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Production and Characterization of Mucoadhesive Membranes for Anesthetic Vehiculation

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Studies on release systems produced from natural polymers such as chitosan (CS) have intensified in the pharmaceutical area. Polymeric membranes for the topical administration of drugs are being developed for the controlled release of drugs. Thus, the present study aimed to develop mucoadhesive polymer membranes for topical administration of the anesthetic, lidocaine hydrochloride (LID), for topical use. The methodology was done by a casting method using CS, propylene glycol (PPG) as the plasticizer in the concentrations of 0.2, 0.4 and 0.8% and lidocaine hydrochloride (LID). The membranes were characterized by mechanical properties, swelling index, thermal analysis and cell viability *in vitro*. Results showed membranes containing PPG containing the plasticizing agent was greater flexibility, better swelling profile and higher thermal resistance. In the cell viability assay with HaCat and 3T3 cells, it was possible to observe that all samples showed viability higher than 60 %. Thus, according to the results obtained in this study, it was possible to conclude that this pharmaceutical form is promising for the therapeutic use in topical administration aiming at the anesthetic action.

1. Introduction

The biomaterial is any substance or combination of materials, synthetic or natural, that can be used for a period, fully or partially as part of a system that treats, increases or replaces any tissue, organ or body function. Biomaterials based on polymers constitute an emerging class of applications in the most diverse areas that encompass the biomedical field, such as tissue regeneration, controlled drug delivery devices and gel cell immobilization systems (Chen and Liu, 2016).

The search for new release systems has been an alternative to obtain more efficient therapies compared to conventional treatments, offering greater safety and with diminished side effects. These systems can therapeutically maintain sufficient concentrations of the active principle in the blood and/or skin without generating oscillations at the levels typical of a multiple dose regimen (Kim et al., 2016). Mucoadhesive polymer membranes are dosage forms for administrated in the skin or mucosa. The structure must be adaptable to the local of application, must be stable in the presence of moisture, resistant to traction and easily stored. High contact area, comfort for the patient and its easy handling (Menzel et al., 2016)

Chitosan is a biopolymer of the type polysaccharide with versatility that allows several applications in the field of biomaterials, in the most varied forms, due to its great technological potential. The interest of scientists in CS as a biomaterial in biomedical applications is that these polysaccharides have relevant technological and economic characteristics. The best-known applications of natural biopolymers in the medical and pharmaceutical fields comprise wound treatment and controlled drug release (Bedian et al., 2017). Membranes of chitosan are pharmaceutical forms which dissolve and drug delivery. For this, this type of structure must be adaptable to the application site to be stable in the presence of moisture, to be tensile

resistant and easily stored. High contact area, comfort for the patient and its easy handling are among the main advantages of membranes (Ahsan et al., 2017).
LID is a hydrophilic drug, has a rapid onset, low systemic toxicity and is widely used in skin lesions and surgeries (Runyon et al., 2017). Topical administration of LID has the advantages of patient compliance, drug delivery and reduction of side effects, but its poor permeability and slow absorption still limit its medical applicability. However, it is necessary to develop a formulation capable of circumventing these improved characteristics (Wang et al., 2013). With this, the objective of this work was to develop a mucoadhesive polymer membrane using chitosan as polymer and incorporating lidocaine as anesthetic.

2. Methodology

2.1 Production of chitosan membranes

The membranes were produced by the casting method according (Dhanikula and Panchagnula, 2004). A 2% solution of chitosan in acetic acid was prepared with magnetic stirring. Propylene glycol (0.2%, 0.4%, and 0.8%) and lidocaine hydrochloride (0.006 %) was incorporated. Hydrogel was poured into a polyethylene dish under drying at room temperature

2.2 Mechanical properties

Mechanical properties were evaluated using a TA-XT Plus (Stable Micro Systems) texturometer, where the samples were placed between two clips and pulled until the film ruptured to obtain the strength of stretching. The mucoadhesion was evaluated using mucin discs. Parameters such as adhesion work (area under the force X stretching curve) and maximum force (N) to separate the film from the mucin disc were observed.

2.3 Swelling index

The swelling was performed with 1 cm² samples of the membranes. It was weighed and then moistened in water for a period of 1, 10, 30 and 60 min. Subsequently, the excess water was removed from each of them with filter paper, and they have weighed again (Kim et al., 2008). The swelling index was obtained by the following equation:

$$SI\% = \frac{Final\ weight - initial\ weight}{Final\ weight} \times 100$$

Eq (1): Calculation to evaluate the degree of swelling.

2.4. Thermogravimetry Analysis (TG)

TG of the samples were performed with a TG TA 2920 (Newcastle, DE, USA). The samples were placed in hermetic aluminum pans, and the experiments were performed under a nitrogen gas flow at a heating rate of 10°C/min over a temperature range of 25–500°C.

2.5 Infrared spectroscopy with fourier transforms (FTIR)

The characterization of the membranes by FTIR was performed with the objective of determining a possible interaction between the components of the formulation. Analysis of the membranes was performed on the spectrophotometer (IR-Prestige-2, Shimadzu) and analyzed in the range of 400 - 4000 cm^{-1} in KBr pellets.

2.6 Scanning Electron Microscopy (SEM)

The surface and cross-sectional images of the Chito membrane samples, Chito/LID membrane, Chito/PPG membrane and Chito/PPG/LID membrane were obtained with 500 to 5000 fold magnifications. SEM tests were performed on a scanning electron microscope 4401 (Leo Electron Microscopy).

2.7. Cell viability in 3T3 and HaCat cells *in vitro*

Cell lines from rat fibroblasts (3T3) and human keratinocytes (HaCat) were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 1% antibiotic, in a humid oven, 5% CO2 and 37°C. For the experiment, the cells were plated in 96-well plates containing 1 x 106 cells/mL in each well, after adherence the cells were treated with the membranes. After 24 hours of treatment, the wells were washed, and the cells were exposed to 10 µL of the 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution, which was left for 3 h. After the exposure time, the MTT medium was removed and 80 µL of dimethylsulfoxide was added to each well. The plates were shaken gently for 10 min and then the absorbance was measured in the ELISA apparatus at 570 nm.

4. Results and discussion

4.1 Mechanical properties

The results of mechanical properties are shown in Table 1. It was observed the samples containing PPG showed higher perforation, resilience and tensile strength that is caused by PPG promote molhability of the polymer chain (Rivero et al., 2016). These factors are essential requirements for the patient to handle the membrane without breaking it. Membranes without PPG showed the high value of mucoadhesion because free CS shows more amine group livre that can interact with mucin.

Table 1: Mechanical properties of chitosan membranes.

Sample	Resilience (%)	Perforation	Mucoadhesion (N)	Traction
CS	1.518 ± 0.01	8.218 ± 3.901	1.715 ± 1.078	9.652 ± 0.159
CS+PPG0.2%	3.794 ± 0.874	3.794 ± 0.847	0.593 ± 0.035	15.512 ± 1.168
CS+PPG0.4%	3.097± 0.998	5.901 ± 0.858	0.649 ± 0.043	17.472 ± 0.898
CS+PPG0.8%	2.224 ± 0.076	4.303 ± 0.324	0.326 ± 0.498	16.001 ± 1.234
CS+LID	2.239 ± 0.584	7.238 ± 1.305	2.727 ± 0.008	9.670 ± 0.520
CS+LID+PPG0.2%	3.039 ± 0.016	4.646 ± 0.088	1.499 ± 0.416	18.347 ± 0.409
CS+LID+PPG0.4%	3.059 ± 0.092	6.988 ± 2.595	0.289 ± 0.422	15.045 ± 0.141
CS+LID+PPG0.8%	2.770 ± 0.270	7.872 ± 1.999	1.367 ± 0.061	10.293 ± 0.071

4.2 Swelling index

The degree of swelling is related to the ability of the mucoadhesive polymer membrane to absorb water (Marques et al., 2016). The evaluation of the degree of swelling of membranes was demonstrated in Figure 1. It was observed that the samples with PPG, LID, and PPG+LID showed a higher degree of swelling compared with membranes containing the only chitosan. All samples showed a swelling equilibrium after one hour. This behavior may be related to the moisture absorption capacity of propylene glycol, resulting in a higher degree of swelling of the membranes containing this plasticizer (Rasool and Khan, 2010). The swelling behavior of membranes containing lidocaine hydrochloride may also be related to the hydrophilic characteristic of this drug, a factor observed in mucoadhesive devices containing salbutamol sulfate, a water-soluble drug (Puratchikody et al., 2011).

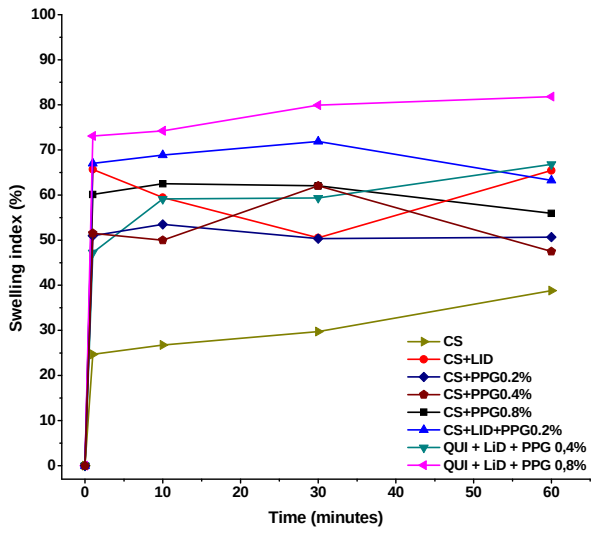

Figure 1. Profile of swelling obtained for the chitosan membranes.

4.3 Thermogravimetry Analysis (TG)

TG studied the thermal degradation of membranes. Figure 2A shows the three steps of thermal decomposition for the CS membrane. The first stage refers to a loss of residual water present in the sample, which started at the temperature of 30°C and followed up to 150°C, having a mass loss of about 20%. The second stage, between 150 and 330°C, can be attributed to the onset of degradation of the polymer, with a mass loss of approximately 45%. The third stage of thermal degradation refers to depolymerization and decomposition of the polysaccharide structure, occurring in the temperature range of 300 to 500°C. CS+LID membrane the initial stages of decomposition presented a lower rate of mass loss at temperatures of 50 to 150ºC.
Figure 2B and 2C shows CS+PPG the mass loss was lower when compared to the CS membranes. CS membranes with LID and PPG at various concentrations (Figure 2C) showed a higher thermal in the temperature range of 50 to 280°C. Then, samples with 0.8% of PPG was more stable when compared to other membranes. Therefore, it was possible to observe the relationship between the presence of the plasticizing agent and a higher thermal resistance to the membranes.

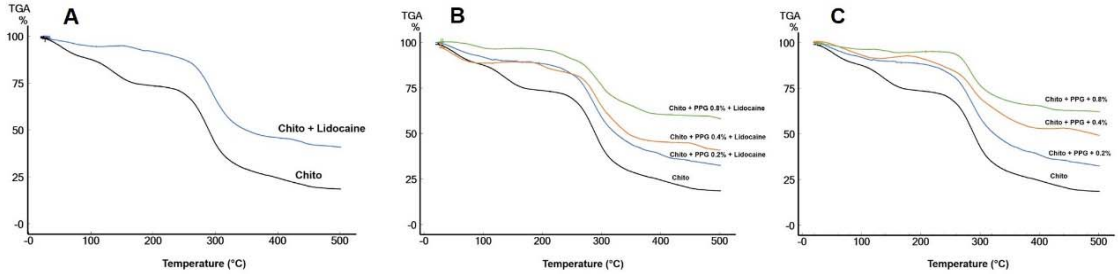

Figure 2. Thermogravimetric analysis of membranes.

4.4 Infrared spectroscopy with fourier transforms (FTIR)

The FTIR spectra of the chitosan membranes are shown in Figure 3. FTIR spectra presented similar regions, most of them related to chitosan. For the CS membrane, a broad absorption attributed to the presence of the N - H group and the stretching of the O-H group is observed in the region between 3600 and 3150 cm^{-1}. In the range of 2900 to 2800 cm^{-1}, there is a weak band, referring to the symmetrical and asymmetrical stretches of the C - H group. In the 1650 cm^{-1} region, the stretching of the C = O (amide I) and 1560 cm^{-1} groups are shown in the stretches -NH_2 (amide II). Vibrations corresponding to C - O stretch at C - OH and C – O - C bonds (saccharide structure) are observed in the 1160 to 890 cm^{-1} regions (Aburahma, 2011; Carvalho, 2014). All samples containing PPG and LID showed a small change is observed for the bands observed in the CS membrane, which may suggest the existence of a possible interaction between the components of the formulation (Hermans et al., 2014).

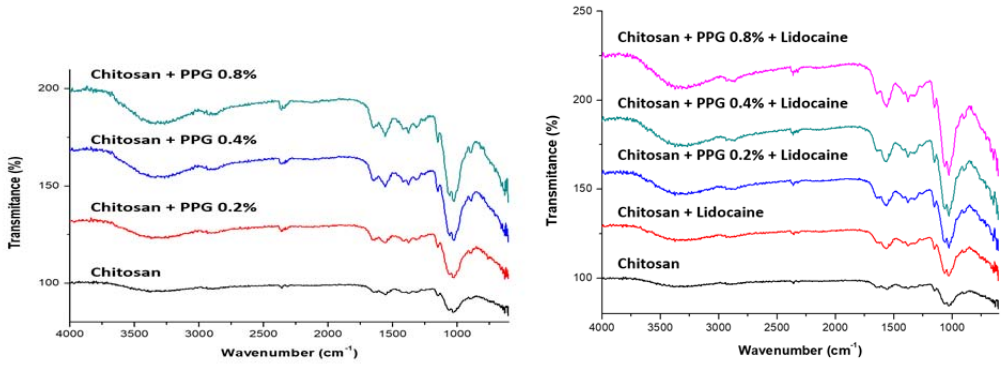

Figure 3. FTIR spectra of the membranes

4.5 Scanning Electron Microscopy (SEM)

SEM shows information about the morphology of material. CS+LID, CS+PPG0.8%, and CS+LID+PPG0.8% that were used to perform morphological study. Figure 4 shows the surface images (1000x) and crosses

sections of the obtained membranes (5000x). It was observed that all the samples had a partially homogeneous structure with some cracks and side cuts. These changes may be attributed when membranes were removed from Petri dishes. The images of the cross-section were observed fibrous polymer structure. (Hosseini et al., 2016) Attributed to the structure of CS. LID membranes showed small crystals confirm the presence of drug (Khan et al., 2016). The membranes with PPG+LID were observed as a rougher structure with a larger number of crystals.

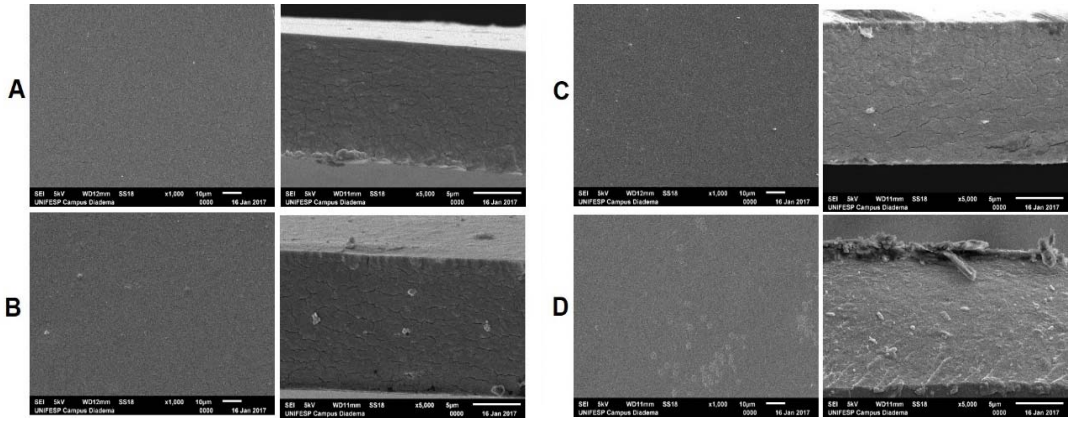

*Figure 4. SEM images of membranes **a**) CS membrane, **b**) CS+LID membrane, **c**) CS+PPG0.8% membrane and **d**) CS+PPG0.8%+LID membrane.*

4.6 Cell viability in 3T3 and HaCat cells *in vitro*

To assess the biocompatibility of membranes for potential dermatological applications. MTT assay is a great model to evaluate the response of biological systems towards biopolymer networks. HaCaT and NIH-3T3 cell lines were chosen because they are cellular components of the skin. Results are pointed out in Figure 5. All samples showed viability greater than 60%, thus suggesting lack of cytotoxicity of the material. Similar results were found by (Dash et al., 2011) that explain CS shows characteristics such as non-toxicity, biocompatibility, and biodegradability, characteristics that prevent its rejection and allow its change in products to carry out its elimination from the organism.

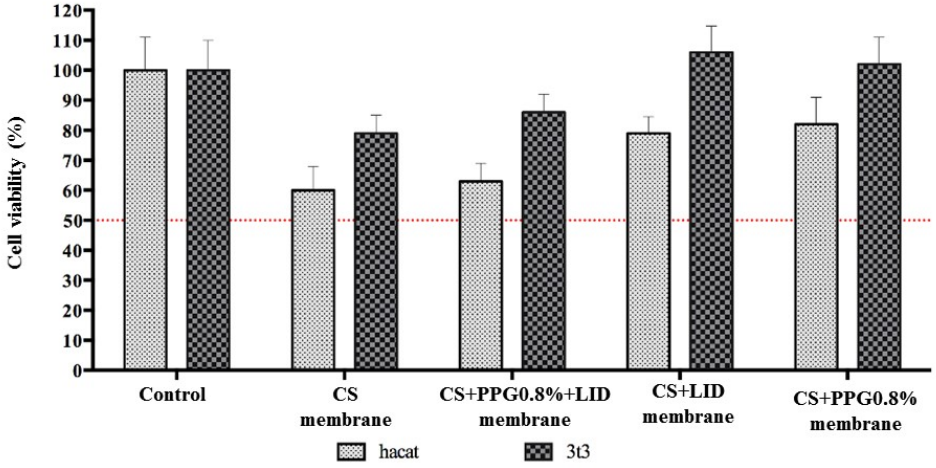

Figure 5. Percentage of cell viability obtained in MTT cytotoxicity assays using HaCaT and 3T3.

5. Conclusion

In the present paper, a CS membrane was prepared by a casting method. Membranes with 0.8% PPG showed proved to flexibility, homogenous morphology and biocompatibility, demonstrating the importance of the use of plasticizers in the production of this type of pharmaceutical form. Finally, the biomaterial is a promising for topical application.

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