Novel Mucoadhesive Extended Release Tablets for Treatment of Oral Candidosis: "*In Vivo*" Evaluation of the Biopharmaceutical Performance

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ABSTRACT: Mucoadhesive tablets containing nystatin (10 mg) were evaluated *in vivo*. The assays were carried out with 12 healthy volunteers and the concentration of nystatin in saliva was determined at different times. Tablets remained attached to the buccal mucosa during 270 min ± 30 min. No evidence of ulceration or bleeding was observed. Typical appearance of intact human buccal mucosa was seen before and after contact with the tablet. The tablets were well accepted by the volunteers, although most of the volunteers reported a light bitter taste, probably due to nystatin. Concentration of nystatin in saliva was several times higher than MIC over a period of approximately 4.5 h, which was in agreement with the behavior observed *in vitro*. These results allow us to infer that the administration of these mucoadhesive tablets could be advantageous compared to conventional formulations and mucoadhesive extended-release tablets might produce better therapeutic performance than conventional formulations in the treatment of oral candidosis.
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Keywords: formulation; solid dosage form; bioavailability; buccal; polymeric drug delivery systems

INTRODUCTION

The frequency and clinical importance of buccal affections provoked by fungi have increased as a consequence of the use of potent immune suppressor drugs in transplants, anticancer therapy, and of diseases where the host defenses are defective (AIDS).[1] Also, elderly denture wearers may suffer denture stomatitis.[2]

Candidosis is one of the most common pathologies occurring in the oral cavity, which is usually caused by *Candida albicans*.[3,4] Clinical treatment of this pathology requires long-term administration, because conventional pharmaceutical dosage forms —such as solutions, gels, suspensions, and mouthwashes— are not usually effective, principally due to the fact that drugs are quickly removed from the oral cavity. Consequently, the two main problems associated to the treatment of oral candidosis are the discontinuation of the required drug concentration in the saliva and potential side effects caused by swallowing of large quantities of the drug.

Nystatin (N) is the main antifungal agent recommended for the treatment of oral candidosis[5] and it is normally administered through oral suspensions (100,000 UI/mL) or tablets (100,000 UI) four times daily for 7–14 days.[4] However, these standard dosages imply the administration of large quantities of drug over very long periods of time. Furthermore, in spite of its efficacy *in vitro*, treatment with N can fail *in vivo*.[6,7]

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The design of mucoadhesive forms to retain the device in the oral cavity during the period of delivery, together with a sustained release of the drug to keep its concentration within the therapeutic range, are valid approaches in order to overcome the shortcomings of conventional treatments.[8,9] Also, the use of antifungal mucoadhesive systems has been investigated aiming to prolong the life time of polymeric prosthesis in laryngectomised patients.[10] In a previous work, we designed mucoadhesive tablets containing N which showed good pharmaceutical performance *in vitro* (good "*in vitro*" mucoadhesion, high water uptake and were able to modulate the release of N).[11,12] The design of these systems was based on polymeric matrices which were able to adhere to the mucosa, and at the same time, to modulate drug release, with the mucoadhesive tablets being compounded by carbomer (C), lyophilized carbomer sodium salt (CNa_L) and N.[12]

In this work, we evaluated the *in vivo* biopharmaceutical behavior of this formulation. To carry this out, mucoadhesive tablets containing N were administered to healthy volunteers and the salivary concentration of N was measured.

MATERIALS AND METHODS

Materials

Materials used were N USP (Parafarm, Buenos Aires, Argentina). C 934P (Acritamer® 934, a gift from RITA Corporation, Woodstock, IL), Lyophilized sodium carbomer (CNa_L, prepared as described in Methods Section). Sodium Sacharin (Parafarm) and Magnesium Stearate (Parafarm). All chemicals and solvents used were of analytical grade.

Methods

Attainment of CNa_L

This was prepared by dispersing C in an aqueous solution of NaOH (2 M) to obtain 100% neutralization. Then, the dispersion was homogenized in order to achieve a homogeneous semisolid, which was frozen and lyophilized using the Freeze Dry System Freezone 6 Labconco (Labconco Corporation, Kansas City, MI). The lyophilized material was subjected to particle size reduction (mesh 50) with mortar and pestle. All chemicals and solvents were of analytical grade.

Tablet Manufacture

Tablets were manufactured through a dry granulation process (DG). The powdered materials (N 10%, C 43.75%, CNa_L 43.75% and a portion of saccharin 0.75%) were blended for 16 min by tumbling. The blend thus obtained was compressed using a conventional tablet machine (Talleres Sanchez, Buenos Aires, Argentina, SP1) at a low compaction pressure in order to produce slugs of 13 mm in diameter and 5 mm thick. The slugs were milled to obtain the granules, which were then sieved. The fraction with particle size between 425 and 210 μ (#40) showed the most convenient rheological behavior, and this was therefore selected to be compressed. The prepared granulate was finally blended with the remaining saccharin (0.75%) and magnesium stearate (1%), and compressed in a single punch eccentric tablet machine (Talleres Sanchez, Buenos Aires, Argentina, SP1). The tablets, weighing 100 mg, were 7 mm in diameter and 2 mm thick.

Evaluation of Tablets

Tablet Weight Variation. This was evaluated by weighing 20 tablets on an electronic analytical balance, with a sensitivity of 0.1 mg (Mettler H35AR, Mettler-Toledo, Inc., Columbus, Ohio).

Drug Content Uniformity. Ten tablets were weighed and ground with mortar and pestle. An aliquot amount of this powder equivalent to 10 mg of N was accurately weighed, dissolved in MeOH, and analyzed using the UV-Vis spectrophotometric method at 306 nm (Shimadzu UV 160-A, Shimadzu Corporation, Kyoto, Japan).

Tablet Hardness. Ten tablets were randomly selected and tested for tablet hardness (Hardness tester DU4, AVIC, Buenos Aires, Argentina).

Tablet Friability. This was determined by testing 10 tablets in an electronic friabilator (Equipos Farmacéuticos, Buenos Aires, Argentina) at 25 rpm for 4 min.

All results were expressed as mean ± standard deviation.

In Vitro *N Release*

In vitro N release assays were carried out using a USP dissolution apparatus (USP XXIV) type 2 (paddle method, Hanson SR II 6 Flask Dissolution Test Station Hanson Research Corporation,

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Chatsworth, CA) at 37 ± 1°C, 75 rpm, with distilled water as the medium (900 mL). Tablets were fixed with a cyanoacrylate adhesive to a metallic disk placed at the bottom of each vessel. At predetermined time intervals, 5 mL samples were withdrawn and replaced with fresh dissolution media. After appropriate dilution and filtration, the UV absorption of the samples was measured at 306 nm (UV-Vis spectrophotometer Shimadzu UV 160-A, Shimadzu Corporation).

Determination of N Salivary Concentration in Healthy Volunteers

Assay. A panel of twelve healthy subjects (four male, eight female, aged 20–30 years) was used in this study. The informed consent of the volunteers, as well as of the Ethics Committee of Hospital Nacional de Clínicas approbation, were obtained. To determinate the best place in the buccal cavity for tablet adhesion, we made a prior *in vivo* study (data not shown). The results revealed that the area of greatest comfort was the cheek (left or right). So that, 1 h after breakfast, one mucoadhesive tablet was applied to the right or left cheek, in the region of the second molar with the help of a slight pressure with a finger for 30 s. No drinking was allowed 20 min before sample collection. Care was taken that the tongue did not make contact with the tablet for at least 10 min before sampling, in order to avoid an abnormally high drug release. Prior to application, a blank sample of saliva was collected. Further samples were subsequently collected at predetermined intervals of time while the tablet was attached to the mucosa. Two additional samples were collected at 15 and 30 min after tablet detachment. Samples of approximately 1 mL were collected each time. These were then placed in a vial and stored at −20°C. All volunteers were examined pre and posttablet (24 h) administration, with the aim of evaluating any possible alteration in mucosa attributable to tablet administration. All observations were documented by taking photographs.

Analytical Determination (HPLC). The N concentration in saliva samples was measured by using a HPLC method developed *ad hoc*.[13] Briefly, the chromatographic system consisted of a Waters 1525 pump, a Waters 717 plus autosampler, a Waters 1500 series column heater, a Waters 2475 multy λ Fluorescence detector (λ_{ex} 290 nm, λ_{em} 410 nm, with detection being performed in emission), and a Waters 2996 photo array detector (PDA; Waters Corp. Milford, MA). The wavelength was set at 305.6 nm. Data acquisition was performed by Empower Software data registration™. The analytical column was a reversed-phase Luna™ C_{18} (250 × 4.6 mm I.D., 100 Å, 10 μm particle size, Phenomenex™), maintained in the column oven at 25°C and protected by a SecurityGuard® precolumn. The mobile phase consisted of methanol:water:*N*-dimethylformamide (70:20:10 v/v/v). Elution was performed isocratically at 25°C, at a flow-rate of 0.8 mL/min. The mobile phase was filtered through a 0.45 μm Millipore® Durapore filter and degassed by vacuum prior to use. The assay was validated for inter- and intra-day precision (6.3% SD), linearity (0.75–50 μg/mL, $r^2 = 0.991$), recovery (more 90%), limit of detection (0.7 μg/mL) and specificity for N (no interfering peaks were observed).

RESULTS AND DISCUSSION

Rationality in Matrix Design

It is well known that powdered solid materials need to have adequate physical–mechanical properties so that the compression process can be performed without problems, in order to obtain a pharmaceutical dosage form with the required pharmaceutical quality.[14] For DC, it is indispensable that the powder blend to be compressed has a good flow, compressibility and compactability.

The selection of the polymeric matrix used in the design of the mucoadhesive tablets was based on two principal properties of the materials, the mucoadhesion and the capacity for release modulation. These properties were evaluated in a earlier work and consequently, we defined the incorporation of C and CNa_L as matrix components. The latter was incorporated to reduce the acidic properties of polymeric blend.

In this case, the polymeric matrix showed bad flow properties (angle of repose >40°, Hausner Index = 1.78, Carr Index = 43.8). Also, the incorporation of auxiliary excipients was judged to be inconvenient, because the mucoadhesion and drug release could be adversely affected. Therefore, it was concluded that some granulation process would be necessary. On the other hand, wet granulation implied the addition of a binder solution, which may be able to change the physical structure of the solids. This would be detrimental for solid performance, especially for CNa_L, and affect polymer water uptake, mucoadhesion and

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Table 1. Composition of Mucoadhesive Tablets

Component	%
Nystatin	10
Carbomer	43.75
Carbomer sodium salt	43.75
Sacharin	1.5
Magnesium stearate	1.0

drug release. Taking these facts into consideration, we utilized DG process for the manufacture of mucoadhesive tablets, as described in the Methods Section. The composition of the formula is detailed in Table 1.

Granules obtained in this way possessed better physical–mechanical properties compared to those of powder blend, when considering flow and compressibility parameters (see Tab. 2). Therefore, the #40 mesh fraction (210–425 μm) was selected for tablet manufacture. Tablets were easily made, and the pharmaceutical properties such as appearance, content uniformity, friability, hardness, and disintegration were then evaluated. The results are presented in Table 3 and showed that the properties of the tablets were found to be satisfactory.

In Vitro Nystatin Release

The mechanism of drug release from swellable matrices is governed by several physico–chemical characteristics. Among these, polymer water uptake, gel layer formation and polymeric chain relaxation are regarded as primarily involved in the modulation of drug release. Eq. (1) is usually used for the analysis of the drug release process in order to categorize the predominant mechanism:[15]

$$\frac{M_t}{M_\infty} = kt^n \quad (1)$$

M_t/M_∞ is the ratio of drug released at time t, k is the kinetic constant, and the exponent n has been

Table 2. Physical–Mechanical Properties of Granulates

	#40	#50	#70	>70
CARR	6.14863	6.17558	8.49351	26.49691
HAUSSNER	1.06551	1.06582	1.09282	1.36049
α	35.014051	36.8007	38.1782	40.8350

Table 3. Pharmaceutical Properties of Mucoadhesive Tablets

	Mean	SD	%E
Hardness (kg/cm^2)	4.42	1.08	24.5
Friability (%)	0.11		
Weight uniformity (g)	0.0991	0.0022	2.3
Content uniformity (g)	0.0118	0.0009	8.2

proposed to be indicative of the release mechanism. In this context, $n = 0.5$ indicates Fickian release (diffusionaly controlled release) and $n = 1$ indicates a purely relaxation controlled delivery which is referred as Case II transport. Intermediate values indicate an anomalous behavior (nonFickian kinetics corresponding to coupled diffusion/polymer relaxation).[16] Occasionally, values of $n > 1$ has been observed, which has been regarded as Super Case II kinetics.[17–19] N release from mucoadhesive tablet showed a biphasic mechanism (see Fig. 1). During the first stage of the release, an anomalous mechanism ($n < 1$) was observed. After this period, the release changes to a Super Case II mechanism ($n > 1.0$), where a process of plasticization occurs due to N dissolution.

In our case, 100% of N was released in a modulated fashion in approximately 5 h (see Fig. 1). It was expected and found that this behavior would allow the N salivary concentration to be maintained above the minimal inhibitory concentration (MIC) in the oral cavity during that period (see next section).

Salivary Concentration of N in Healthy Volunteers

The comparative bioavailability of N suspensions and tablets has been published elsewhere.[20] In that study, formulations containing N were administered by 5 min, then taken away from the oral cavity with the N concentration being quantified over the time. The low amount of drug in saliva in the suspensions was evident, since after 2 h no N was detected. In the case of tablets, it was reported that the N concentration remained above MIC (0.78 μg/mL) for 5 h. This observation was not well justified, being attributed to the formulation. Furthermore, it is not expected that immediate release tablets can provide enough drug concentration for a period longer than that in which the formulation remains in the administration site. This is especially true in the oral

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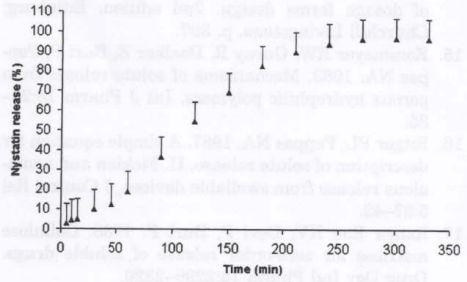

Figure 1. *In vitro* nystatin release from mucoadhesive tablets.

cavity, where the drug is quickly removed due to the fast salivary renovation and swallowing.

In the present study, mucoadhesive tablets (one dose) were administered to healthy volunteers located at the cheek. A variation in antifungal salivary concentration was reported depending on the application site of tablets.[21] In that study was observed higher drug concentrations in saliva when the formulation was attached to the gingiva instead of the cheek. However, in our case, we have previously determined that the check was the most comfortable place to attach the tablets.

Tablets remained attached to the buccal mucosa for 270 ± 30 min. Although light reversible irritation as a consequence of tissue occlusion was detected, no evidence of ulceration or bleeding was observed. The typical appearance of intact human buccal mucosa was seen, both before and after contact with the tablet. These tablets were well accepted by the volunteers, although most of the volunteers reported a light bitter taste, probably due to the presence of N.

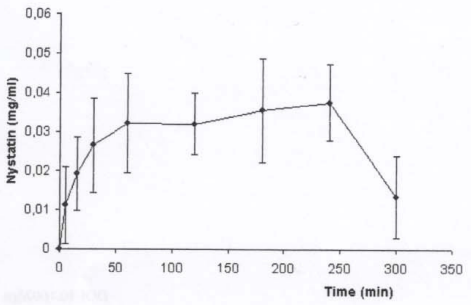

Figure 2. *In vivo* nystatin release from mucoadhesive tablets (in saliva).

With regard to the salivary concentration of N, the study revealed that the drug concentration was several times higher than MIC[22] over a period of approximately 4.5 h (see Fig. 2), which was well correlated with the N release observed "*in vitro*." Similar behavior has been observed for miconazole (10 mg) formulated in mucoadhesive tablets compounded by thermally modified maize starch and C 934, although higher amount of drug was released.[23] In this case, a longer time of adhesion was also observed, owed probably to the high mucoadhesive properties of modified starch derivatives.

All these findings permit us to infer that the administration of these mucoadhesive tablets could be advantageous compared to conventional formulations, even more so when considering that a much lower dose was used in this novel formulation. These tablets showed an acceptable bioavailability in saliva, good acceptance by volunteers, and were able to remain attached to the buccal mucosa for a long period of time thus modulating the drug release. Further studies will be now carried out with the aim of evaluating their clinical efficacy.

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