras®, Brazil). Each tape was uniformly pressed three times on the skin with the aid of a glass stick, and then placed in test tubes (3 tapes per tube) containing 4 mL of methanol. The dermis and epidermis underwent a thermal bath (60 °C for 45 s) and were separated using a spatula. After separation, the layers were transferred to test tubes containing methanol (1 mL for epidermis and 2 mL for dermis). All samples were submitted to vortex (2 min) and ultrasound (15 min), for the complete I3C extraction. Finally, the samples were filtered through a 0.45 µm membrane and analyzed in HPLC according to the previous described methodology (section 2.3.2).

Statistical analysis

Data was expressed as mean ± standard deviation. The statistical analysis was performed through *t* test or one-way analysis of variance (ANOVA) followed by Newman-Keuls test. Values of $p < 0.05$ were considered significant. All analyses were performed using the GraphPad Prism® version 6 statistical program (San Diego, USA).

RESULTS AND DISCUSSION

Natural polysaccharides have been extensively explored for formulations of pharmaceutical products. LBG is an attractive excipient with interesting functional properties for the development of pharmaceutical formulations. It is important to emphasize that there are reports in the literature on the development of films and hydrogels for the cutaneous route using LBG in association with other polysaccharides; however, there are no studies about the preparation of hydrogels only with LBG. Therefore, a concentration range of 1% to 5% was tested to obtain hydrogels, where it was found that the lowest concentration (1%) was not efficient to obtain a semi-solid with adequate viscosity for cutaneous application. On the other hand, the concentration of 5% led to a very consistent hydrogel with the presence of lumps.

This way, concentrations of 2%, 3% and 4% were chosen to work. Even in the established range, LBG was resistant to disperse in water, so an overnight process was necessary to make this polysaccharide swell, as well as the use of propylene glycol, applied to help with LBG solubility. Observing its compatibility with the formulation, the same solvent was used for the active ingredient.

Hydrogels are semi-solid bases that have become advantageous because of their hydrophilic character, easy application and removal that gives a refreshing aspect when applied on the skin (Mayba, Gooderham, 2018). After preparation, the formulations remained macroscopically homogeneous without the presence of precipitates and with a translucent visual aspect, as shown in Figure 1. This aspect agrees with other studies that used natural gums to obtain semi-solid formulations (Ferreira *et al.*, 2020; Osmari *et al.*, 2020).

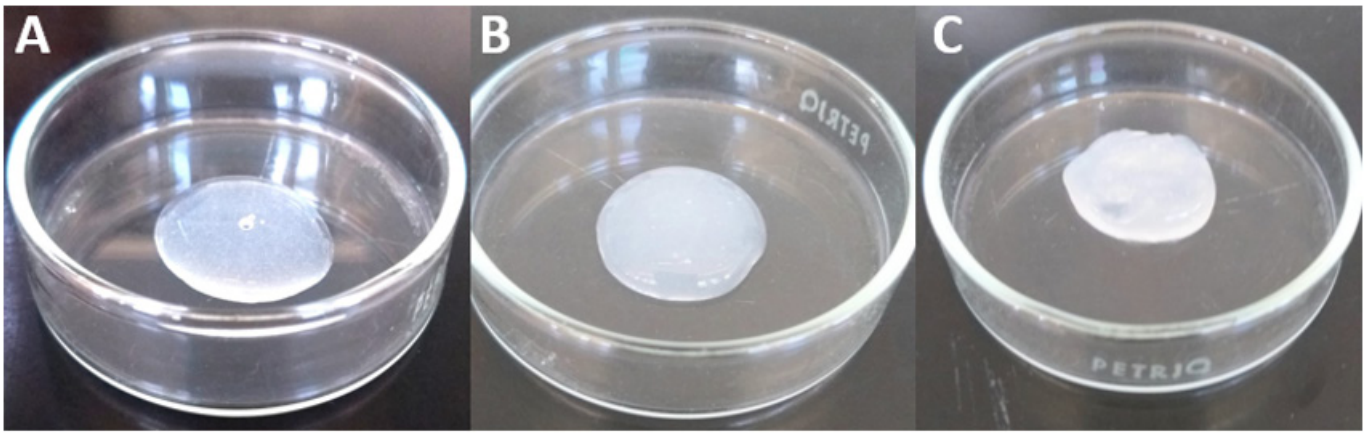

FIGURE 1 - Macroscopic appearance of hydrogels: HG-LBG-2% (A); HG-LBG-3% (B) e HG-LBG-4% (C).

The pH of formulations containing I3C is a parameter that requires caution, since this bioactive under acidic conditions undergoes oligomerization, resulting in condensation products (Banerjee *et al.*, 2011). The developed hydrogels presented pH values in the neutrality range (Table I), ensuring the I3C stability. Similar results have been reported for hydrogels obtained from natural gums or synthetic polymers (Ahmad, *et al.*, 2019; Ferreira *et al.*, 2020; Dalla Lana *et al.*, 2020; Sari *et al.*, 2020).

TABLE I - Characterization of locust bean gum hydrogels in terms of pH, I3C content, spreadability, total amount of I3C retained in the skin and in the receptor medium

	pH	I3C content (mg/g)	Spreadability factor (mm^2/g)	Total I3C retained skin ($\mu g/cm^2$)	Total I3C receptor medium (µg/mL)
HG-LBG-2%	7.4 ± 0.2	0.56 ± 0.04	7.26 ± 0.94 $^{++}$	33.6 ± 9.9	10.35 ± 4.05 $^{+}$
HG-LBG-3%	7.3 ± 0.1	0.51 ± 0.07	6.52 ± 0.84*	30.1 ± 3.1	8.44 ± 1.23
HG-LBG-4%	7.3 ± 0.1	0.55 ± 0.06	4.02 ± 0.74	22.2 ± 4.9	4.43 ± 2.31

Mean ± standard deviation (n=3). One-way ANOVA followed by Newman-Keuls. (+) $p < 0.05$, (++) $p < 0.01$: statistical difference between HG-LBG-2% and HG-LBG-4%. (*) $p < 0.05$: statistical difference between HG-LBG-3% and HG-LBG-4%.

In addition, Sharadha *et al.* (2020) comment on how vehicles with acidic or basic pH can damage the skin and, therefore, formulations with moderate pH are more suitable for cutaneous application. Chin and collaborators (2019) also reported that pH values in a more neutral range may be compatible with chronic skin injuries. Regarding the I3C content in the hydrogels, it remained close to theoretical value, which indicates that there were no I3C losses or degradation during the preparation, keeping the concentrations close to 100% (Table I).

Spreadability is an important assessment in the development of semi-solid formulations, as it verifies the spreading ability of a formulation on a surface. In addition, this parameter is related to the adequate transfer of the dose to the application area, determining the product acceptability (Debebe *et al.*, 2018). Hydrogels spreadability profiles (Figure 2), as well as the

spreadability factor (Table I), show that LBG influenced on the formulations ability to spread over the surface, since HG-LBG-2% and HG-LBG-3% had a greater spreadability factor compared to HG-LBG-4% ($p < 0.05$). This result was expected because higher proportions of polymer can increase the interaction between polymer chains, increasing viscosity and reducing spreadability (Barakat, 2010).

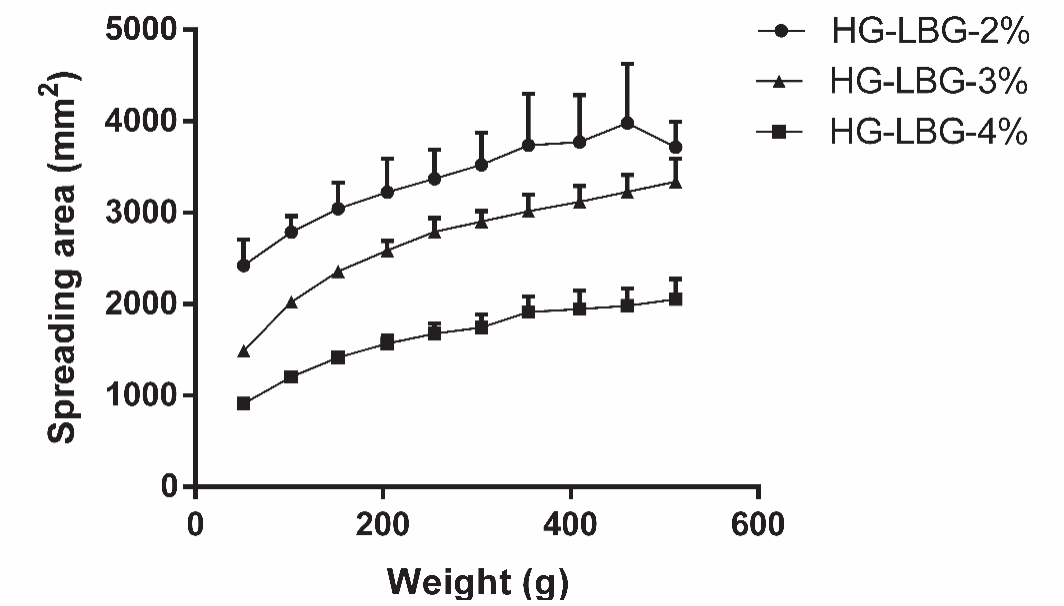

FIGURE 2 - Spreadability profiles of hydrogels. Mean ± standard deviation ($n = 3$).

Another parameter to be considered is the rheological evaluation, which indicates the flow properties. The hydrogels showed non-Newtonian flow, since the viscosity reduces (Figure 3) and the shear stress increases (Figure 4) with the increase in the applied shear rate (Teixeira *et al.*, 2017). According to the correlation coefficients (r) shown in Table II, all formulations were better fitted to the Ostwald-de-Waele model, indicating pseudoplastic flow, which does not need a minimum initial force to start flowing (Osmari *et al.*, 2020). The flow index values (n) confirm the pseudoplastic behavior because they were less than 1 (Table II) (Teixeira *et al.*, 2017). Similar profiles have already been reported in other studies that used LBG (He *et al.*, 2017) and hydrogels from other natural gums (Osmari *et al.*, 2020; Sari *et al.*, 2020). This behavior is interesting for the intended administration route as it indicates adequate flow on the skin and it is easy to apply, especially considering skin disorders, where the skin may be injured, avoiding pain and discomfort to the patient (Pegoraro *et al.*, 2020).

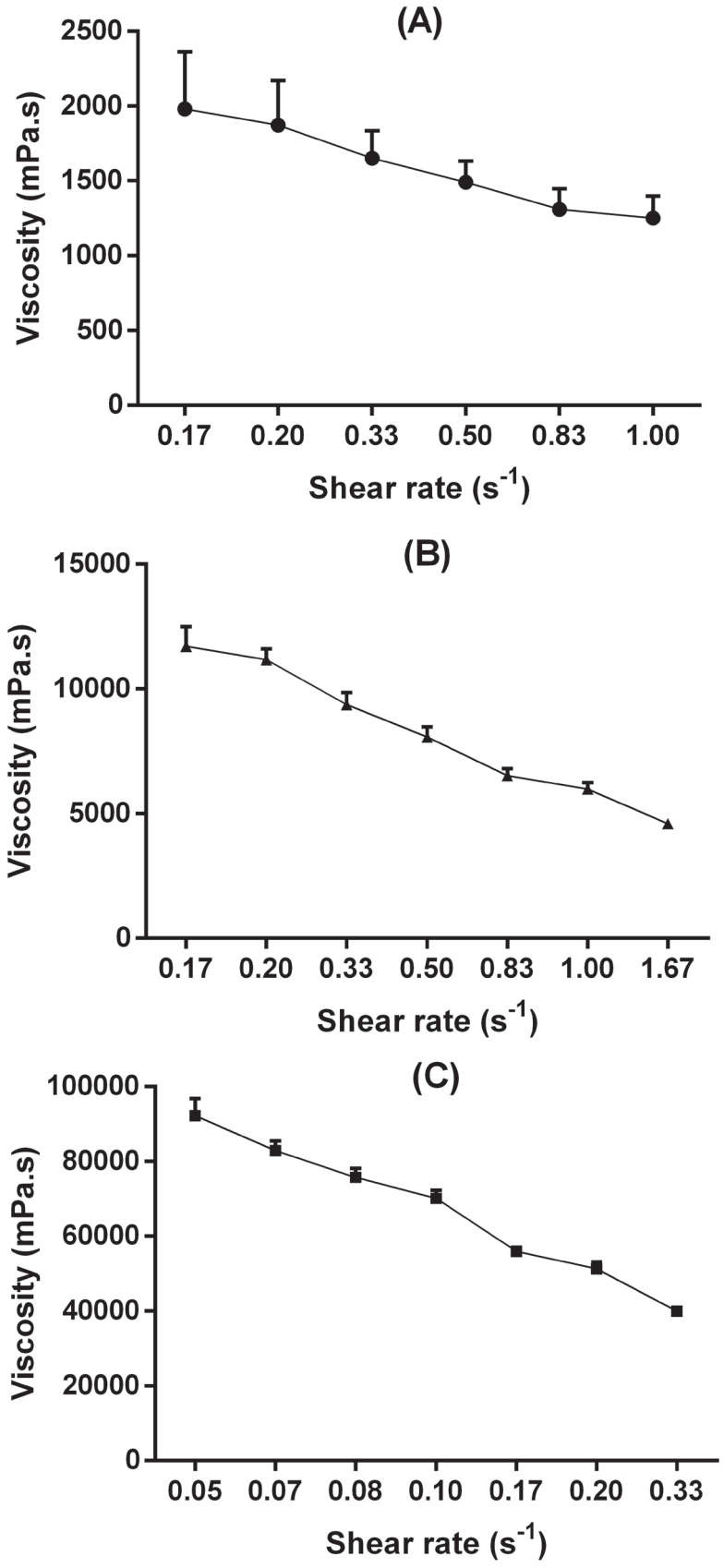

FIGURE 3 - Rheograms of hydrogels: HG-LBG-2% (A), HG-LBG-3% (B) and HG-LBG-4% (C). Mean ± standard deviation (*n* = 3).

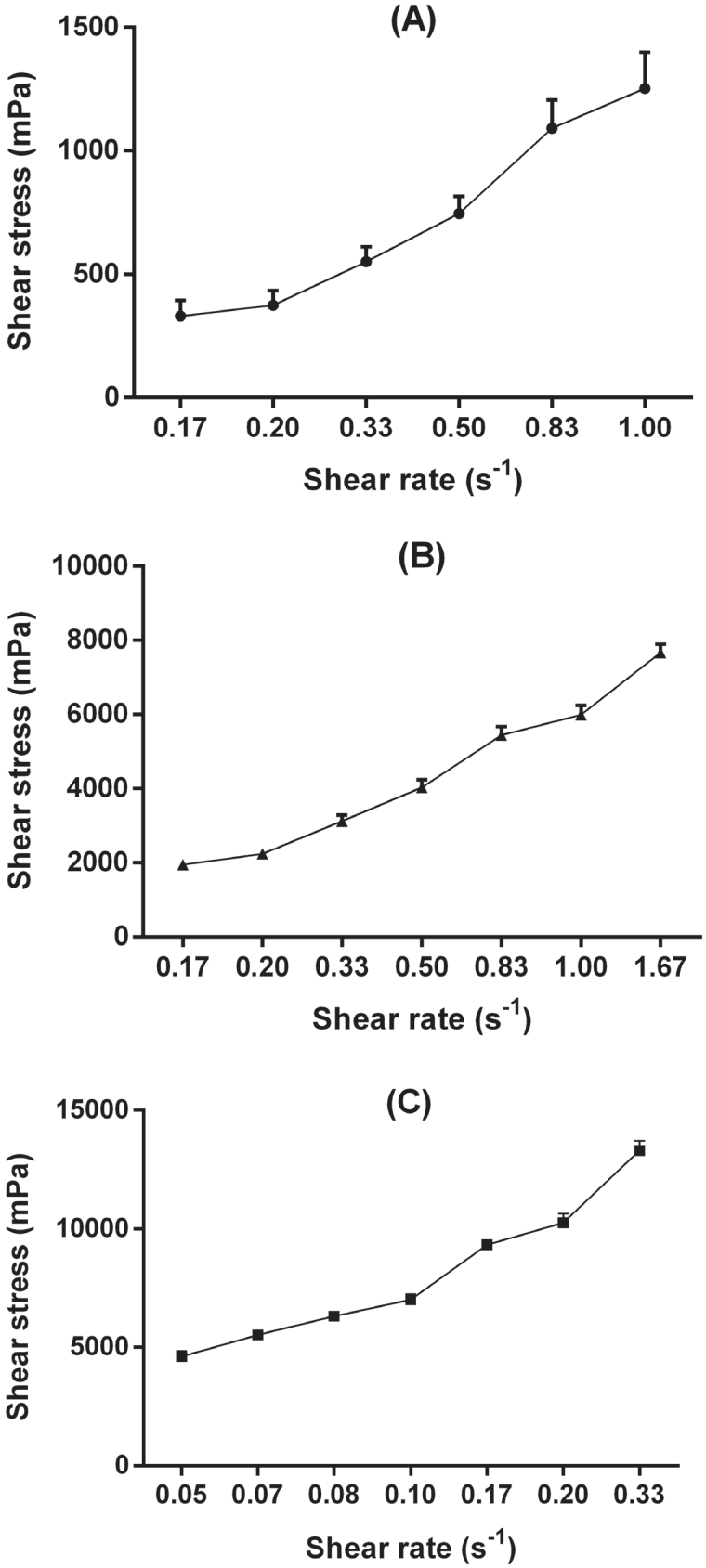

FIGURE 4 - Flow curves of hydrogels: HG-LBG-2% (A), HG-LBG-3% (B) and HG-LBG-4% (C). Mean ± standard deviation (*n* = 3).

TABLE II - Correlation coefficients (*r*) obtained for different mathematical models, flow index (*n*) and consistency index (K) obtained for different hydrogels

	HG-LBG-2%	HG-LBG-3%	HG-LBG-4%
Binghan	0.993 ± 0.003	0.961 ± 0.006	0.977 ± 0.002
Casson	0.997 ± 0.001	0.982 ± 0.003	0.990 ± 0.002
Ostwald-de-Waele	0.999 ± 0.000	0.996 ± 0.001	0.996 ± 0.004
Flow index *(n)* and consistency index (K)			
n	0.750 ± 0.042	0.600 ± 0.012	0.558 ± 0.015
K	59.17 ± 15.91	507.1 ± 41.7 ***	2549.2 ± 132.1

Mean ± standard deviation (*n* = 3). Unpaired t-test: (***) statistical difference between HG-LBG-3% and HG-LBG-4% ($p < 0.001$).

From the rheological measures, the consistency index (K) was calculated, which is related to the resistance to flow, that is, to the product's own viscosity. It was possible to notice that the highest K value occurs in the formulation where the LBG concentration was higher, indicating an increase in the consistency of HG-LBG-4% when compared with HG-LBG-3% ($p < 0.05$). In relation to HG-LBG-2%, it was not possible to carry out a direct comparison of K value with other values because this formulation was analyzed under different conditions, consequently making the comparison impossible. However, analyzing the values represented in Table II, it may be suggested that the formulation suffers the same influence of the gum concentration in relation to viscosity.

K data corroborate the data that were observed for spreadability, since the hydrogels fluidity behaves inversely proportional to the polymer concentration, which promotes increased resistance to deformation. This property is important, as it may directly influence on the intimate contact between the hydrogel and skin, favoring the permanence of the formulation on the application site (Calixto *et al.*, 2015).

The safety profile investigation and their constituents are something of great relevance. CAM model has been increasingly used to assess the irritation potential of formulations developed for application by different routes, including skin (Gehrcke *et al.*, 2021; Prado *et al.*, 2021). Thus, all developed hydrogels were tested in CAM, as shown in figure 5. There were no changes in CAM observing the images that were recorded at zero time and after 300 s of exposure. The same procedure was performed with the positive and negative controls, and they were classified as moderate irritant (IS = 8.0 ± 0.1) and non-irritant, respectively.

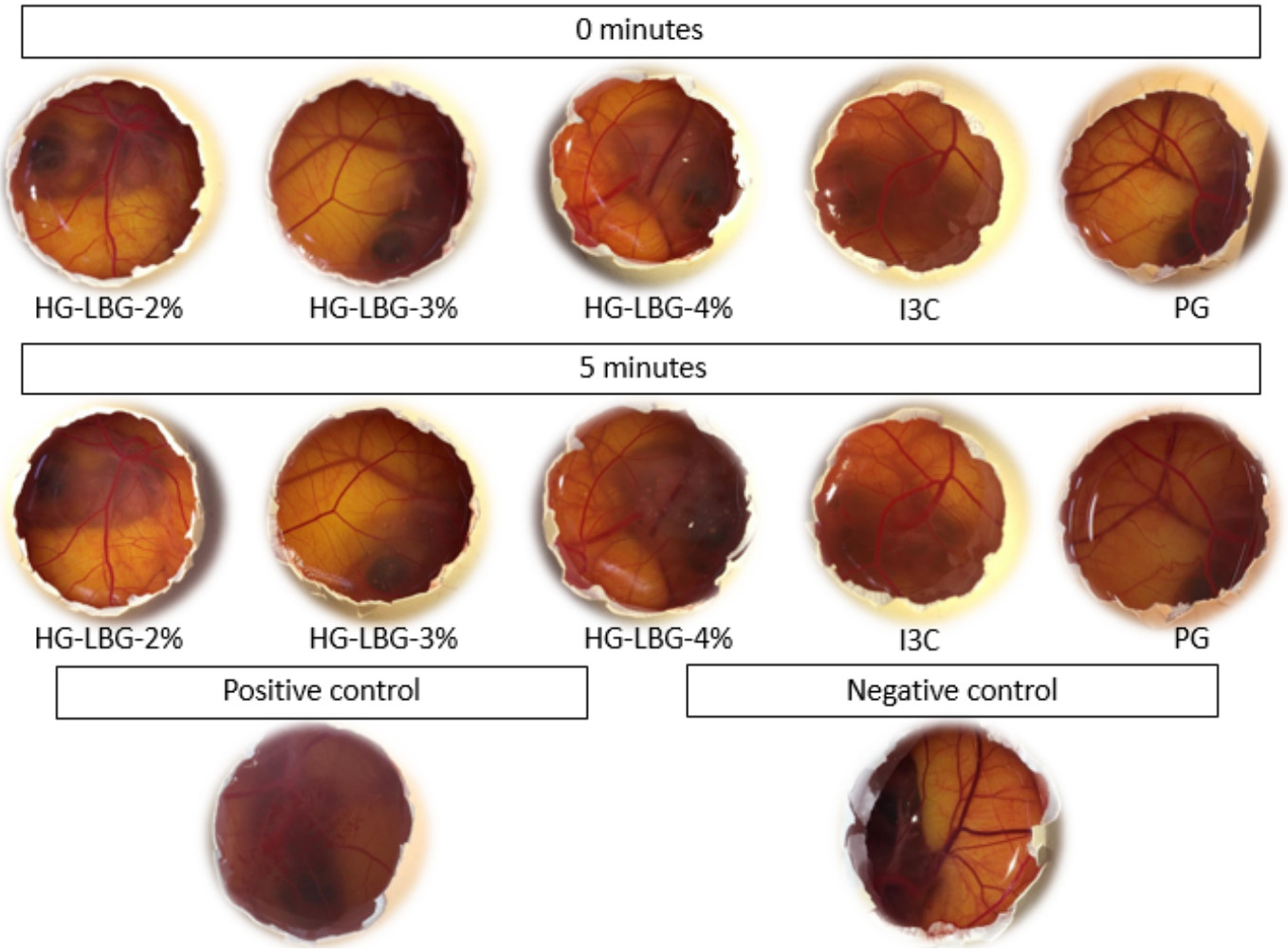

FIGURE 5 - Chorioallantoic membrane test. Images at time zero and after 300 seconds of exposure.

This way, the developed hydrogels were classified as non-irritant. In addition, different assessments also indicated the LBG biosafety. Meena *et al.* (2018) developed a cryogel from LBG and chitosan to obtain a hemostatic dressing, which proved to be cyto- and hemocompatible. Other studies have also evaluated the cytotoxicity of formulations containing LBG in fibroblasts cell lines, demonstrating their biocompatibility (Akkaya *et al.*, 2020; Pettinelli *et al.*, 2020).

It is known that an effective drug delivery through the skin can be limited by the time that a formulation remains on skin. In this way, LBG-HGs bioadhesive properties were evaluated in comparison to HG-CARBOPOL, a gel-forming agent frequently used because of its bioadhesion capacity (Kumar *et al.*, 2014). Observing the results, in Figure 6, it can be seen the bioadhesive capacity in increasing order ($p < 0.05$): HG-LBG-2% <HG-LBG- 3% <HG-LBG-4%. The last hydrogel has a potential similar to HG-CARBOPOL ($p > 0.05$). Therefore, the increasing concentration of the gum proportionally increased the hydrogels bioadhesion.

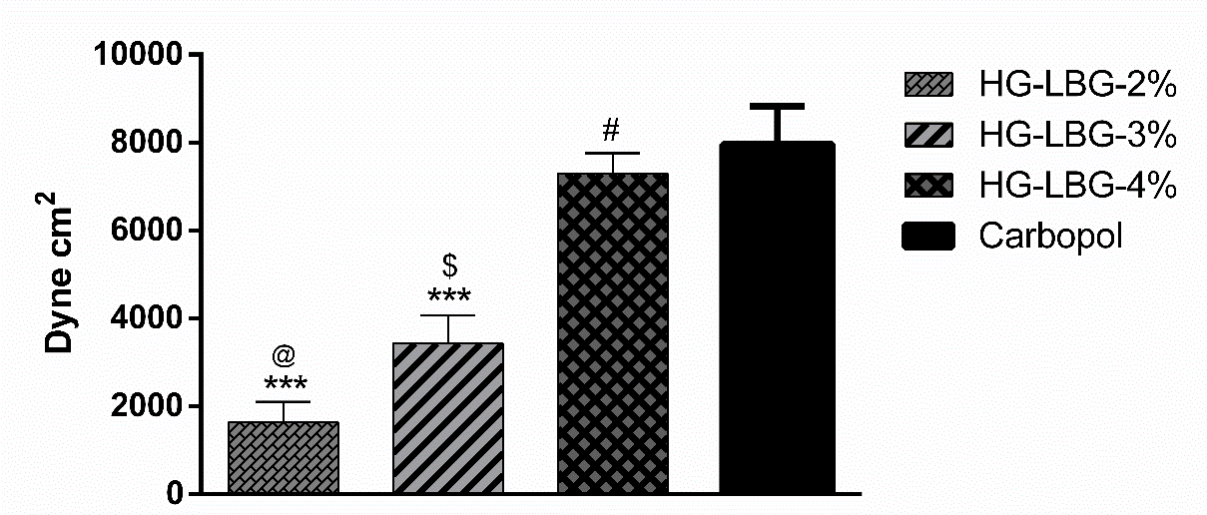

FIGURE 6 - Bioadhesive profile of hydrogels on the skin. Mean ± standard deviation (n = 3). One-way ANOVA followed by Newman-Keuls: (***) indicates difference between Carbopol and other hydrogels ($p < 0.001$), (@) indicates difference between HG-LBG-2% and other LBG hydrogels ($p < 0.05$), ($) indicates difference between HG-LBG-3% and other LBG hydrogels ($p < 0.05$) and (#) indicates difference between HG-LBG-4% and other LBG hydrogels.

Previous studies have investigated LBG tablets bioadhesive potential when combined with other gums for oral or vaginal use (Cazorla-Luna *et al.*, 2019; Deshmukh, Jadhav, Sakarkar, 2009; Vijayaraghavan, Vasanthakumar; Ramakrishnan, 2008). LBG structural characteristics, such as high molecular weight and the presence of carbonyl groups, could contribute to a greater interaction with the skin (Zhu *et al.*, 2019). The result may indicate that if the accumulation of polymer on the biological surface is higher, more units of polymer chains are available to interact with the tissue by Van der Waals forces and hydrogen bonds, strengthening the adhesion and contributing to retain the formulation on the chosen site (Timur *et al.*, 2019).

Skin permeation studies are performed to assess the substances penetration through the skin and I3C distribution in the different layers. After 8-hour incubation, the total I3C permeation in the skin from different hydrogels showed no significant difference ($p > 0.05$) (Table I). However, different amounts of LBG influenced on the bioactive distribution profile in skin layers.

Hydrogels caused the retention of a greater amount of active in the *stratum corneum* regardless of the polymer concentration ($p < 0.05$). Regarding the 2% concentration of LBG in the hydrogels, there is no difference among other layers ($p > 0.05$). On the other hand, at 3% and 4% it is possible to observe that there is a longer permanence of I3C in the epidermis in relation to dermis (Figure 7). Concerning the receptor medium, it was observed that the HG-LBG-4% presented a lower amount of I3C when compared to the HG-LBG-2% ($p<0.05$) (Table I).

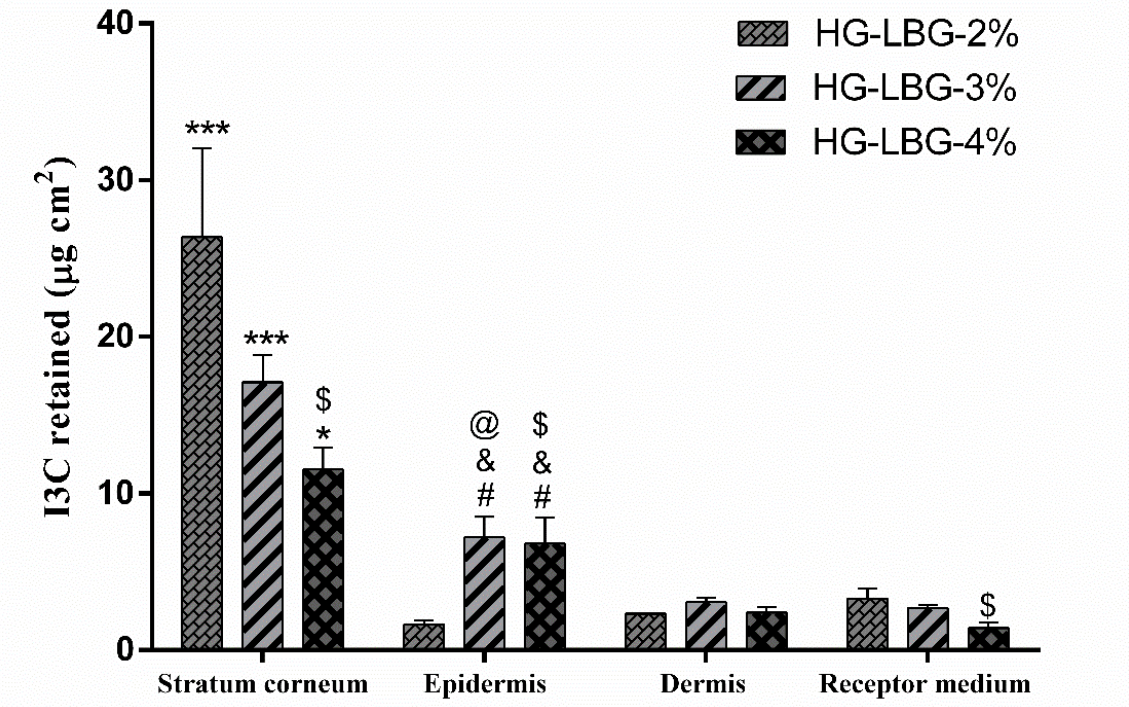

FIGURE 7 - Amount of I3C (µg/cm²) found in the different layers of the skin. Mean ± standard deviation ($n = 4$). One-way ANOVA followed by Newman-Keuls: (*) $p < 0.05$, (***) $p < 0.001$: difference between stratum corneum and other layers exposed to the same hydrogel. (#) $p < 0.05$: difference between epidermis and dermis exposed to the same hydrogel. (@) $p < 0.05$: difference HG-LBG-2% and HG-LBG-3% in the same skin layer. ($) $p < 0.05$: difference between HG-LBG-2% and HG-LBG-4% in the same skin layer.

The results found for skin permeation seem to be related to a bioadhesive profile, where HG-LBG-4% showed superior bioadhesive potential, which may have increased the interaction of this hydrogel with the skin, promoting greater active retention in the epidermis and less in the receptor medium. Dalla Lana and collaborators (2020) developed hydrogels from Pemulen® TR-2, a polymer with known bioadhesive potential, for dermatomycoses treatment. The results showed a significant active substance retention in the epidermis and its absence in the receptor medium.

It is known that a greater amount of active in viable layers (epidermis or dermis) is something desirable in most skin pathologies, allowing an improved therapeutic effect (Sari *et al*., 2020; Yuan *et al*., 2020). Besides, as it is a local therapy, it is important that the active does not reach the systemic circulation (Yuan *et al*., 2020). Such characteristics are found with the LBG increase in the hydrogels preparation, being the HG-LBG-4% the one that best meets these requirements.

CONCLUSION

LBG hydrogels showed bioadhesivity and skin permeation profile of I3C in a concentration-dependent manner, with higher LBG concentrations resulting in a bioadhesive potential increase. The hydrogels bioadhesivity may have contributed to an improved skin distribution profile with greater I3C amounts in the viable skin layers, while decreased the possibility of systemic absorption. In this way, the LBG hydrogel with higher polysaccharide concentration may be a promising vehicle for I3C delivery through the skin.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Ahmad A, Sakr WA, Wahidur Rahman KM. Mechanisms and therapeutic implications of cell death induction by indole compounds. Cancers (Basel). 2011;3(3):2955-2974. doi:10.3390/cancers3032955

Ahmad S, Ahmad M, Manzoor K, Purwar R, Ikram S. A review on latest innovations in natural gums based hydrogels: Preparations & applications. Int J Biol Macromol. 2019;136:870-890. doi:10.1016/j.ijbiomac.2019.06.113

Akkaya NE, Ergun C, Saygun A, Yesilcubuk N, Akel-Sadoglu N, Kavakli H, et al. New biocompatible antibacterial wound dressing candidates; agar-locust bean gum and agar-salep films. Int J Biol Macromol. 2020;155:430-438. doi:10.1016/j.ijbiomac.2020.03.214

Banerjee S, Kong D, Wang Z, Bao B, Hillman GG, Sarkar FH. Attenuation of multi-targeted proliferation-linked signaling by 3,3'-diindolylmethane (DIM): From bench to clinic. Mutat Res - Rev Mutat Res. 2011;728(1-2):47-66. doi:10.1016/j.mrrev.2011.06.001

Barakat NS. Evaluation of glycofurol-based gel as a new vehicle for topical application of Naproxen. AAPS PharmSciTech. 2010;11(3):1138-1146. doi:10.1208/s12249-010-9485-x

Calixto G, Yoshii AC, Silva HR, Cury BSF, Chorilli M. Polyacrylic acid polymers hydrogels intended to topical drug delivery: Preparation and characterization. Pharm Dev Technol. 2015;20(4):490-496. doi:10.3109/10837450.2014.882941

Cazorla-Luna R, Martín-Illana A, Notario-Pérez F, Bedoya LM, Bermejo P, Ruiz-Caro R, et al. Dapivirine bioadhesive vaginal tablets based on natural polymers for the prevention of sexual transmission of HIV. Polymers (Basel). 2019;11(3):1-17. doi:10.3390/polym11030483

Chin JS, Madden L, Chew SY, Becker DL. Drug therapies and delivery mechanisms to treat perturbed skin wound healing. Adv Drug Deliv Rev. 2019;149-150:2-18. doi:10.1016/j.addr.2019.03.006

Coviello T, Trotta AM, Marianecci C, Carafa M, Di Marzio L, Rinaldi F, et al. Gel-embedded niosomes: Preparation, characterization and release studies of a new system for topical drug delivery. Colloids Surfaces B Biointerfaces. 2015;125:291-299. doi:10.1016/j.colsurfb.2014.10.060

Dalla Lana DF, Lavorato SN, Giuliani LM, Cruz L, Lopes W, Vainstein MH, et al. Discovery of a novel and selective fungicide that targets fungal cell wall to treat dermatomycoses: 1,3-bis(3,4-dichlorophenoxy)propan-2-aminium chloride. Mycoses. 2020;63(2):197-211. doi:10.1111/myc.13027

Debebe D, Gabriel T, Brhane Y, Temesgen A, Nigatu M, Marew T. Comparative in vitro evaluation of brands of clotrimazole cream formulations marketed in Ethiopia. J Drug Deliv Ther. 2018;8(1):17-22. doi:10.22270/jddt.v8i1.1546

Deshmukh VN, Jadhav JK, Sakarkar DM. Formulation and in vitro evaluation of theophylline anhydrous bioadhesive tablets. Asian J Pharm. 2009;3(1):54-58. doi:10.4103/0973-8398.49176

Ferreira LM, Sari MHM, Azambuja JH, Silveira EF, Cervi VF, Marchiori MCL, et al. Xanthan gum-based hydrogel containing nanocapsules for cutaneous diphenyl diselenide delivery in melanoma therapy. Invest New Drugs. 2020;38(3):662-674. doi:10.1007/s10637-019-00823-2

Gehrcke M, Brum TB, Rosa LS, Ilha BD, Soares FZM, Cruz L. Incorporation of nanocapsules into gellan gum films: A strategy to improve the stability and prolong the cutaneous release of silibinin. Mater Sci Eng C. 2021;119:111624. doi:10.1016/j.msec.2020.111624

Gehrcke M, Giuliani LM, Ferreira LM, Barbieri AV, Marcondes MH, Silveira EF, et al. Enhanced photostability, radical scavenging and antitumor activity of indole-3-carbinol-loaded rose hip oil nanocapsules. Mater Sci Eng C. 2017;74:279-286. doi:10.1016/j.msec.2016.12.006

He H, Ye J, Zhang X, Huang Y, Li X, Xiao M. κ-Carrageenan/locust bean gum as hard capsule gelling agents. Carbohydr Polym. 2017;175:417-424. doi:10.1016/j.carbpol.2017.07.049

Jana S, Gandhi A, Sheet S, Sen KK. Metal ion-induced alginate-locust bean gum IPN microspheres for sustained oral delivery of aceclofenac. Int J Biol Macromol. 2015;72:47-53. doi:10.1016/j.ijbiomac.2014.07.054

Kaur R, Sharma A, Puri V, Singh I. Preparation and characterization of biocomposite films of carrageenan/locust bean gum/montmorrillonite for transdermal delivery of curcumin. BioImpacts. 2019;9(1):37-43. doi:10.15171/bi.2019.05

Kim JK, Park SU. Current results on the biological and pharmacological activities of Indole-3-carbinol. EXCLI J. 2018;17:181-185.

Kumar K, Dhawan N, Sharma H, Vaidya S, Vaidya B. Bioadhesive polymers: Novel tool for drug delivery. Artif Cells, Nanomed Biotechnol. 2014;42(4):274-283. doi:10.3109/21691401.2013.815194

Mayba JN, Gooderham MJ. A guide to topical vehicle formulations. J Cutan Med Surg. 2018;22(2):207-212. doi:10.1177/1203475417743234

Meena LK, Raval P, Kedaria D, Vasita R. Study of locust bean gum reinforced cyst-chitosan and oxidized dextran based semi-IPN cryogel dressing for hemostatic application. Bioact Mater. 2018; 3(3):370-384. doi:10.1016/j.bioactmat.2017.11.005

Osmari BF, Giuliani LM, Reolon JB, Rigo GV, Tasca T, Cruz L. Gellan gum-based hydrogel containing nanocapsules for vaginal indole-3-carbinol delivery in trichomoniasis treatment. Eur J Pharm Sci. 2020;151:105379. doi:10.1016/j.ejps.2020.105379

Pegoraro NS, Camponogara C, Gehrcke M, Giuliani LM, Silva DT, Maurer LH, et al. Oleic acid-containing semisolid dosage forms exhibit in vivo anti-inflammatory effect via glucocorticoid receptor in a UVB radiation-induced skin inflammation model. Inflammopharmacology. 2020;28(3):773-786. doi:10.1007/s10787-019-00675-5

Pettinelli N, Rodríguez-Llamazares S, Farrag Y, Bouza R, Barral L, Feijoo-Bandin S, et al. Poly(hydroxybutyrate-co-hydroxyvalerate) microparticles embedded in κ-carrageenan/locust bean gum hydrogel as a dual drug delivery carrier. Int J Biol Macromol. 2020;146:110-118. doi:10.1016/j.ijbiomac.2019.12.193

Prado VC, Sari MHM, Borin BC, Pinheiro RC, Cruz L, Schuch A, et al. Development of a nanotechnological-based hydrogel containing a novel benzofuroazepine compound in association with vitamin E: An in vitro biological safety and photoprotective hydrogel. Colloids Surfaces B Biointerfaces. 2021;199:111555. doi:10.1016/j.colsurfb.2020.111555

Rigo LA, Julia W, Silva CB, Beck RCR. Evaluation of the spreadability of pharmaceutical or cosmetic semisolid formulations using scanned images. Lat Am J Pharm. 2012;31(10):1387-1391.

Rigon C, Marchiori MCL, Jardim FS, Peggoraro NS, Chaves PS, Velho MC, et al. Hydrogel containing silibinin nanocapsules presents effective anti-inflammatory action in a model of irritant contact dermatitis in mice. Eur J Pharm Sci. 2019;137:104969. doi:10.1016/j.ejps.2019.104969

Saidin NM, Anuar NK, Meor Mohd Affandi MMR. Roles of polysaccharides in transdermal drug delivery system and future prospects. J Appl Pharm Sci. 2018;8(3):141-157. doi:10.7324/JAPS.2018.8320

Sari MHM, Fulco CWB, Ferreira LM, Pegoraro NS, Brum ES, Casola KK, et al. Nanoencapsulation potentiates the cutaneous anti-inflammatory effect of p,p′-methoxyl-diphenyl diselenide: Design, permeation, and in vivo studies of a nanotechnological-based carrageenan gum hydrogel. Eur J Pharm Sci. 2020;153:105500. doi:10.1016/j.ejps.2020.105500

Sharadha M, Gowda DV, Vishal Gupta N, Akhila AR. An overview on topical drug delivery system – updated review. Int J Res Pharm Sci. 2020;11(1):368-385. doi:10.26452/ijrps.v11i1.1831

Teixeira MC, Severino P, Andreani T, Boonme P, Santini A, Silva AM, et al. D-α-tocopherol nanoemulsions: Size properties, rheological behavior, surface tension, osmolarity and cytotoxicity. Saudi Pharm J. 2017;25(2):231-235. doi:10.1016/j.jsps.2016.06.004

Timur SS, Yüksel S, Akca G, Şenel S. Localized drug delivery with mono and bilayered mucoadhesive films and wafers for oral mucosal infections. Int J Pharm. 2019;559:102-112. doi:10.1016/j.ijpharm.2019.01.029

Ullah F, Othman MBH, Javed F, Ahmad Z, Akil HM. Classification, processing and application of hydrogels: A review. Mater Sci Eng C. 2015;57:414-433. doi:10.1016/j.msec.2015.07.053

Verma A, Tiwari A, Panda PK, Saraf S, Jain A, Jain SK. Locust Bean Gum in Drug Delivery Application. Elsevier Inc.; 2019. doi:10.1016/B978-0-12-817055-7.00008-X

Vijayaraghavan C, Vasanthakumar S, Ramakrishnan A. In vitro and in vivo evaluation of locust bean gum and chitosan combination as a carrier for buccal drug delivery. Pharmazie. 2008;63(5):342-347. doi:10.1691/ph.2008.7139

Yuan L, Pan M, Lei M, Zhou X, Hu D, Liu Q, et al. A novel composite of micelles and hydrogel for improving skin delivery of hydrocortisone and application in atopic dermatitis therapy. Appl Mater Today. 2020;19:100593. doi:10.1016/j.apmt.2020.100593

Zepon KM, Martins MM, Marques MS, Heckler JM, Morisso FDP, Moreira MG, et al. Smart wound dressing based on κ–carrageenan/locust bean gum/cranberry extract for monitoring bacterial infections. Carbohydr Polym. 2019;206(2018):362-370. doi:10.1016/j.carbpol.2018.11.014

Zhu BJ, Zayed MZ, Zhu HX, Zhao J, Li SP. Functional polysaccharides of carob fruit: A review. Chinese Med (United Kingdom). 2019;14(1):1-10. doi:10.1186/s13020-019-0261-x

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