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Design and Evaluation In Vitro of Controlled Release Mucoadhesive Tablets Containing Chlorhexidine

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ABSTRACT This investigation deals with the development of buccal tablets containing chlorhexidine (CHX), a bis-bis-guanide with antimicrobial and antiseptic effects in the oral cavity, and able to adhere to the buccal mucosa to give local controlled release of drug. A mucoadhesive formulation was designed to swell and form a gel adhering to the mucosa and controlling the drug release into the oral cavity.

Some batches of tablets were developed by direct compression, containing different amounts of hydroxypropylmethylcellulose (HPMC) and carbomer; changing the amount ratio of these excipients in formulations, it is possible easily modulate the mucoadhesive effect and release of drug. The in vitro tests were performed using the USP 26/NF paddle apparatus, a specifically developed apparatus, and a modified Franz diffusion cells apparatus. This last method allows a simultaneous study of drug release rate from the tablets and drug permeation through the buccal mucosa.

Similar tests have also been carried out on a commercial product, Corsodyl gel®, in order to compare the drug release control of gel with respect to that of the mucoadhesive tablet, as a formulation for buccal delivery of CHX. While the commercial formulation does not appear to control the release, the formulation containing 15% w/w methocel behaves the best, ensuring the most rapid and complete release of the drug, together with a negligible absorption of the active agent as required for a local antiseptic action in the oral cavity.

KEYWORDS Mucoadhesive tablets, Buccal chlorhexidine controlled release, Methocel, Carbopol, Local action/permeation

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INTRODUCTION

Chlorhexidine (CHX) is a bis-bis-guanide widely used to treat skin and mucosa infections (Lafforgue et al., 1997) efficient against a wide range of microbial species (Salem et al., 1987). In particular, CHX reduces adhesion of *candida*

albicans to oral mucosal cells (Audus et al., 1992; Darwazeh et al., 1994) and has a topical efficacy against *staphylococcus epidermidis* (Lboutounne et al., 2002; Emilson, 1994; Bowden, 1996).

Many formulations containing CHX, like mouthwashes (Golasan®, Neoxene®) and dental gels (Broxodin®, Corsodyl gel®) are commercially available for the treatment of oral infections. However, these formulations may prove difficult to be used and have the major drawback of a short in situ time due to the constant flow of saliva and the mobility of the tissues involved. Chlorheridine (CHX) preparations are not suitable for long-term therapy as drug release is not controlled and is therefore usually fast. A recognized local side effect of CHX is reversible brown staining of teeth (Newton et al., 2004), which can be reduced by using small chips inserted directly into the periodontal pockets (Greenstein & Polson, 1998). Mucoadhesive tablets were found to limit staining (Maffei et al., 2004; Sköld et al., 1998) while prolonging the drug release for a long period of time, providing targeted drug delivery to the oral cavity. Therefore, in this paper, we designed and evaluated tablets containing CHX associated to mucoadhesive and swelling agents to control both the residence time in the oral cavity and the release rate of the drug.

The mucoadhesive property was developed using two polymers, polyacrylic acid (Carbopol 940) and hydroxypropylmethyl cellulose (HPMC, Methocel) in different percentages (Maffei et al., 2004). Polyacrylic acid can give an effective long-lasting controlled release of CHX, since the polymer adsorbs the drug on its surface (Ceschel et al., 2001). Hydroxypropylmethyl cellulose (HPMC) swells in contact with a liquid medium forming a gel that controls the release and reduces irritation in the mouth (Maffei et al., 2004).

Three tests were carried out to evaluate the controlled release of CHX from the tablets. The first test was carried out following a standard method, which uses the USP 26/NF paddle apparatus. The second test was carried out with a new apparatus, developed in our lab, devised to better mimic the tablet's physiological usage conditions. The third test used modified Franz diffusion cells to study at the same time both the drug release rate from the tablet and the drug permeation through the buccal mucosa, simulated by a porcine buccal mucosa.

The same tests were also applied to a commercial formulation, Corsodyl gel®.

MATERIALS AND METHODS

Materials

Chlorhexidine diacetate monohydrate was supplied by Sigma Aldrich (Milan, Italy); carbomer (Carbopol 940P), hydroxypropylmethylcellulose (HPMC, Methocel), lactose (200 Mesh), and talc were supplied by Eigenmann & Veronelli S.p.A (Milan, Italy). Corsodyl gel was a commercial sample.

Preparation of the Tablets

Six batches (M1–M6) of tablets were developed using different percentages and type of components (Table 1), while the composition of Corsodyl gel® is shown in Table 2, as declared by the producer.

All the tablets were designed to contain 1% of CHX (corresponding to 2.5 mg of chlorhexidine diacetate monohydrate for each tablet, that is the amount of drug of a single application of Corsodyl gel® to the area of the mouth).

Drug and additives were mixed with mortar and pestle. The physical mixture was compressed in a Korsch (mod. EKO) single punch tableting machine. Some technological parameters for the final tablets are listed in Table 3.

TABLE 1 **Composition (mg) of the Different Formulations (Total Weight 60 mg)**

	M1	M2	M3	M4	M5	M6
Chlorhexidine	2.5	2.5	2.5	2.5	2.5	2.5
Carbomer 974 P	/	/	/	1.5	4	12
Hydroxypropylmethyl Cellulose	9	12	20	10.5	8	/
Talc	1.2	1.2	1.2	1.2	1.2	1.2
Lactose monohydrate	47.3	44.3	36.3	44.3	44.3	44.3

TABLE 2 **Composition of Corsodyl Gel®**

Chlorhexidine Gluconate 1%
Hydrogenated Polyoxyethylencastroil
Hydroxypropylcellulose
Sodium Acetate

Tissue Preparation

Porcine buccal mucosa, with a fair amount of underlying connective tissue, was surgically removed from the oral cavity of a freshly killed male pig obtained, on each study day, from a local slaughter house (CLAI Imola, Bologna). The buccal mucosa was placed in ice-cold phosphate buffer 0.15 M. The connective tissue of the mucosa was carefully removed using fine point forceps and surgical scissors. The cleaned buccal mucosa membrane was then placed in ice-cold pH 7.4 phosphate buffer 0.06 M until it was mounted in the diffusion cells. The thickness of the porcine buccal mucosa was measured (Ceschel et al., 2000) by means of an electronic calliper: 1.0 ± 0.1 mm.

Spectrophotometer UV/VIS Analysis

Chlorhexidine (CHX) was determined using a Spectrophotometer device (UV/VIS Spectrophotometer model Jasco V-530).

HPLC Analysis

Chlorhexidine (CHX) was determined using an HPLC apparatus (Model 305, Gilson) equipped with UV detector set at 239 nm (Model Spectra 200, Spectra-Physics Optimize Technologies, Oregon City, USA). A Nova-Pak C18 (150×3.9 mm, 4 mm, Waters) column was used. Elution was carried out at room temperature with a mobile phase consisting of phosphate buffer pH 3 (50%) and acetonitrile (50%); the injecting volume was 20 µl. The flow rate was 1.0 mL/min. In these conditions, retention time of CHX was 5.00 min.

In Vitro Release Test Using a USP Paddle Apparatus

Tablets were evaluated for in vitro CHX dissolution in 900 mL of pH 7.4 phosphate buffer using the USP 26/NF paddle method at 50 rpm and 37 ± 0.2°C. Aliquots of the solution were withdrawn at appropriate time intervals and assayed for CHX using the HPLC method. All experiments were carried out in triplicate.

In Vitro Release Test Using a New Developed Apparatus

Tablet release test was carried out using an unofficial method, specifically developed for the case. The method was designed in order to simulate the physiological conditions of the tablets in the gingiva and mimic the action of saliva (Maffei et al., 2004).

The system is shown in Fig. 1. It consists of a pump that extracts a pH 7.4 buffer solution from a thermostated beaker with a Gilson 305 membrane pump (37 ± 0.2°C) and drips the solution over the test tablet from a distance of 2 cm with a flux of 1 mL/min. The dropping solution reproduces the action of saliva in the mouth. During the control a tablet adheres on a square pig's mucosa placed on a PVC holder settled at an angle of 45 degree. On the holder there are two holes of 1 cm in diameter for dripping and withdrawing the buffer solution that drips. At regular intervals of time, the dripped buffer solution is tested using UV spectrophotometer.

In Vitro Release and Permeation Test

This test was carried out using a previously modified Franz method (Fig. 2) (Ceschel et al., 2001).

The inferior compartment, filled with 4.8 mL of artificial saliva, simulated the oral cavity while the superior compartment, filled with 3 mL of a pH 7.4 phosphate buffer, simulated the blood circulation. A porcine buccal mucosa was clamped with the external surface turned versus the inferior compartment. The tablet adhered to

TABLE 3 **Technological Parameters of Tablets of Different Formulations (Mean for 10 Tablets)**

	Formulation					
Parameter	M1	M2	M3	M4	M5	M6
Diameter (mm)	7.04 ± 0.3	7.08 ± 0.7	7.14 ± 0.6	7.02 ± 0.9	7.03 ± 0.4	7.03 ± 0.8
Thickness (mm)	1.19 ± 0.6	1.22 ± 0.4	1.13 ± 0.3	1.17 ± 0.5	1.24 ± 0.7	1.15 ± 0.9
Total weight (mg)	58.6 ± 1.67	59.1 ± 2.04	57.2 ± 1.88	57.5 ± 2.13	59.2 ± 2.24	55.8 ± 2.41

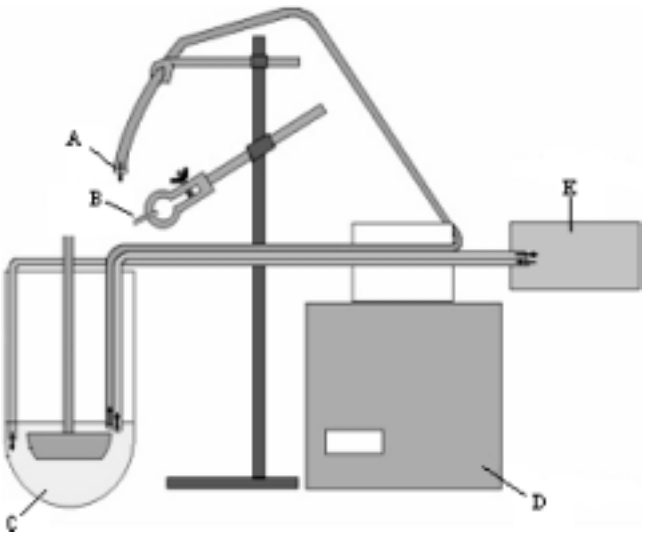

FIGURE 1 **New Apparatus Developed for In Vitro Release Test. A) Dropper; B) Porcine Mucosa and Tablet; C) Receiving Compartment; D) Pump; E) UV Detector.**

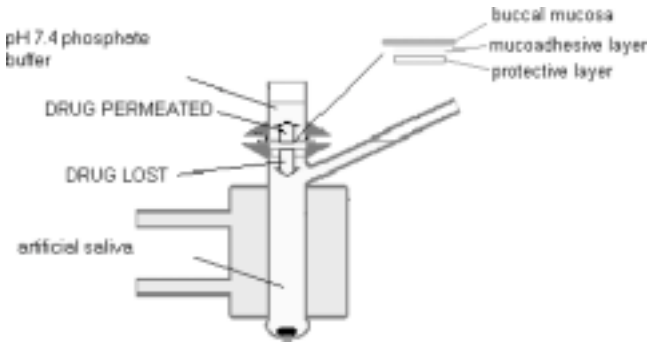

FIGURE 2 **In Vitro Release and Permeation Test Carried Out in Standard Franz Diffusion Cells.**

the external mucosal surface into the inferior compartment with the mucoadhesive layer while other parts of the tablet were in contact with the artificial saliva. The tablet fit perfectly in the inferior compartment circumference (diameter 0.9 cm) where the solution was continuously stirred at 600 rpm using a Teflon coated magnetic stirrer to simulate the mechanical movements of the mouth. The amount of CHX released in the simulated oral cavity layer was determined removing 4 mL aliquots from the inferior compartment every 30 min. while the amount of drug permeated through porcine buccal mucosa was determined removing the total amount of the superior compartment at 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h. Samples thus obtained were transferred in volumetric flasks and stored in a refrigerator until they were analyzed by HPLC (Franz, 1975; Friend, 1992).

All experiments were carried out in triplicate. Data are reported in Fig. 3, 4, 5, and 6.

In Vitro Permeation Study from a CHX Suspension

A CHX suspension of 1 mL was placed in the donor compartment. This suspension was previously heated at 50°C to saturation and then equilibrated at 37°C ± 0.5°C for 24 h. The receptor compartment contained 4.8 mL of a continuously stirred aqueous solution. The whole apparatus was maintained at 37°C by means of a thermostated jacket surrounding the whole apparatus.

The amount of CHX permeated through porcine buccal mucosa was determined by removing 2 mL aliquots from the receptor compartment using a syringe and immediately replacing the same volume of solution (kept at 37°C). The samples were transferred to volumetric flask and stored in a refrigerator until they were analyzed. Sampling schedule was 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 24 h.

All experiments were carried out in triplicate.

Data Analysis

The Higuchi and Peppas model was used to evaluate the kinetic release rate of the drug from the mucoadhesive layer towards the mucosa and to clarify the release mechanism:

$$M_t / M_{\inf} = Kt^n \qquad (1)$$

where $M_{t/}M_{\inf}$ is the fraction of drug released at time t, k is a kinetic constant, and n is indicative of release order kinetic.

Values for n and k for each formulation were obtained plotting the logarithm of the fractional release against the logarithm of time, considering data between the first withdrawal and the one corresponding to the release of the 60% of the dose:

$$\ln\left(M_t / M_{\inf}\right) = \ln K + \ln t \qquad (2)$$

The slope of the line is n while ln k is the intercept on y axis (Ritger & Peppas, 1987).

Considering that the permeation through porcine buccal mucosa is a passive diffusion process, the effective permeability coefficients (Papp [cm/s]) was evaluated, using the following equation (Richter & Keipert, 2004):

TABLE 4 ***N*** **Diffusional Exponent Values and** ***k*** **Kinetic Constants of the Release Rate of the Different Experiments**

		M1	M2	M3	M4	M5	M6	Corsodyl gel®
New in vitro	n	0.96	0.62	0.51	0.86	0.71	0.67	0.83
method	k	$1.26.10^{-4}$	$2.62.10^{-4}$	$1.87.10^{-4}$	$3.85.10^{-5}$	$8.37.10^{-5}$	$6.59.10^{-5}$	$1.74.10^{-4}$
USP 26 paddle	n	0.87	0.74	0.50	0.76	0.68	0.42	0.62
Standard method	k	$2.83.10^{-4}$	$2.48.10^{-4}$	$2.94.10^{-4}$	$3.59.10^{-5}$	$7.25.10^{-5}$	$2.47.10^{-5}$	$1.16.10^{-4}$
Modified	n	0.94	/	0.79	/	/	0.76	0.63
Franz method	k	$5.84.10^{-4}$	/	$3.37.10^{-4}$	/	/	$4.24.10^{-5}$	$8.23.10^{-4}$

In vitro release test using a new developed apparatus (new in vitro method), in vitro release test using a USP Paddle apparatus (USP 26 paddle Standard method), and in vitro release test in Franz cells (modified Franz method).

$$\text{Papp} = (\text{dc/dt})(V / A\,\text{Co}60) \qquad (3)$$

where dc/dt (mg/min) is the increase of permeated cumulative drug amount versus time, V is the volume of the receiver compartment, A is the surface area of the membrane (0.6 cm^2), Co is the initial drug concentration in the donor compartment, and 60 is the conversion factor from minute into second. The steady-state flux (dc/dt) was determined from the slope of the linear portion of cumulative permeated drug amount versus time. The lag time was also determined from this graph, extrapolating the linear portion to the x-axis. Results are shown in Table 4.

RESULTS AND DISCUSSION

Buccal delivery offers a simple and non-invasive route for systemic delivery of drug. However, when a local action of the drug is required, absorption across the oral mucosa must be avoided and permeability enhancers must be absent in the formulation (Zhang and Robinson, 1996). On the contrary, due to the peculiar environment of the mouth (mechanical movements, deglutition, dilution with saliva, speaking, etc.), an increased residence time of the formulation is needed for an efficient local action. Moreover, morphological similarities and component water permeability suggested considering porcine buccal mucosa as a model for CHX permeability studies.

In Vitro Release Test Using the Paddle Apparatus

Stable tablets were obtained with present formulations (Table 1), which allow a long residence time inside the oral cavity, and therefore a prolonged release of CHX at the same time, preventing its absorption since CHX must act locally on the mouth surface as antiseptic. Mucoadhesive property was developed by Carbomer 940P and/or HPMC, together with lactose, as a filler. The presence of HPMC and Carbomer 940P inside solid dosage forms is largely used to achieve mucoadhesion and to control the release of the drug (Anlar et al., 1994; Taylan et al., 1996) also for buccal formulations. These polymers, when hydrated with water, adhere to the mucosa and control the drug release. Their mucoadhesion is so largely described that in the present paper we did not measure adhesion of the tablets.

The second point was achieved by suitably changing their amount and weight ratio inside the tablets. Since HPMC and carbomer are known to exert mucoadhesion, in this paper we mainly aimed to study a controlled release and the absence of absorption since this aspect is more important. Chlorhexidine (CHX) is, in fact, a local aseptic agent and its absorption could become not negligible during the long residence time designed for these CHX releasing tablets in the oral cavity.

Figures 3 and 4 show the release profiles obtained according to the USP standard paddle method and those obtained by the newly developed method. Some points can be outlined.

- The method developed in our lab appears to discriminate better the six formulations as far as CHX release is concerned; moreover, the profiles appear more regular and the rank order observed with USP method is maintained and made clearer. Using this second method, release profiles appear almost linear as a function of time.

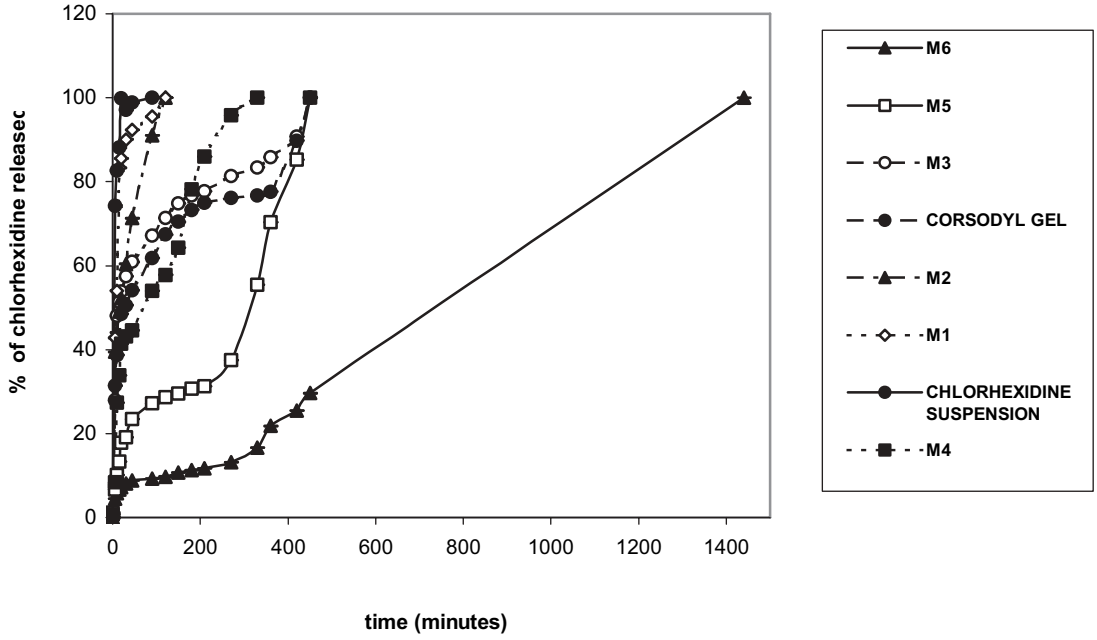

FIGURE 3 **CHX Release Profiles Obtained Following USP 26 Standard Paddle Method.**

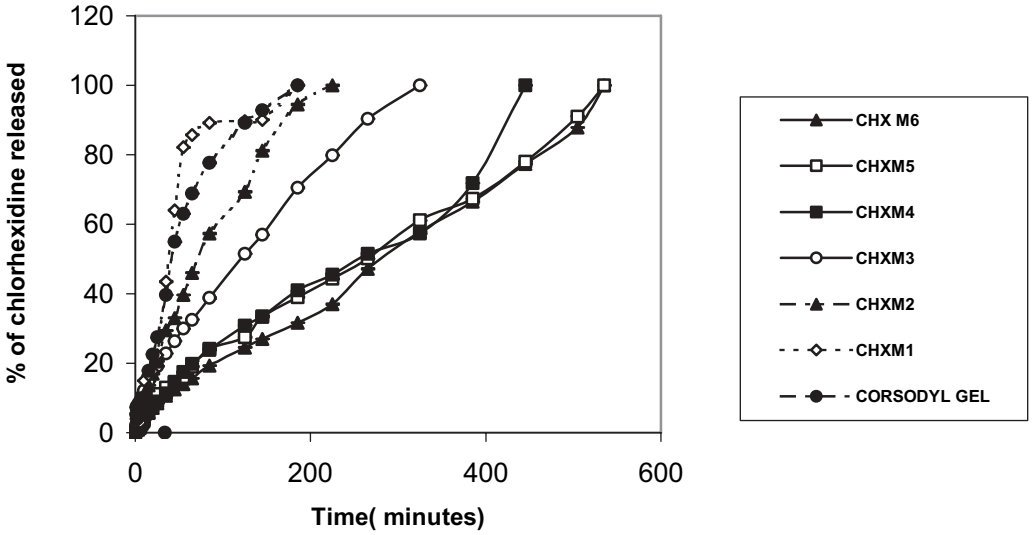

FIGURE 4 **CHX Release Profiles Obtained Using New Developed Apparatus.**

- M1 and M2 formulations do not provide any control of the release since it is complete after a short time, differently from M4, M5 and M6, where the release is complete after 5–6 h and their profiles overlap. An increasing HPMC concentration slows down the release rate: M1 > M2 > M3 for the slope of the linear portion. The formation of a gel of increasing viscosity, as HPMC concentration increases, limits the release providing a control. Formulations, where carbomer content is the highest one, release CHX very slowly.
- The commercial formulation behavior is intermediate between M1 and M2 formulations.
- Carbomer, independently of the concentration, levels the release profile for M4, M5, and M6 (Fig. 4). These results can be attributed the chemical

structure of carbomer, containing carboxylic moieties, which ionize in the presence of a dissolution medium at pH 7.4. This fact, swelling the tablet, should increase the release, but the possibility that positively charged CHX forms ion-pairs with this negatively charged polymer (Vilches et al., 2002) appears dominant since these profiles display the lowest slope.

Analysis of the experimental data for CHX release in the terms of the Peppas equation reveals that *n* values, that is the diffusional exponents, vary in the range 1–0.5, suggesting that increasing HPMC (in M1, M2, and M3) and carbomer concentrations (in M4, M5, and M6) modify the release mechanism. The observed trend appears independent of the method used to follow the release. *N* values decrease as the polymer concentration increases, suggesting a dominant diffusion mechanism of the release for M3 and M6, where the polymer concentration is the highest one. Possibly, these polymers ensure higher mechanical stability to the tablets since when concentration is low, an almost zero-order kinetic is observed which is in accord with a possible erosion of the tablets. According to these results, further tests were designed only for M1, M3, and M6 formulations together with the commercial gel.

In Vitro Release and Permeation Test

Permeability and release were examined using the device previously described. When the tablet was inserted inside the Franz cell, Fig. 5 shows the amount of drug found in the inferior compartment as a function of time, that is, drug released in the simulated oral cavity. In these conditions, the results showed that M1 releases the drug completely. The release profile of Corsodyl gel®, close to that of M3, is much lower than that of M1. M6 tablets showed a very low CHX release. The order M1 > Gel > M3 > M6 is the same as that observed in the dissolution test previously described even though the release in the experimental conditions of a Franz cell is more limited.

The permeation test was also carried out on a CHX suspension to evaluate the permeation characteristics of the non-formulated drug. Figure 6 shows the permeation profiles of CHX from the selected tablets compared with the CHX suspension.

The permeation profiles and the corresponding Papp values are listed in Table 5. They range from 5.10^{-6} cm/s for the suspension that is the highest value to $1.4.10^{-8}$ for M1 formulation. These values suggest that the excipients used are able to control mucosa permeation of CHX from the tablets. It is known from the literature that when the release compartment contains agents able to interact with the permeating drug, in these conditions, reduction of Papp is expected. This can explain the low Papp for M6 formulation since the presence of carbomer retains the drug, thereby reducing mucosal permeation. M1 shows the lowest drug permeation profile. The low content of HPMC negatively affects the swelling and thus the release extent, and this fact together with the best release, suggests that M1 is an appropriate formulation for a mucoadhesive buccal tablet where release

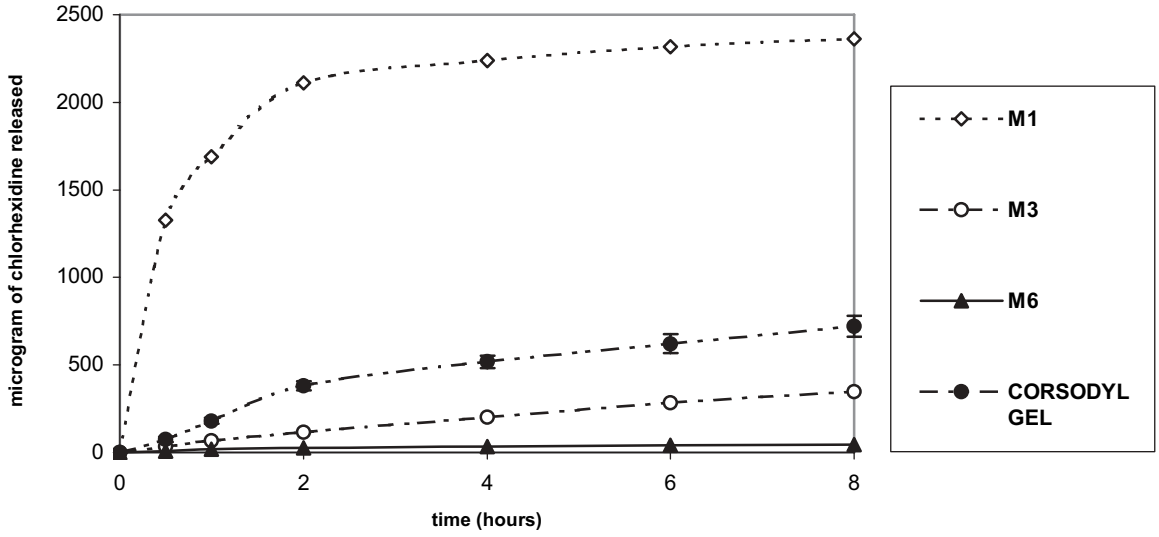

FIGURE 5 **CHX Released in the Simulated Oral Cavity of Franz Cell.**

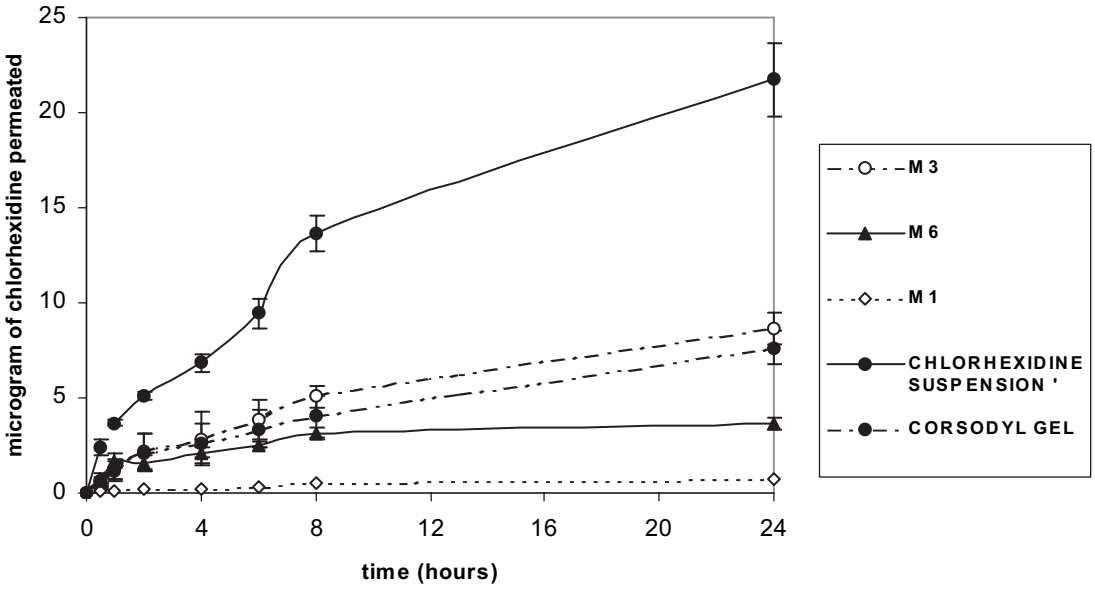

FIGURE 6 **CHX Permeation Profiles for Different Tablets and from Suspension.**

TABLE 5 **Permeability Coefficients of CHX Across the Mucosa from the Different Tablets and from the CHX Suspension**

	M1	M3	M6	Corsodyl gel®	Chx suspension
Papp (cm/s)	$1.4.10^{-8} \pm 0.36$	$2.10^{-7} \pm 0.89$	$3.0.10^{-8} \pm 0.97$	$2.10^{-1} \pm 0.72$	$5.10^{-6} \pm 0.56$

occurs rapidly in the oral cavity without any degree of absorption of the active drug.

CONCLUSIONS

In this study, different mucoadhesive tablets were formulated to adhere to the buccal mucosa for a local CHX release and compared with a commercial formulation.

The tests carried out highlighted the importance of nature and amount of the excipients in CHX formulations to guarantee controlled local drug release from the tablets.

M1 tablet yielded the highest CHX release in association with the lowest mucosal permeation. The M6 tablet had the slowest release profiles and a low permeation profile. The M3 tablet had release and permeation results comparable to Corsodyl gel®. This result is related to the presence of a high percentage of HPMC in M3 than in the M1 formulation.

M1 formulation is suitable formulation for local oral administration of CHX because it guarantees efficient controlled and high release of CHX in the oral cavity with the lowest mucosal permeation of the drug.

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