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# Preparation and Characterization of a Topical Delivery System for Nanoemulsions Using a Composite Film of Pectin and Tapioca

**Luciana Agostinho and Pedro Rocha-Filho ***

School of Pharmaceutical Sciences of Ribeirão Preto, Department of Pharmaceutical Sciences, University of São Paulo, Ribeirão Preto 14040-903, SP, Brazil; luagostinho@yahoo.com.br
* Correspondence: pedranjo@fcfrp.usp.br

**Abstract:** Nanoemulsions represent a remarkable class of colloidal systems that play a pivotal role in the encapsulation, protection, and targeted delivery of active ingredients to the skin. One of the primary objectives in skincare science is to maximize the interaction between the active ingredients and the skin. This can be achieved through various mechanisms, two of which are occlusion and extended contact of the formulation with the skin. Cosmetic masks can be used to improve the skin's hydration by creating a barrier that minimizes transepidermal water loss while acting as a repository of the active ingredient, increasing the skin's absorption of the formulation's active ingredients. In order to diminish the environmental impact, natural-polymeric-material masks are being used, as an alternative to synthetic materials, for its biocompatibility and biodegradability. In this work pectin and tapioca starch were used to develop a polymeric mask to deliver a rice bran oil nanoemulsion and study some characteristics of the final product. The results show that the association of pectin and tapioca starch can be used to produce a film that can be molded and shows occlusive effects, besides being flexible and compatible with the skin.

**Keywords:** nanoemulsion; pectin; tapioca starch



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## 1. Introduction

The skin is a complex and specialized organ that covers the surface of the human body and is constantly exposed to environmental conditions. In the quest for radiant and healthy skin, proper hydration and protection against environmental aggressions are critical points [1,2]. One of the application systems of interest in the cosmetic field is nanoemulsion (NE), due to its characteristics such as kinetic stability and the inherent ability to facilitate the optimal absorption of active ingredients by the skin [3]. Vegetable oils are prized in cosmetics for their natural, nourishing, and versatile properties, natural-emollient nature, nourishing properties and easy absorption by the skin, making them a preferred choice for both skincare enthusiasts and manufacturers in the beauty industry. The difference in the percentage composition of fatty acids in vegetable oils can also provide beneficial biochemical effects on the skin. Rice oil has an unsaponifiable portion composed of high levels of tocopherols, tocotrienols, phytosterols, and, most importantly, the natural antioxidant γ-oryzanol, which presents protective properties against damage caused by UVB radiation. This potent blend of constituents furnishes rice oil with exceptional protective properties, rendering it an invaluable ingredient for pharmaceutical and cosmetic formulations [4,5]. The administration of active ingredients in the skin is one of the challenges in the pharmaceutical field, given the effectiveness of the skin's barrier function. Strategies that aim to disrupt or alter the skin's structure have been studied with the objective of enhancing the release and retention of these valuable active ingredients within the skin. Some of the commercial products that have increased interest are cosmetic masks, such as sheet masks, peel-off masks and hydrogel masks. Sheet masks are the most used for their

convenience and easy application. They are made out of a fabric, generally not biodegradable, soaked in a solution, that contains the active ingredient. However, as our collective environmental consciousness grows, there is an increasing focus on creating sustainable and more eco-friendly solutions. The use of bioactive films produced with biodegradable raw materials has garnered significant attention thanks to their compatibility with the skin and their non-toxic nature. Pectin, a water-soluble polysaccharide that is easily obtainable, is one of the raw materials of interest in biofilm production. Pectin gels, an intriguing subject in material science, are formed through crosslinking that forms a crystallin web that entraps water within its structure [6]. The gel formation mechanism depends on molecular structure, the intramolecular forces that hold the network together and the nature of the junction zones of the polymer structure. Low methoxyl pectin (LMP) gels exhibit a distinct stabilizing mechanism. They rely on the formation of calcium bridges between different polymer chains, a phenomenon that is further reinforced by hydrophobic interactions. This structural arrangement is often referred to as the "egg-box" model, wherein the calcium ions act as pivotal anchors holding the polymer chains in a precisely organized fashion. Films prepared with LM pectin and tapioca starch are moldable and, when well reticulated, are found to be ideal candidates for use as masks in cosmetic applications. By serving as carriers for nanoemulsions, these films introduce an occlusive effect to the skin, thereby reducing the loss of moisture through the epidermis. Moreover, they offer the additional advantages of being sourced from natural materials and, crucially, being biodegradable, aligning with sustainability goals. In this work, a film was prepared with pectin and tapioca starch and the influence of the plasticizer used and concentration of calcium chloride necessary to crosslink was studied. Additionally, the study examined how the film responded to the presence of nanoemulsion, particularly in terms of swelling, shedding light on its suitability as a vehicle for active ingredients. The occlusive effect of the film, as well as its swelling when in contact with NE and the humidity loss in controlled conditions were determined in vitro, providing valuable insights into the film's ability to maintain a favorable microenvironment for the skin. By delving into these aspects, this research not only contributes to the understanding of pectin and tapioca starch-based films but also opens up new horizons for the development of sustainable, effective, and customizable cosmetic products designed to enhance the health and appearance of the skin.

## 2. Materials and Methods

### *2.1. Materials*

For the aqueous phase, purified water obtained from reverse osmosis was used. The oil phase used for the preparation of the NE was rice bran oil (supplied by Campestre) and surfactants Sorbitan Monooleate (supplied by Croda) and PEG-30 castor oil (supplied by Oxiteno). For the preparation of the films, low molecular (LM) pectin (by CP Kelco) was used as a film formation polymer; tapioca starch (TS) (by Upchef) was used as rheological modifier. Glycerin (G) and propylene glycol (PG) (both supplied by Synth) were used as plasticizers and calcium chloride ($CaCl_2$) (supplied by Cinetica Química) as a crosslinking agent.

### *2.2. Methods*

#### 2.2.1. Nanoemulsion Preparation

Nanoemulsions (NE) were prepared using the Phase Inversion Composition (PIC) method [7]. The aqueous phase was composed of purified water, obtained by reverse osmosis (80.0%). The oily phase was composed of rice bran oil (10.0%), PEG-30 castor oil and Sorbitan Monooleate surfactants (10.0%). The hydrophilic–lipophilic balance (HLB) value for the NE was 8. The aqueous phase was heated until 75 ± 5 °C and poured over the oily phase containing surfactants, also heated up at the same temperature. The mixture was stirred at 600 rpm, in an electric stirrer, for 15 min until the emulsion reached room temperature (25 ± 5 °C).

2.2.2. Preliminary Stability Tests for NE

Stability tests were conducted after 24 h of preparation as follows: (i) Centrifugation test: Samples (5.0 g) were taken and submitted to a 3000 rpm rotation speed for 30 min [8]. Only the formulations that remained stable after the centrifuge test were submitted to thermal stress and heating/cooling cycles. (ii) Thermal stress test: Vials containing a 5.0 g sample were submitted to a range of temperature (from 50 up to 80 ± 2 °C, increasing by 5 °C intervals, for 30 min at each condition. The temperature was adjusted using a thermostatic bath [9]. (iii) Heating/cooling cycles: Heating/cooling cycles were performed to assess the samples' stability to extreme changes in temperature. This test consisted in submitting the samples at 40 ± 5 °C temperature, in a heating chamber, for 24 h and then at 4 ± 2 °C temperature, in a refrigerator, for another period of 24 h, thus completing one cycle (2 days). Six cycles (12 days) were performed in all [10]. Physicochemical characteristics (pH, electrical conductivity, color and aspect) were evaluated before and after thermal stress and heating/cooling cycles (in triplicate).

2.2.3. Droplet Size and Polydispersity Index (PdI)

Droplet size and PdI were determined by dynamic light scattering (DLS) (Nanosizer Malvern ZS) at a scattering angle of 173°. The samples were diluted in purified water at a 1:100 ratio, at 25 ± 2 °C.

2.2.4. Pectin- and Tapioca-Starch-Based Film

To prepare the film, pectin, tapioca starch (TS) and plasticizer (G or PG) were mixed until all the powder was dissolved, as shown in Figure 1. Then, the NE was poured over and the mix was heated until 75 ± 2 °C for 10 min, under agitation. The solution was cast into a Petri dish and then submerged in the calcium chloride solution, for 5 min, for crosslinking by film immersion [11,12]. The reticulated film was removed from the Petri dish and dried in absorbent paper. The films were kept in plastic wrap, to avoid drying out, and analyzed after 24 h.

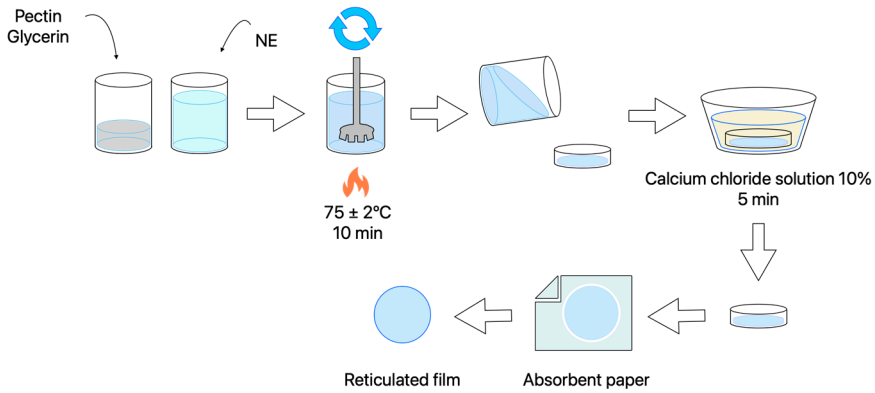



**Figure 1.** Method for preparing pectin/tapioca starch films.

2.2.5. Macroscopical Analysis of the Films

The films were analyzed against a light source, to ensure that the films exhibited a consistent and uniform composition throughout, without any irregularities or structural flaws. As Petri dishes were used as a mold, the ease of unmolding and the fragility upon manipulation of the films were analyzed as well.

2.2.6. Influence of the Plasticizer

The film was prepared as described in item 2.1.4.; using different concentrations of plasticizer (G or PG), ranging from 3.0 to 10.0%, the resulting films were macroscopically analyzed.

2.2.7. Influence of Calcium Chloride Concentration

Pectin and tapioca films were prepared and reticulated with solutions of calcium chloride with concentrations of 1, 5, 7 and 10.0%. The resulting films were macroscopically analyzed, as described in item 2.1.5.

2.2.8. In Vitro Evaluation of Occlusive Effect on Gelatin Support Cells

The emulsions' hydration power was determined using the methodology proposed by Rocha- Filho (1997). The films were applied on support gelatin cells (Figure 2) and the loss of water was evaluated as a function of time [13].

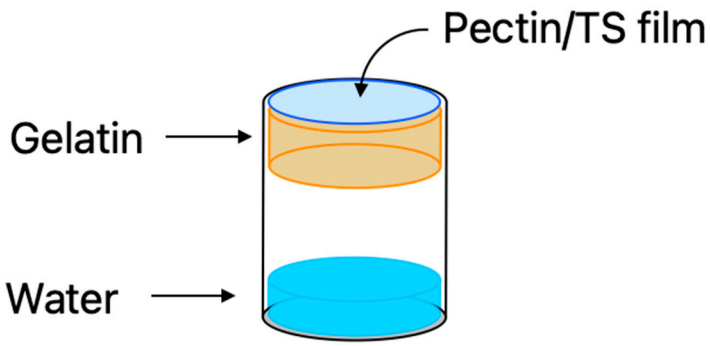



**Figure 2.** Representation of the gelatin cell.

The test films were applied over the surface of the gelatin and the water loss (WL) determined after 25, 50, 100 and 150 min is calculated using Equation (1).

$$WL\left(\mathrm{mg/cm^2}\right) = \frac{P_{t0} - P_{tx}}{7} \times 1.000 \quad (1)$$

where $P_{t0}$ = initial weight of the gelatin cell (g), $P_{tx}$ = weight of gelatin cell after x minutes (g), and the denominator (7) = the cell surface ($cm^2$).

The occlusive power was calculated from the dehydration rate in the stationary phase obtained before and after the test application. The occlusive power of each formulation can be expressed by Equation (2).

$$E(\%) = \frac{a_t - a_p}{a_t} \times 100 \quad (2)$$

where $a_t$ = tangent before application of the test formulation, and $a_p$ = tangent after application of the test product.

2.2.9. Swelling Test

The films were immersed in NE and taken to a desiccator, with controlled temperature and humidity, for a period of 27 h. The samples were weighed before the test and in time intervals of 13, 17, 21, 23 and 27 h to verify if the film would absorb the NE (in triplicate). The swelling of the films was calculated using Equation (3).

$$GI = \frac{m_i - m_f}{m_i} \quad (3)$$

2.2.10. Humidity Loss (HL)

The films were taken to a desiccator, with controlled temperature and humidity, and weighed (in triplicate), to verify the humidity loss, before the beginning of the test, and at 15, 30, 45, 60, 90, 120, 150, 180 and 210 min, and after 48 h.

## 3. Results

### *3.1. Nanoemulsion Preparation*

NEs were prepared with rice bran oil (10.0%) and PEG-30 castor oil and Sorbitan Monooleate surfactants (10.0%), as studied by Bernardi (2011) [14]. The NE showed no signs of instability after centrifugation, thermal stress and the heating/cooling cycles, affirming their suitability for further examination. Conductivity and pH values, droplet size and PdI were measured before and after the heating/cooling cycle, and the results are shown in Table 1. The pH measured is compatible with topical use and droplet size was under 100 nm and PdI remained under 1,0, which shows a narrow size distribution among droplets, even after undergoing the challenging HCC. Conductivity measurements can be used to assess the stability of the NE. Measurements show a slight increase and may indicate some coalescence of droplets after HCC.

**Table 1.** Test results before and after the heating/cooling cycle (HCC).

| | Before HCC | After HCC |
|---|---|---|
| pH | 6.28 ± 0.07 | 5.17± 0.09 |
| Conductivity (μS/cm) | 174.70 ± 0.04 | 194.30 ± 0.10 |
| Size (nm) | 63.56 | 85.97 |
| PdI | 0.174 | 0.385 |

HCC: heating/cooling cycle; PdI: polydispersity index.

### *3.2. Pectin and Tapioca based film*

To produce pectin and TS films, different ratios were tested in an attempt to optimize their formulation. The solutions of pectin above 2.0% produced films that exhibited the desired properties, enabling them to be poured into the mold and subsequently unmold from the Petri dish, after reticulation with calcium chloride solution. The TS concentrations of 2.0, 3.0 and 5.0% were considered ideal for unmolding (Table 2), without tearing or structural damage, a critical factor in the successful production of these films. Above 5.0% of TS, the solutions were too viscous and difficult to pour into the mold. The elevated viscosity hindered the flow and workability of the mixture, making it less suitable for the intended purpose of film production. Glycerin was initially employed as a plasticizer, to verify the pectin and tapioca starch concentrations necessary to produce films able to unmold from the Petri dish without tearing up. The role of glycerin in this context was to enhance the flexibility and pliability of the films, contributing to their overall quality and usability.

**Table 2.** Formulation of the pectin/TS films.

| Formulation | Pectin (%) | Glycerin (%) | NE (%) | TS (%) |
|---|---|---|---|---|
| P2T2G5 | 2.0 | 5.0 | 91.0 | 2.0 |
| P2T3G5 | 2.0 | 5.0 | 90.0 | 3.0 |
| P2T4G5 | 2.0 | 5.0 | 89.0 | 4.0 |

TS: tapioca starch; NE: nanoemulsion.

### *3.3. Influence of the Plasticizer*

Formulations shown in Table 3 were prepared to study the influence of the plasticizer concentration in the macroscopic aspects of the film. Plasticizers are important to improve the flexibility of the film, reducing the molecular forces along the polymer chains, improving the wetting of the dry materials and reducing sticking in molds [15].

The films prepared with 2.0 and 3.0% of glycerin adhered to the Petri dish and teared up when attempting to remove from the mold. This result emphasized the need to adjust the plasticizer concentration for optimal performance. From 5.0% up to 10.0% of glycerin concentration, the films were well reticulated and easy to unmold, without the issue of structural damage during manipulation. So, the 5.0% plasticizer concentration was chosen

to test an alternative for glycerin (Table 4) that could maintain the desirable properties exhibited by the 5.0% glycerin-plasticized films. PG was chosen because, along with glycerin, it is a common plasticizer used in polysaccharide-based films and is compatible with topical use [15].

**Table 3.** Formulations prepared to study the influence of plasticizer concentration.

| Formulation | Pectin (%) | Glycerin (%) | NE (%) | TS (%) |
|---|---|---|---|---|
| P2T2G3 | 2.0 | 3.0 | 93.0 | 2.0 |
| P2T2G4 | 2.0 | 4.0 | 92.0 | 2.0 |
| P2T2G5 | 2.0 | 5.0 | 91.0 | 2.0 |
| P2T2G6 | 2.0 | 6.0 | 90.0 | 2.0 |
| P2T2G7 | 2.0 | 7.0 | 89.0 | 2.0 |
| P2T2G8 | 2.0 | 8.0 | 88.0 | 2.0 |
| P2T2G9 | 2.0 | 9.0 | 87.0 | 2.0 |
| P2T2G10 | 2.0 | 10.0 | 86.0 | 2.0 |

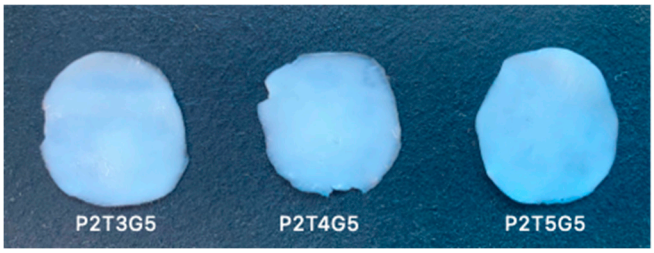
P: pectin; G: glycerin; NE: nanoemulsion; TS: tapioca starch.

**Table 4.** Formulations prepared to study the influence of G and PG as plasticizers.

| Formulation (%) | Pectin (%) | G (%) | PG (%) | NE (%) | TS (%) |
|---|---|---|---|---|---|
| P2T3G5 | 2.0 | 5.0 | - | 90.0 | 3.0 |
| P2T4G5 | 2.0 | 5.0 | - | 89.0 | 4.0 |
| P2T5G5 | 2.0 | 5.0 | - | 88.0 | 5.0 |
| P2T3PG5 | 2.0 | - | 5.0 | 90.0 | 3.0 |
| P2T4PG5 | 2.0 | - | 5.0 | 89.0 | 4.0 |
| P2T5PG5 | 2.0 | - | 5.0 | 88.0 | 5.0 |

G: Glycerin; PG: Propylene glycol; NE: nanoemulsion; TS: tapioca starch.

Macroscopical analysis showed no difference in the characteristics of films produced with G and PG, indicating that the substitution of glycerin with PG is not only feasible but also highly practical, using the preparation method proposed in this work. Figure 3 shows the aspect of the films produced with G and PG.




(**a**)


(**b**)

**Figure 3.** Aspect of films produced with glycerin (**a**) and propylene glycol (**b**).

Both G and PG are humectants, a quality that allows them to attract and retain moisture and reduce transepidermal water loss (TWL), contributing to skin health and maintaining its natural moisture balance. Moreover, PG boasts an additional attribute—it is recognized as a penetration enhancer, which may facilitate the delivery of active ingredients into the deeper layers of the skin [16,17]. The successful substitution of G for PG underscores the adaptability and flexibility of the proposed formulation.

### *3.4. Influence of Calcium Chloride Concentration*

The pectin/tapioca films were prepared and reticulated in calcium chloride solutions of different concentrations, to evaluate its influence in the film reticulation. The concentration of calcium chloride is important because the presence of calcium ions is crucial for creating

the egg-box junction zones, where the calcium ions keep together the pectin chains, forming the gel (Figure 4). These junction zones are the structural bedrock of the films, providing the necessary stability and coherence. A low concentration of calcium ions may result in poor reticulation, resulting in a fragile film, which renders it susceptible to structural weaknesses and tearing [18].

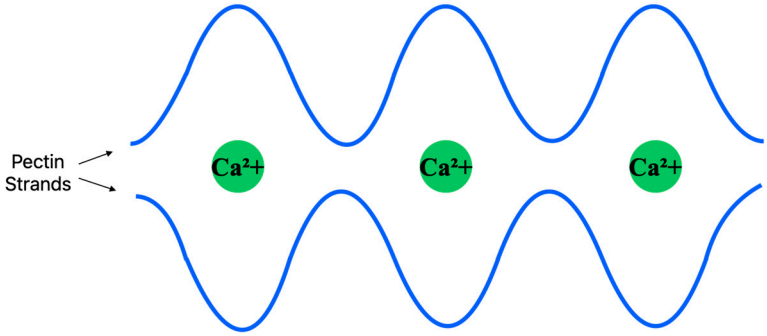



**Figure 4.** Egg box model for LM pectin gels showing the interactions between the calcium ions and the pectin strands.

A series of crosslinking solutions were prepared (1.0, 5.0, 7.0 and 10.0% *w*/*w*). Films were immersed in the solution for 5 min for crosslinking, then removed from the mold and dried in absorbent paper. Films immersed in a 1.0% calcium chloride solution displayed a pronounced fragility and teared up when unmolding was attempted. The films prepared with G demonstrated a stronger adherence to the mold than those prepared with PG. At a 5.0% concentration of calcium chloride solution, a notable shift in behavior was observed. Films were more easily removed from the mold; however, some films retained liquid regions, showing that the crosslink was incomplete and suggesting that a more substantial concentration of calcium chloride might be necessary to secure a more uniform and comprehensive reticulation of the films. At above a 7.0% of calcium chloride concentration solution, all films could be effortlessly removed from their molds and, importantly, there were no lingering liquid regions. These findings underscore the importance of fine-tuning the crosslinking process to achieve the desired balance between mold release, structural integrity, and complete formation of a well-crosslinked gel matrix within the films.

### *3.5. In vitro Evaluation of Occlusive Effect on Gelatin Support Cells*

The pectin/TS films of different concentrations of TS were used to evaluate the occlusive effect in vitro. For comparison, a commercial product (Cial Prod) was used. The commercial mask consisted in a cotton fabric base that had been thoroughly impregnated with a liquid formulation, designed for topical application. The commercial mask and the pectin/TS films were cut to cover the superior extremity of the gelatin cells. The results show that the formulations containing 4.0% TS presented a larger occlusive effect than formulations with 3.0 and 5.0% TS, irrespective of whether glycerin (G) or propylene glycol (PG) served as the chosen plasticizer (Figure 5).

Films with 4.0% TS also showed an increase in occlusive effect in the third step of the test, which may infer that the permanence of the film in the skin may have the potential to enhance the absorption of NE. In essence, the continued occlusive properties of these films could extend the duration during which the skin remains in close contact with the NE, thus improving the delivery and absorption of the active ingredients contained within the NE formulation. The comercial product had a negative occlusive effect in the first stage of the test. It is likely that, as it is a tissue soaked in liquid formulation, it lost humidity to the controlled environment of the dissecator. The instructions for the Cial Prod were to apply and let it sit in the skin for 20 min and, in that time, in this test, the Cial Prod was not functioning as an occlusive product. On the other hand, the pectin/TS films showed an

occlusive effect right from the outset of the test, which may improve the delivery of active ingredients that can be incorporated into the NE.

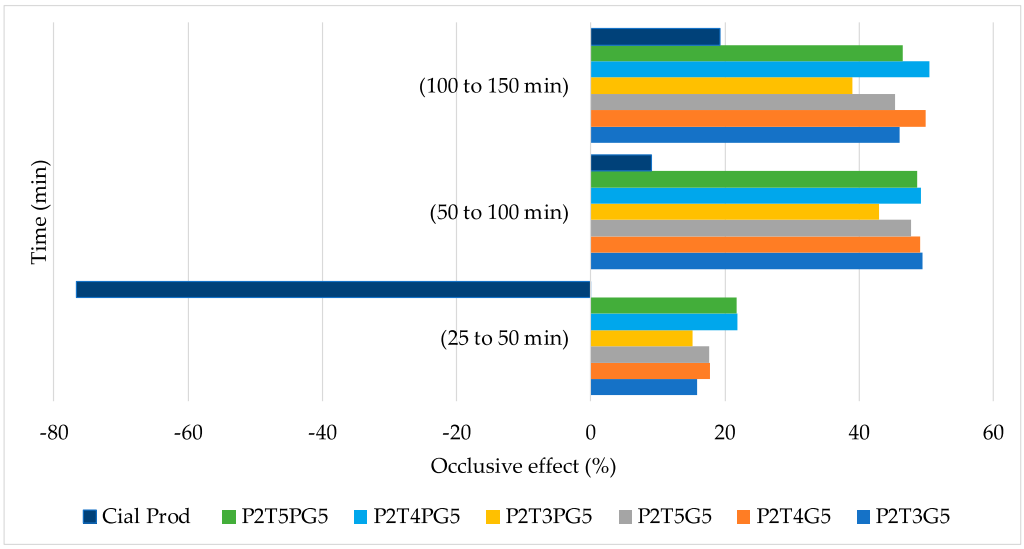



**Figure 5.** Occlusive effect of the films.

### *3.6. Swelling Test*

The films were taken to a dessecator, immersed in NE, with controlled temperature and humidity, to verify if the films would absorb the NE. Hydrogel commercial masks are typically submerged in a cosmetic solution within their packaging, to maximize the absorption of the formulation by the mask and delivery of the formulation through the skin. In a well-crosslinked hydrogel matrix, elastic forces that maintain the structure of the polymeric matrix reduce the swelling capacity of the film, when immersed in an aqueous solution [19]. So, well-reticulated gel will present minimal swelling behavior, an attribute that is valuable in the context of controlled delivery systems. The results of the swelling test for films with G are in Figure 6a and for films with PG, in Figure 6b.

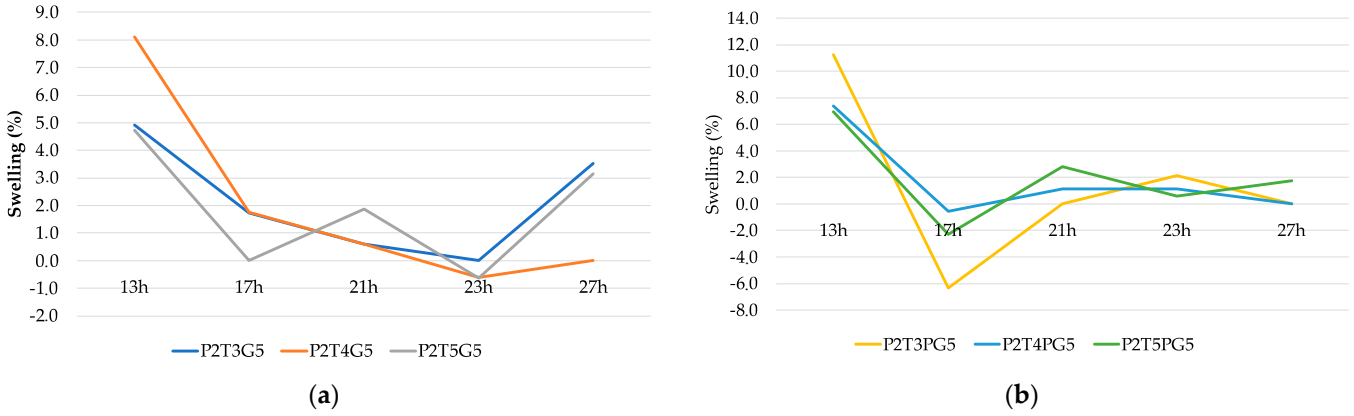
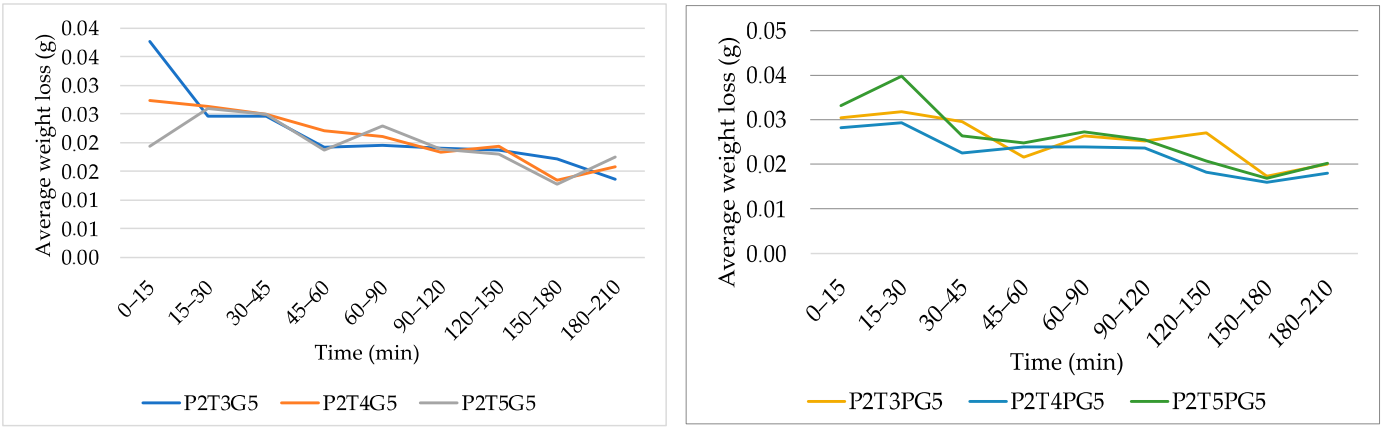



**Figure 6.** Swelling test results for films produced with glycerin (**a**) and propylene glycol (**b**).

Results show that the swelling exhibited by PG-plasticized films was notably lower than that of their G-plasticized counterparts. The films produced with PG presented an initial decrease in swelling, but after 17 h, started to reach a state of equilibrium, eventually stabilizing their swelling capacity. Films prepared with 4.0% TS (with G or PG) displayed a more consistent and regular behavior throughout the duration of the test.

### *3.7. Humidity Loss (HL)*

Evaluation of HL was tested in films produced 24 h before and stored in plastic wrap. Then, the films were placed in an open Petri dish, inside a dissecator, with silica as a desiccant material. The films were weighed before the test and after 15, 30, 45, 60, 90, 120, 150, 180 and 210 min. The humidity loss of the films is shown on Figure 7a,b.




(**a**) (**b**)

**Figure 7.** Humidity loss of the films with glycerin (**a**) and propylene glycol (**b**) as plasticizers.

Films produced with 4.0% TS (with G or PG as plasticizer) showed a more uniform humidity loss over the course of the experiment. The weight of the films showed little variation during the test, which may indicate the formation of a well-crosslinked gel, suggesting that the gel matrix formed within these films was robust and able to withstand external factors, such as changes in environmental conditions or exposure to moisture. After 48 h, the films were weighted again and the total weight loss is shown in Figure 8.

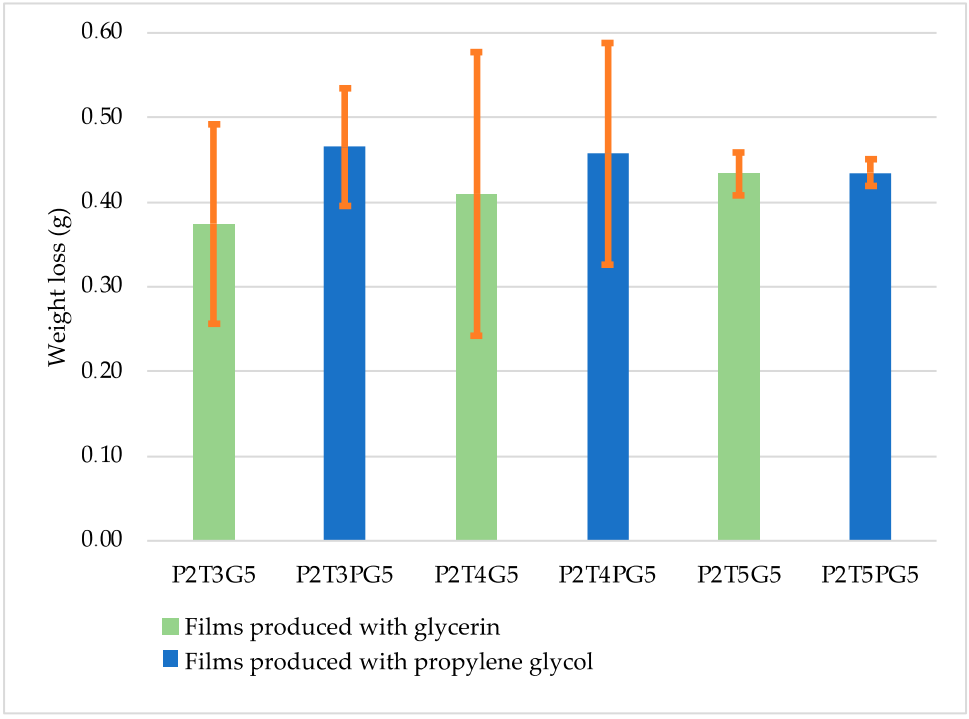



**Figure 8.** Weight loss of the films after 48 h.

After 48 h, the humidity losses of the films produced with 5.0% TS were similar between films with both G or PG as plasticizer and the standard deviation (SD) of the weight measures was small, probably due to a synergistic crosslink effect between pectin and tapioca on the gel matrix formation. The films produced with 3.0 and 4.0% TS showed less humidity loss with G as plasticizer; compared to their counterparts with PG as the plasticizer, they also showed greater variability among samples, as indicated by the increasing SD. Furthermore, when considering the concentration of tapioca starch, a distinct difference emerged between films plasticized with G and those plasticized with PG. Considering the TS concentration, films with G as plasticizer showed increasing HL with increasing TS concentration. In contrast, films plasticized with PG showed an inverse tendency, wherein higher TS concentrations correlated with reduced humidity loss (HL).

## 4. Discussion

The association with LM pectin and TS was used to produce a flexible film for cosmetic application as a mask, for delivering a rice bran oil NE to the skin. The plasticizing agent can significantly influence several critical characteristics, including the ease of unmolding the film, its occlusive properties, the extent of NE penetration into the skin, as well as the overall strength and flexibility of the film. The concentration of the calcium chloride solution, used to reticulate the films is also critical, because the calcium is essential to form the gel matrix and to guarantee its strength. A crosslinking solution with an insufficient calcium chloride concentration may result in fragile films that adhere to the mold and are prone to tearing during handling. The amount of TS in the film solution affects the ability for pouring into the mold and the association of pectin and TS changes the characteristics of the gel, the film's flexibility, its capacity to be unmolded effectively and its capability to retain the NE. The occlusive effect of the films is also affected by the quantity of TS present in the films' composition and the results indicate that films produced with 4.0% TS have a higher occlusive effect than films produced with 3.0 and 5.0%. This effect may be an indicator of a role of TS in the formation of the gel matrix, for starches may enhance the pectin network through ionic forces [20]. It is also worth mentioning that the choice of plasticizer matters significantly. Other hand films plasticized with G showed increased HL as tapioca starch concentration rises, and films plasticized with PG had the opposite behavior: the higher the TS concentration, the lower the HL. Importantly, while the differences in weight are comparatively smaller, these variations in hydrophilicity reflect the capacity to tailor films to leverage the distinctive properties each ingredient offers, contingent upon the intended application of the final product. This nuanced approach allows for the development of films that align precisely with the desired attributes and functions, ensuring their suitability for diverse cosmetic and skincare applications.

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