Physicochemical and antimicrobial evaluation of chitosan and hydroxypropyl methylcellulose films for prolonged release of pilocarpine

Abstract: Introduction: The use of prolonged local drug delivery to the oral cavity offers multiple benefits, such as increasing the pharmacological action in the desirable local site and reducing the usual dose and the adverse effects. Pilocarpine is a cholinergic drug approved by the FDA for the treatment of glandular hypofunction; however, the extent of its adverse effects limits its use. Objective: The main aim of this study was to analyze the physical and chemical properties of films, including pH, thickness, solubility, consistency and the ability to release pilocarpine for a prolonged time. Additionally, the antimicrobial activity in two opportunistic pathogens in hyposalivation (Streptococcus mutans and Candida albicans) was also assessed. Methodology: Chitosan and HPMC (Methocel K4M CR) films were prepared in 1% acetic acid and pilocarpine was added under magnetic stirring. PH, thickness and time of solubility in artificial saliva, as well as diffusion and drug release kinetics per cm² (OD=420nm) were assessed by spectrophotometry. The antimicrobial activity was tested by disk diffusion test against St. mutans ATCC 700610 and C. albicans ATCC 90029 at concentrations of hyposalivation (1.44x1.2x10⁶ CFU and 10³ CFU, respectively). Results: All the films, except for Hydroxypropyl methylcellulose / Pilocarpine formulation, were found to have optimal physical-chemical properties for handling, maintaining drug diffusion in 76% per cm² for four hours extended-release without showing antimicrobial activity at concentrations of hyposalivation. Conclusion: The films had optimum handling properties and a constant drug release; however, antimicrobial activity was not found.

Keywords: Films, Pilocarpine, Hyposalivation, Chitosan.

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Apart from that, it has application for parenteral, oral and ocular administration, tissue engineering, and different mucosal tissues, among others.

Likewise, Hydroxypropyl methylcellulose (HPMC) is a hygroscopic cellulose ether used for prolonged drug release. It has the capacity to form a gel layer in the periphery of the biofilms on aqueous liquids, constantly releasing the drug through pores in the matrix.

The system of HPMC and chitosan polymeric matrices for controlled drug release provides the mechanical properties for the desired application. Also, they have to be biocompatible, biodegradable, and avoid inflammatory, immunological and cytotoxic response.

Physical-chemical properties of the controlled-release systems containing chitosan and HPMC using films for transdermal and oral models, such as pH, tension-elongation, uniformity of drug diffusion and solubility, have been studied proving beneficial for systems as a future application.

Currently, models containing Chitosan and HPMC have been developed for in vivo and in vitro oral administration for prolonged release of drugs like lidocaine, ibuprofen, fluconazole and metronidazole, as promising means of intraoral matrices.

Pilocarpine is the first drug approved by the FDA (Federal Drug Administration) for treating deterioration of salivary glands. However, it has many adverse effects such as sweating, rhinitis, nausea, increased urinary frequency, as well as increased gastrointestinal secretion of hydrochloric acid, and it is contraindicated for many ailments.

An attempted has been made to find an alternative way to deliver pilocarpine in xerostomia using mouthwashes of pilocarpine in saline solution. It gave good results with 2% pilocarpine. However, it was not found to be effective 90 minutes after using the mouthwash.

The aim of this study was to evaluate physical-chemical properties (pH, thickness, solubility, uniformity and drug time release) and antimicrobial properties of films made of chitosan and HPMC biopolymers with pilocarpine with the purpose of proposing an alternative treatment for hyposalivation by increasing salivary flow and inhibiting the growth of the two main opportunistic microorganisms.

**MATERIALS AND METHODS.**

**Preparation of chitosan and Hydroxypropyl methylcellulose biofilms with pilocarpine.**

To carry out the experimental studies, chitosan with a 75% degree of deacetylation, obtained from Sigma-Aldrich Chemical Company (St. Louis, Missouri, U.S.), Hydroxypropyl methylcellulose (Methocel K4M CR) donated by Colorcon from Mexico (Cuajimalpa, Edo. Of Mexico) and pilocarpine obtained from the GLOBAL laboratory (Monterrey, N. L., Mexico) were used.

There were six formulations: chitosan (1g/100ml), HPMC (1.5g/100ml), chitosan/HPMC (0.5gr in every/100ml), which were taken as a basis for the realization of the treatments and added with a weight dose of pilocarpine for rats (0.15gr/mL of Pilocarpine). The formulations were homogenized under magnetic stirring in an aqueous solution of 1% acetic acid at 70ºC for one hour. Then, they were put into Petri dishes for drying in the open air for 24 hours, to later be detached (Figure 1).

**Evaluation of physicochemical properties.**

With the aim to know the physicochemical properties of the biofilm, pH (Beckman potentiometer), thickness (Mitutoyo Digimatic 1”) (Figure 2) and the time of solubility in 24 ml of artificial saliva, which is the amount of saliva secreted by diabetic patients in the course of an hour, were evaluated.

**Determining uniformity of diffusion of Pilocarpine in the films.**

In order to determine uniformity of Pilocarpine in the films, 0.15gr of Pilocarpine were dissolved in 25ml of distilled water under magnetic stirring at 37ºC for an hour, absorbing with a spectrophotometer (BIORAD Smart Spec 3000) at different optical densities (OD) (200nm - 500nm) to obtain a reference value to consider as a control. Thus, the optical density which revealed higher absorbance (420nm) was taken into account.
Once the value of control with pilocarpine was obtained, 1 cm² of the chitosan/HPMC/Pilocarpine film was dissolved in 25 ml of distilled water at 37°C under magnetic stirring for one hour to later measure the absorbance with the purpose of obtaining the value of pilocarpine content in the films.

Assessment of the release time of pilocarpine
To determine the time of release of the drug of the chitosan/HPMC/pilocarpine film, 1 cm² of the film was placed in 25 ml of distilled water at 37°C under magnetic stirring. Besides, absorbance was measured every 15 minutes for 4 hours at an OD of 420 nm, which was determined during the drug uniformity test, with the goal of creating release kinetics.

Evaluation of the antimicrobial activity of the film.
The antimicrobial effect of the films was evaluated against the two opportunistic pathogens causing the main diseases present in hyposalivation, using Streptococcus mutans strains ATCC 700610 in a culture medium of brain heart infusion (BHI) and Candida albicans ATCC 90029 in a culture medium of YPD (yeast peptone Dextrose). The cellular concentrations of the strains, Streptococcus mutans (1.44 x 10⁶ CFU) and Candida albicans (1.2 x 10³ CFU), were adjusted to the concentrations found in diabetic patients with hyposalivation, based on the pattern of 0.5 McFarland with the purpose of obtaining the cellular patterns. The technique of disk diffusion was used with 1 cm² of the films.

RESULTS.
Evaluation of the physicochemical properties.

pH:
Results showed that, given the acidification of the acetic acid, the films showed a variable pH around 3, in which chitosan increased pH, being the HPMC films the more acidic, which maintained a stable pH below 3 (Table 1).

Determining thickness:
The average uniform thickness of each formulation varied in extent depending on the grain of the polymers to form pores. HPMC had a thinner thickness compared with the chitosan film and it was even seen at the moment of solubility, being the thinnest films the first ones to dissolve in artificial saliva (Table 2).

Films Solubility:
HPMC and HPMC/Pilocarpine films dissolved in an average time of 33±0.1 min, and the other formulations, such as Ch and Ch/HPMC and the one supplemented with pilocarpine completely dissolved 48 hours after being placed in artificial saliva.

Drug diffusion per cm²:
For determining the uniformity of the Pilocarpine in Ch/HPMC/P films, the average absorbance of Pilocarpine dissolved in 25 ml of saliva at an OD of 420 nm was considered as a control. It was 0.056±0.014 A. Afterwards, absorbance was made to the film under the same conditions, which showed 0.043±0.004 A, then, it was uniformly 0.11 gr (76%) per cm² out of 0.15 gr (100%).

Drug release kinetics:
Regarding uniformity of the drug, release kinetics of pilocarpine showed that the percentage of absorbance at the time of release was 71%, 89% after 2 hours, 82% after 3 hours and 129% after 4 hours (Figure 3).

Antimicrobial evaluation of the films:
Using the diffusion technique in well with the aim to
The five areas where the thickness of the biofilms was measured using a micrometer are shown (Mitutoyo Digimatic 1”).

### Table 1. PH of films.

| Formulated pH | CH | HPMC | CH/HPMC | CH/P | HPMC/P | CH/HPMCP |
|---------------|----|------|---------|------|--------|----------|
| pH            | 3.98±2.88±3.60±3.95±2.89±3.60± | 0.04640.0050.010.020.01 | 0.04640.0050.010.020.01 |

PH of films. CH=Chitosan, HPMC=hydroxypropyl methylcellulose, P=pilocarpine.

### Table 2. Film thickness (μ).

| Formulated thickness | CH/P | HPMC/P | CH/HPMCP |
|----------------------|------|--------|----------|
| Thickness of the film by micrometer. CH= Chitosan, HPMC= hydroxypropyl methylcellulose, P= pilocarpine. |

evaluate the antimicrobial activity of the cellular patterns found in diabetic patients with hyposalivation, Streptococcus mutans (1.44x10⁶ CFU) and Candida albicans (1.2x10³ CFU), showed that none of the films presented effective antimicrobial activity against the two organisms evaluated.

**DISCUSSION.**

Currently, due to the increase in infectious diseases, there is a need to prevent and eradicate the problems which are very commonly found within the oral cavity with higher prevalence in sufferings which increase these diseases, as hyposalivation, in which Candida albicans and Streptococcus mutans are actively involved²⁸.

In 2011, Souza et al.³ became interested in effectively finding MIC and MBC values of chitosan in gel at different concentrations against Streptococcus mutans which were a p>1.25 and p<2.50, respectively. This is consistent with Tapia et al., who, in 2009⁹, found MIC and MBC values of chitosan against different Candida spp., including Candida albicans, by broth microdilution method. They found that the species were inhibited at concentrations below 1.25mg/ml of chitosan. In comparison with our results, not effective antimicrobial activity of chitosan against strains of Streptococcus mutans and Candida albicans was found using the disk diffusion technique. This can be attributed to the inability of chitosan to diffuse in agar as film, resulting in a negative antimicrobial effect. However, with the increased salivary production which this biofilm would cause by stimulating exocrine glands, the line of defense and the enzyme systems which this fluid has would increase. This would reduce the level of risk for opportunistic infections in hyposalivation.

In 2008, Juliano et al.²⁹ conducted a study in which they tested prolonged release of chlorhexidine through HPMC and chitosan films and, using in vitro tests, determined the uniformity of the drug in the films, which was found in 72% of the drug expected. Then, it was tested in vivo in the oral cavity of healthy patients performing release kinetics of the drug using saliva samples,
finding the highest concentration (33.18gr/mL) 120 min after the application, still above MIC of *Candida albicans* (7.8mg/mL) 15 min after the application. This is consistent with our result in which we found a uniformity of 76% of pilocarpine. However, the time when it shows the greatest concentration of pilocarpine was at 240 min (0.07338 A), during the realization of the *in vitro* release kinetics. This offers a constant release of the drug, which when placed locally in the oral cavity may persistently stimulate salivary flow and be an alternative treatment for hyposalivation, decreasing oral diseases and reducing the adverse effects of pilocarpine.

Nowadays, new treatment alternatives at eye level with nanoparticles chitosan have been developed. Due to their optimum properties of biodegradability, biocompatibility, non-toxicity and mucoadhesiveness, and given the high bacterial resistance to ophthalmological level, they promise an alternative delivery treatment for drugs, which has been compared in the treatment of glaucoma, so that by comparing the conditions that are found in the oral cavity and the best conditions encountered in this study through the films. It would be important to assess mucosa of the oral cavity for their in vivo study.

**CONCLUSION.**

All the films were found to have optimal physicochemical properties for handling, with an adequate diffusion (72%) and a constant release of the drug for 4 hours after their placement in an aqueous medium. However, although the films did not showed antimicrobial activity, when stimulating salivation it would be expected the enzyme systems increase, which would help the natural inhibition of these microorganisms. Given the results obtained from the films, they can be considered an alternative choice to alleviate the symptoms and reduce the adverse effects of the usual administration of pilocarpine.

Evaluación físico-química y antimicrobiana de biopelículas de quitosán e hidroxipropilmetilcelulosa para liberación prolongada de pilocarpina.

**Resumen:** Introducción: El uso local de administración prolongada de fármacos en la cavidad oral proporciona múltiples ventajas, aumentando la acción farmacológica en el sitio local deseable, reducción de la dosis usual y disminución de los efectos adversos. La pilocarpina es una droga colinérgica aprobada por la FDA para el tratamiento de la hipofunción glandular, sin embargo la amplitud de sus efectos adversos limitan su uso. Objetivo: Con el objetivo de analizar las propiedades físico-químicas de las biopelículas se evaluó el pH, grosor y el tiempo de solubilidad en saliva artificial, así como la uniformidad de difusión y cinética de liberación de la droga por cm² mediante espectrofotometría (OD=420nm). Mediante difusión en disco se evaluó la actividad antimicrobiana ante *Streptococcus mutans* ATCC 700610 y *Candida albicans* ATCC 90029 en concentraciones encontradas en hipoosalivación (1.44 x 10⁶ UFC y 1.2 x 10³ UFC respectivamente). Resultados: Todas las biopelículas, a excepción de la formulación Hidroxipropilmetilcelulosa e Hidroxiolpilmetilcelulosa/Pilocarpina resultaron tener las propiedades físico-químicas óptimas de manipulación, manteniendo una uniformidad de difusión de la droga en 76% por cm² con liberación prolongada por 4 horas, sin mostrar actividad antimicrobiana en concentraciones de hipousalivación. Conclusión: Las películas obtuvieron las propiedades óptimas de manipulación, y una constante liberación del fármaco, sin embargo, ninguna formulación presentó actividad antimicrobiana

**Palabras clave:** Biopelículas, Pilocarpina, Hiposalivación, Quitosán
REFERENCES.

1. Cavallari C, Fini A, Ospitali F. Mucoadhesive multiparticulate patch for the intrabuccal controlled delivery of lidocaine. Eur J Pharm Biopharm. 2013;83(3):405–14.

2. Nagpal K, Singh S, Mishra D. Chitosan nanoparticles: a promising system for buccal administration of ibuprofen. J Controlled Release. 2004;99(1):73–82.

3. Park Y, Lee Y, Park S, Sheen S, Chung C, Lee S. Platelet derived growth factor regeneration. Biomaterials. 2000;21:153–9.

4. Verma A, Kumar A, Kumar S. Preparation and swelling controlled-release floating matrix tablets containing hpmc and chitosan. Int J Pharm Pharm Sci. 2012;4:82–7.

5. De Carvalho M, Stamford T, Pereira 2012;4:82–7.

6. Sánchez R, Damas R, Domínguez P, Rodríguez J, Sánchez R, Garza M, Nakagoshi M, Solís J, Arévalo K & Garza E. Pysicochemical and antimicrobial evaluation of chitosan and hydroxypropyl methylcellulose films for prolonged release of pilocarpine. J Oral Res 2015; 4(1):25-31. DOI: 10.17126/joralres.2015.007

7. De Moura M, Avena R, McHugh T, dos Santos P, Sampaio F. Chitosan as an oral antimicrobial agent. Formatex 2011. 2012 1 (13): 542-550

8. Sánchez R, Damas R, Domínguez P, Cerezo P, Salcedo I, Aguzzi C. Uso de la HidroxiPropilMetilCelulosa (HPMC) en liberación modificada de fármacos. Farmaespaña Ind. 2010:48–51.

9. De Moura M, Avena R, McHugh T, Krocha J, Mattoso L. Properties of Novel Hydroxypropyl Methylcellulose Films Containing Chitosan Nanoparticles. J Food Sci. 2008 8;73(7):31–37.

10. Olivas I, García P, Martel A, Martínez R, Martínez A, Martínez C. Preparación y caracterización de compuestos de quitosana/nanotubos de carbono. Rev Mex Ing Quím. 2009;8(2):205–11.

11. Rotta J, Ozório R, Kehrwald A, de Oliveira Barra G, de Melo R, Barreto P. Parameters of color, transparency, water solubility, wettability and surface free energy of chitosan/hydroxypropylmethylcellulose (HPMC) films plasticized with sorbitol. Mater Sci Eng C. 2009;29(2):619–23.

12. Archana D, Dutta J, Dutta PK. Evaluation of chitosan nano dressing for wound healing: Characterization, in vitro and in vivo studies. Int J Biol Macromol. 2013;57:193–203.

13. Lavertu M, Darras V, Buschmann MD. Kinetics and efficiency of chitosan recetylation. Carbohydr Polym. 2012;87(2):1192–8.

14. Siddaramaiah, Kumar P, Divya K, Mhemavathi B, Manjula D. Chitosan/HPMC Polymer Blends for Developing Transdermal Drug Delivery Systems. J Macromol Sci Part A. 2006;43(3):601–7.

15. Kim T, Ahn J, Choi H, Choi Y, Cho C. A novel mucoadhesive polymer film composed of carbopol, poloxamer and hydroxypropylmethylcellulose. Arch Pharm Res. 2007;30(3):381–6.

16. Ofori K, Fell J. Biphasic drug release: the permeability of films containing pectin, chitosan and HPMC. Int J Pharm. 2001;226(1):139–45.

17. Ofori K, Fell J. Leaching of pectin from mixed films containing pectin, chitosan and HPMC intended for biphasic drug delivery. Int J Pharm. 2003;250(1):251–7.

18. Perioli L, Ambrogli V, Angelici F, Ricci M, Giovagnoli S, Capuccella M. Development of mucoadhesive patches for buccal administration of ibuprofen. J Controlled Release. 2004;99(1):73–82.

19. Yehia S, El-Gazayerly O, Basalious E. Design and In Vitro/In Vivo Evaluation of Novel Mucoadhesive Buccal Discs of an Antifungal Drug: Relationship Between Swelling, Erosion, and Drug Release. AAPS PharmSciTech. 2008;9(4):1207–17.

20. Wong C, Yuen K, Peh K. An in-vitro method for buccal adhesion studies: importance of instrument variables. Int J Pharm. 1999;180(1):47–57.

21. Aframian D, Helcer M, Livni D, Robinson S, Markitziu A, Nadler C. Pilocarpine treatment in a mixed cohort of xerostomic patients. Oral Dis. 2007;13(1):88–92.

22. Babaei N, Zahedpasha S, Zamaninejad S, Gholizadehpasha A, Moghadamnia Y, Moghadamnia A. Effects of milk curd on saliva secretion in healthy volunteer compared to baseline, 2% pilocarpine and equivalent pH adjusted acetic acid solutions. Indian J Dent Res. 2011;22(4):547.

23. Sawaya A, Abreu I, Andreazza N, Eberlin M, Mazzafera P. Pilocarpine and related alkaloids in Pilocarpus Vahl (Rutaceae). Nova Sci Publ Inc. 2010:63–80.

24. Bernardi R, Perin C, Becker FL, Ramos GZ, Gheno GZ, Lopes LR. Effect of pilocarpine mouthwash on salivary flow. Braz J Med Biol Res. 2002;35(1):105–10.

25. Nakamura N, Sasano N, Yamashita H, Igaki H, Shiraishi K, Terahara A, Asakage T, Nakao K, Ebihara Y, Ohtomo K, Nakagawa K. Oral pilocarpine (5mg t.i.d.) used for xerostomia causes adverse effects in Japanese. Auris Nasus Larynx. 2009;36(3):310–3.

26. Dirix P, Nuyts S, Van den Bogaert W. Radiation-induced xerostomia in patients with head and neck cancer: A literature review. Cancer. 2006 1;107(11):2525–34.
27. Hua L, Kawasaki P, Pokala V, Hayes J. An Interprofessional Study of the Effects of Topical Pilocarpine on Oral and Visual Function. Health Interprofessional Pract. 2012;1(3):1-10.

28. Gallardo J. Xerostomía: etiology, diagnosis and treatment. Rev Med Inst Mex Seguro Soc. 2008;46(1):109–16.

29. Juliano C, Cossu M, Pigozzi P, Ras-su G, Giunchedi P. Preparation, In Vitro Characterization and Preliminary In Vivo Evaluation of Buccal Polymeric Films Containing Chlorhexidine. AAPS Pharm-SciTech. 2008;9(4):1153–8.

30. Zhou H, Hao J, Wang S, Zheng Y, Zhang W. Nanoparticles in the ocular drug delivery. Int J Ophthalmol. 2013;18;6(3):390–6.