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

Jesús Rodríguez[1].
Rosa Sánchez[1].
Marianela Garza[1].
María Nakagoshi[1].
Juan Solís[1].
Katiushka Arévalo[2,3].
Emmanuel Garza[1].

1. Facultad de Odontología, Universidad Autónoma de Nueva León. México.
2. Facultad de Ciencias Biológicas, Universidad Autónoma de Nueva León. México.
3. Instituto de Biotecnología, Universidad Autónoma de Nueva León. México.

Corresponding author: Emmanuel Garza. Depto de Histología, Facultad de Odontología, Universidad Autónoma de Nuevo León. México. E. Aguirre Pequeño y Silao, Mitras Centro, Monterrey, N.L. México, CP 64460. Phone: 52 81 83294000 Extensión UANL: 3300, 3161. E-mail: emmanueljc4@hotmail.com - emmanuel.garza@uanl.mx

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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 hyposialia (*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 cm^2 (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 ($1.44x1.2x10_6$ CFU and 10_3 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 cm^2 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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INTRODUCTION.

The use of prolonged local drug delivery to the oral cavity offers greater advantages in comparison with the systemic administration. It increases pharmacological action at the specific site, reduces the usual dose and the adverse effects[1].

Polymeric matrices of chitosan, which is a carbohydrate derived from chitin obtained from the exoskeletons of crustaceans, are active as absorption enhancers at low and high molecular weights[2] because of their low degree of deacetylation. Chitosan has haemostatic properties, serves as osteoconductive material for bone regeneration[3] and as vehicle for extended drug release[4]. It also has antimicrobial activity against *Streptococcus mutans* and *Candida*

spp.[5]. Apart from that, it has application for parenteral, oral and ocular administration, tissue engineering, and different mucosal tissues, among others[2].

Likewise, Hydroxypropyl methylcellulose (HPMC) is a hygroscopic cellulose ether[6] used for prolonged drug release[7]. 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[8].

The system of HPMC and chitosan polymeric matrices for controlled drug release provides the mechanical properties for the desired application[6,9]. Also, they have to be biocompatible, biodegradable, and avoid inflammatory, immunological and cytotoxic response[10].

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[11,12,13,14], have been studied proving beneficial for systems as a future application[15].

Currently, models containing Chitosan and HPMC[16,17] have been developed for in vivo and in vitro oral administration for prolonged release of drugs like lidocaine[1], ibuprofen[18], fluconazole[19] and metronidazole, as promising means of intraoral matrices[20].

Pilocarpine is the first drug approved by the FDA (Federal Drug Administration) for treating deterioration of salivary glands[21,22,23]. However, it has many adverse effects such as sweating, rhinitis, nausea, increased urinary frequency, as well as increased gastrointestinal secretion of hydrochloric acid[24,25], and it is contraindicated for many ailments[26,27].

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[24].

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, US.), 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, 1cm^2 of the chitosan/HPMC/Pilocarpine film was dissolved in 25ml 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 chiitosan/HPMC/pilocarpine film, 1cm^2 of the film was placed in 25ml of distilled water at 37°C under magnetic stirring. Besides, absorbance was measured every 15 minutes for 4 hours at an OD of 420nm, 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 (ICC) and *Candida albicans* ATCC 90029 in a culture medium of YPD (yeast peptone Dextrose). The cellular concentrations of the strains, *Streptococcus mutans* (1.44x10^6 CFU) and *Candida albicans* (1.2x10^3 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 1cm^2 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 cm^2:

For determining the uniformity of the Pilocarpine in Ch/HPMC/P films, the average absorbance of Pilocarpine dissolved in 25ml of saliva at an OD of 420nm 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 0.11gr (76%) per cm^2 out of 0.15gr (100%).

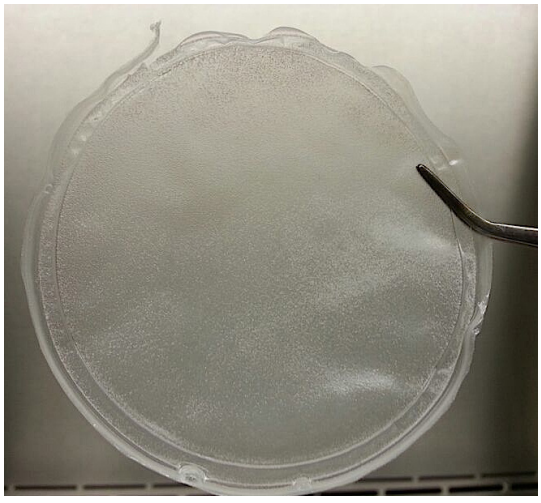
Drug release kinetics:

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:

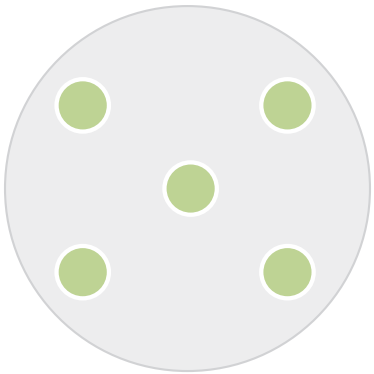
Using the diffusion technique in well with the aim to

Figure 1. Ch/HPMC/P films



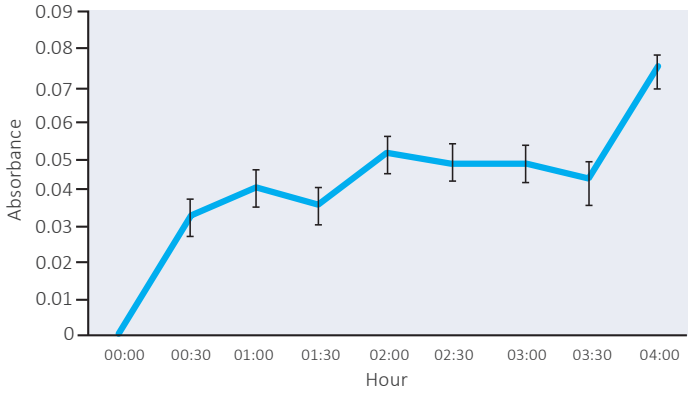
Film detachment after 24 hours drying

Figure 2. Film thickness measurements.



The five areas where the thickness of the biofilms was measured using a micrometer are shown (Mitutoyo Digimatic 1").

Figure 3. Extended release of pilocarpine.



Prolonged release kinetics of pilocarpine of Ch/HPMC/P films per time.

Table 1. PH of films.

	CH	HPMC	CH/HPMC	CH/P	HPMC/P	CH/HPMCP
Formulated pH	3.98± 0.04	2.88± 64	3.60± 0.005	3.95± 0.01	2.89± 0.02	3.60± 0.01

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

Table 2. Film thickness (µ).

Formulated thickness	CH/P	HPMC/P	CH/HPMC/P
	0.064+0.007µ	0.067+0.005µ	0.053+0.011µ

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* ($1.44x10^6$ CFU) and *Candida albicans* ($1.2x10^3$ 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 involved[28].

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 2009[9], 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[2] 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[30].

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 fisico-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, solubilidad, uniformidad y la capacidad de liberar prolongadamente pilocarpina, así como su actividad antimicrobiana ante los dos microorganismos patógenos oportunistas en la hiposialia (*Streptococcus mutans* y *Candida albicans)*. Metodología: Se elaboraron biopelículas de Quitosán e Hidroxipropilmetilcelulosa (Methocel K4MCR) en ácido acético al 1%, adicionadas con pilocarpina bajo agitación magnética, evaluando 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 cm^2 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 hiposalivación (1.44×10^6 UFC y 1.2×10^3 UFC respectivamente). Resultados: Todas las biopelículas, a excepción de la formulación Hidroxipropilmetilcelulosa e Hidroxipropilmetilcelulosa/Pilocarpina resultaron tener las propiedades físico-químicas óptimas de manipulación, manteniendo una uniformidad de difusión de la droga en 76% por cm^2 con liberación prolongada por 4 horas, sin mostrar actividad antimicrobiana en concentraciones de hiposalivación. 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*

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