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Research Article

Preparation, *In Vitro* Characterization and Preliminary *In Vivo* Evaluation of Buccal Polymeric Films Containing Chlorhexidine

Claudia Juliano,[1] Massimo Cossu,[1] Paola Pigozzi,[1] Giovanna Rassu,[1] and Paolo Giunchedi[1,2]

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Abstract. The aim of this work was to investigate the suitability of some polymeric films as buccal systems for the delivery of the antiseptic drug chlorhexidine diacetate, considered as a valid adjunct in the treatment of oral candidiasis. Six different film formulations, mono- or double-layered, containing 5 or 10 mg of chlorhexidine diacetate, respectively, and alginate and/or hydroxypropylmethylcellulose and/or chitosan as excipients, were prepared by a casting-solvent evaporation technique and characterized in terms of drug content, morphology (scanning electron microscopy), drug release behavior, and swelling properties. Moreover, the *in vivo* concentrations of chlorhexidine diacetate in saliva were evaluated after application of a selected formulation on the oral mucosa of healthy volunteers. The casting-solvent evaporation proved to be a suitable technique for preparing soft, flexible, and easily handy mono- or double-layered chlorhexidine-loaded films. Some prepared formulations showed favorable *in vitro* drug release rates and swelling properties. The behavior of a selected formulation, chosen on the basis of its *in vitro* release results, was preliminarily investigated *in vivo* after application in the oral cavity of healthy volunteers. The films were well tolerated and the salivary chlorhexidine concentrations were maintained above the minimum inhibitory concentration for *Candida albicans* for almost 3 h. These preliminary results indicate that polymeric films can represent a valid vehicle for buccal delivery of antifungal/antimicrobial drugs.

KEY WORDS: buccal mucoadhesive; chitosan; chlorhexidine; polymeric films; sodium alginate.

INTRODUCTION

Although *Candida albicans* is a normal commensal in as many as 40% to 65% of healthy adult mouths, it causes a common infection in people wearing dentures, and severe oropharyngeal candidiasis is reported with increasing frequency in patients immunosuppressed or receiving anticancer radiotherapy (1,2).

In addition to a number of effective antifungal drugs available for topical and systemic therapy, chlorhexidine has been used as adjunctive supplement in oral candidosis treatment (3). Traditional dosage forms, such as solutions and semisolid formulations, have been used as vehicles to topically deliver antifungal drugs in the oral cavity. However, the major difficulty for the successful eradication of oral fungal infections is the short time of residence of the drugs due to their rapid dilution by saliva and the swallowing reflex, which usually leads to a rapid decline in their concentration (4).

In order to prolong antimicrobial drug retention in the oral cavity at inhibitory levels, various bioadhesive dosage forms have been recently proposed, such as gels, tablets, and films (5–7). Although buccal films have not yet been widely investigated, they seem to be preferable to mucoadhesive gels or tablets in terms of duration of drug release and flexibility and comfort, respectively (8). Some bioadhesive polymers have been investigated as excipients for formulation of antifungal drug delivery systems (9–11); in particular, alginate and chitosans appear to be of great interest because they effectively combine physicochemical and functional properties. Indeed, first of all, their well-known mucoadhesive properties make them particularly useful for the development of prolonged drug delivery systems (12,13); in addition, they are able to inhibit both growth and bioadherence of *C. albicans*, alginate being more effective (14,15).

The objective of our work was the development of mono- and double-layered buccoadhesive films made of alginate and/or hydroxypropylmethylcellulose and/or chitosan and containing chlorhexidine diacetate aimed to the controlled release of this drug into the buccal cavity. The films were prepared with a casting-solvent evaporation technique and characterized for their swelling and mucoadhesive properties and for their *in vitro* and *in vivo* drug release behavior.

MATERIALS AND METHODS

Materials

Chlorhexidine diacetate and sodium alginate [high viscosity, 2.0% (*w*/*v*) aqueous solution at 25°C with viscosity of

[1] Department of Drug Sciences, University of Sassari, 07100, Sassari, Italy.
[2] To whom correspondence should be addressed. (e-mail: pgiunc@uniss.it)

approximately 14,000 cPs, manufacturer value] were purchased from Sigma Chemicals (St. Louis, MO, USA). Hydroxypropylmethylcellulose (HPMC; Methocel® K100-LV Premium) was from Dow Chemical Company (Midland, MI, USA). Chitosan, deacetylation degree 75–85%, viscosity (Brookfield, 1% solution in acetic acid) 200–800 cps, was supplied by Aldrich (Milwaukee, WI, USA). Glycerol (Sigma) was used as plasticizer. All solvent used were of analytical grade; water was of MilliQ grade.

Chlorhexidine diacetate was used as 0.1% (*w*/*v*) aqueous solution to prepare alginate and alginate/chitosan films and as 0.06% (*w*/*v*) ethanolic solution for HPMC films. Sodium alginate was dissolved at 0.5% and 1% (*w*/*v*) in MilliQ water. Chitosan was dissolved in hydrochloric acid 0.1 M at 1% (*w*/*v*), and the resulting solution was evaporated to dryness; the residual was then redissolved in 100 mL of water. Finally, HPMC solution was prepared at 0.75% (*w*/*v*) in dichloromethane.

Preparation of Polymeric Films

Six different film formulations were prepared, four mono-layered containing 5 mg of chlorhexidine diacetate and two double-layered containing 10 mg of the drug. Table I shows the theoretical compositions (% *w*/*w*) of the six formulations obtained.

Mono-layered films were produced by a casting-solvent evaporation technique. Briefly, suitable volumes of glycerol and of chlorhexidine, alginate and/or HPMC and/or chitosan solutions were mixed under constant magnetic stirring at room temperature. Resulting solutions were left to stand to allow the entrapped air bubbles to be removed, then poured on plastic or glass molds (50-mm depth, 50-mm inside diameter, casting area 19.63 cm^2) equipped with removable plastic bottoms (Fig. 1a) and completely dried in an oven at 37±1°C for a week, except for HPMC solutions which needed only 24 h. The plastic bottoms were then removed (Fig. 1b) and the resulting films, translucent and flexible (Fig. 1c), were carefully detached and stored at room temperature until further analyses.

Double-layered films were prepared in an attempt to obtain formulations with an outer layer able to deliver a first dosage of chlorhexidine and an inner buccoadhesive layer suitable for achieving sustained release of the same drug; they were obtained by overlapping two selected films and introducing them into a pocket (6.5×6.5 cm) made out of an inert plastic net (mesh aperture 4 mm; Fig. 2b). Pockets were placed in a moist chamber at 37°C for 90 min to allow the films to stick each other; the resulting double films were finally dried at 37°C for 24 h and stored at room temperature until further analyses.

Morphology Observation

The morphological characteristics of the bottom and upper surface of mono-layered films were studied by scanning electron microscopy (SEM). Film samples were mounted on aluminum stubs using double-sided adhesive tape and then analyzed with a Zeiss DSM 962 electron microscope (Zeiss, Germany) at 20-kV acceleration voltage, after gold sputtering, under an argon atmosphere.

Drug Content Determinations

Chlorhexidine diacetate content in the mono-layered films was determined as follows: each film was dissolved in 200 mL of MilliQ water in a volumetric flask, under magnetic stirring, and the drug concentration was then evaluated spectrophotometrically at the wavelength of 262 nm, with a Lambda 3 UV/vis spectrophotometer (Perkin-Elmer, Germany).

The amount of effectively incorporated drug (real drug content) was calculated as the experimentally detected amount of chlorhexidine diacetate with respect to the theoretical amount (5 mg) of the drug used for the preparation of films and expressed as percentage.

Each drug content determination was the average of six determinations.

The interference of the polymers on the chlorhexidine absorbance was checked.

In Vitro Drug Release Tests

In vitro chlorhexidine diacetate release tests of buccal films were carried out using the USP dissolution apparatus 2 (paddle). The dissolution medium was USP phosphate buffer pH 7.0, 1,000 mL (sink conditions) at 37±0.1°C, and at a stirring rate of 100 rpm. Each film was introduced in the plastic pocket described above (Fig. 2a), which was suspended immediately beneath the surface of the dissolution medium. At appropriate time intervals, 1 mL samples were withdrawn and then replaced with the same volume of buffer, and drug concentrations were determined spectrophotometrically at the wavelength of 262 nm. For each film formulation, at least six samples were examined.

Swelling Ratio Measurements

The swelling behavior of the polymeric films was evaluated according to Peh and Wong (9) by measuring the weight increase after contact with USP phosphate buffer, pH 7.0. Samples (1.5×1.5 cm, 2.25 cm^2) of each film, cut with

Table I. Composition of Buccal Films (% *w*/*w*)

Formulation	A	B	C	D (A+C)	E (B+C)	F
Sodium alginate	58.69	73.96	–	10.61	19.19	70.82
HPMC	–	–	64.77	53.06	47.97	–
Chitosan	–	–	–	–	–	4.25[a]
Glycerol	29.58	18.64	34.64	32.09	29.01	17.85
Chlorexidine diacetate	11.74	7.40	2.59	4.24	3.84	7.08

[a] Express as chitosan base

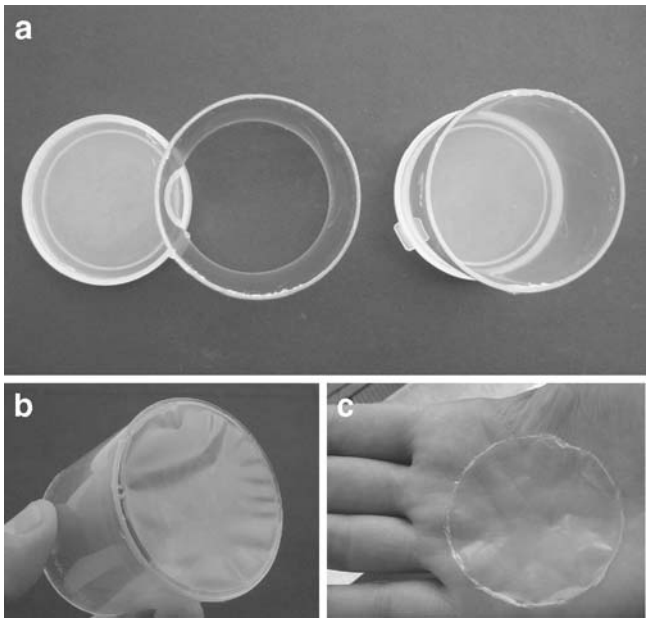

Fig. 1. Plastic mould with removable plastic bottom (detached, *left*, and inserted, *right*) used to prepare polymeric films by a casting-solvent evaporation technique (**a**). Mold with a dry film after bottom removal (**b**); alginate-based film (formulation B) (**c**)

a lancet blade, were weighed and placed on a pre-weighed plastic net (2×2 cm, mesh aperture approximately 500 μm). The support holding the film sample was then placed in a Petri plate (inner diameter 5.5 cm, height 1.2 cm) containing 10 ml of phosphate buffer, removed at appropriate time intervals, and weighed after wiping excess water with blotting paper. This operation was repeated until a constant weight was observed. Double-layered films were then analyzed by exposing both the surfaces to the buffer. At least six samples per formulation were examined.

The degree of fluid uptake was calculated as swelling index using the following equation:

$$\text{Swelling index} = (W_t - W_0)/W_0$$

where W_0 is the initial weight of the sample and W_t its weight at t time.

Determination of Film Thickness

Thickness evaluation was performed on three films of the formulation F, chosen for the *in vivo* test, according to Juliano *et al.* (16). From each film, whose lower surface had previously been marked with black ink, three extremely narrow stripes (length 3 cm) were cut by using a lancet blade, placed on glass slides, and blocked at their ends. For the analysis of thickness of these transversal cuts, at least ten measurements were performed at different points of each stripe using a light microscope Standard Universal Carl Zeiss (Berlin, Germany) equipped with a micrometric ocular.

Mucoadhesive Properties

The mucoadhesivity of the formulation F was evaluated using a modified precision balance (17) according to Sandri *et al.* (18) (modified) by measuring the detachment forces between samples of the films and a cellulose membrane (Sartorius, Goettingen, Germany) conditioned with 4% mucin solution (from porcine stomach, type II, Sigma) or with phosphate buffer, pH 7.0 (blank) 120 s after the contact is established. Results were expressed as detachment force (in mN/cm^2) required to detach the two systems (polymeric film/cellulose membrane; average of nine samples obtained from three films).

In vivo Preliminary Evaluation of Buccal Films

In vivo tests were performed on film formulation F (five replicas) chosen on the basis of the results of *in vitro* tests. The experiments were carried out after approval of the protocol by the scientific ethics committee of the University of Sassari. The films were placed (with the alginate side) on the buccal mucosa (cheek) of three volunteers (healthy males, 30–35 years old). Films placed on the buccal mucosa of the volunteers swelled and then dissolved very slowly; at the end of the test, only some fragments were present into the oral cavity. At each time point of the experiment, samples of saliva (about 1 ml) were collected and chlorhexidine concentrations were immediately determined by a high-performance liquid chromatography (HPLC) assay as described by Pesonen *et al.* (19). The following instruments were used: Hewlett-Packard 1050 series quaternary pump and variable wavelength detector operating at 257 nm (Hewlett-Packard, Germany). The standards and the samples were injected through a 20-μL autosampler injection. The peak areas, determined with a 3390 integrator (Hewlett-Packard, USA), were used for quantitation. Reverse-phase HPLC was performed at room temperature. The column used was a Nucleosil RP-18 (150× 4.6 mm I.D.) from Alltech with a 5-μm average particle diameter. The mobile phase was acetonitrile buffer (0.1 M disodium hydrogen phosphate, 0.005 M 1-heptanesulfonic acid, and 0.05 M triethyleneamine) 35:65 (*v*/*v*). The pH of the buffer was adjusted to 2.5 with phosphoric acid. The flow rate was 1 mL/min.

An extraction procedure of chlorhexidine from saliva was set up and carried out as follows: a 200 μL sample of clear saliva was introduced into a test tube, and 400 μL of 4.5 M sodium hydroxide and 500 μL of acetonitrile were added. The tube was vortex-mixed for 1 min and centrifuged for 1 min at 14,000 rpm and the solution filtered by

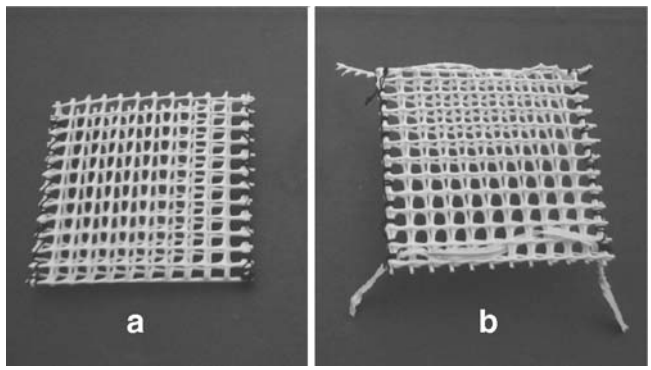

Fig. 2. Plastic pocket used to suspend films into the dissolution vessel (**a**). The same plastic pocket, equipped with short lifting rests, was used to prepare double-layered films (**b**)

polytetrafluoroethylene filter (0.2 μm). Twenty microliters of the organic phase was injected into the HPLC system.

RESULTS AND DISCUSSION

Morphological Characteristics of Mono-layered Films

The mono-layered formulations, which were studied on both sides by scanning electron microscopy, are characterized by two surfaces with a quite different appearance. In particular, upper surfaces of the alginate-based films showed a very smooth and uniform aspect (Fig. 3a), with no substantial differences depending on the different polymer concentrations used for the preparation; the bottom surfaces appeared relatively rough, probably in consequence of the forces applied during the film detachment. Upper surfaces of films made out of HPMC showed a finely granular structure (Fig. 3b) not present on the bottom surfaces and then probably depending on a possible drug crystallization during the rapid evaporation of the solvent. Finally, alginate–chitosan films appeared always characterized by short parallel cracks (Fig. 3c).

Determination of Drug Content of Mono-layered Films

The real chlorhexidine amount was close to the theoretical in formulations A and F (about 89.6% and 95.1%, respectively) and of about 82.6% for B formulation. On the contrary, HPMC-based films (formulation C) contained 72.0% of the expected drug amount, probably due to the fact that large volumes of drug–polymer solution had to be cast, with a partial loss of chlorhexidine on the mold walls during the drying. The drug content of double-layered films was assumed to be the sum of the mono-layered films utilized. Polymers did not interfere with chlorhexidine absorbance at the specified wavelength.

In Vitro Drug Release Tests

The results of the *in vitro* assays are reported in Figs. 4 and 5, expressed both as percentage and as amount (μg/mL) of chlorhexidine released.

The *in vitro* release profiles of mono-layered formulations show differences depending on their composition (Fig. 4). In particular, HPMC was not able to modulate the chlorhexidine release because more than 80% of the drug was delivered from HPMC films within only 30 min; the remaining part of the drug was delivered in about 3 h of dissolution. When chlorhexidine was incorporated in alginate and alginate/chitosan-based films, the drug delivery was delayed because the percent released at 30 min was only 30–35%; after 3 h, A and B released more than 80% of chlorhexidine, while F formulation reached 93%. The release profiles of B and F formulations were almost superimposed, while A films, characterized by a lower alginate content, gave lower drug concentrations.

Figure 5 shows the patterns of chlorhexidine release from the double-layered films. At 30 min, D formulation was able to produce a higher drug concentration (54%) compared to formulation E (33%), but in both cases, more than 90% chlorhexidine was released in 4 h.

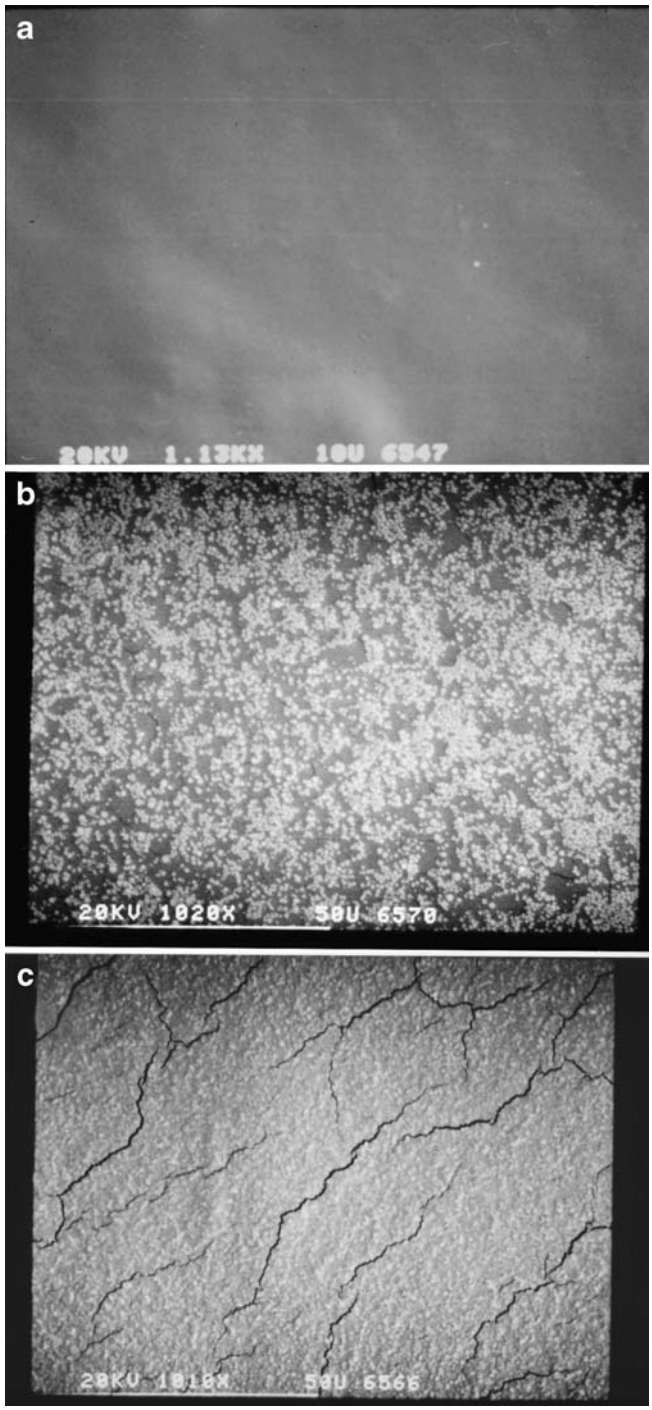

Fig. 3. SEM micrographs of polymeric films. **a** Formulation B (*upper surface*); **b** formulation C (*upper surface*); **c** formulation F (*upper surface*)

Swelling Ratio Measurements

Figure 6 shows the swelling behavior of polymeric films. As far as mono-layered films are concerned, the highest buffer uptake was observed with alginate-based formulations (A and B); their samples reached their maximum swelling indexes at 45 s, then these values started to decrease, probably because of the incipient film dissolution. HPMC

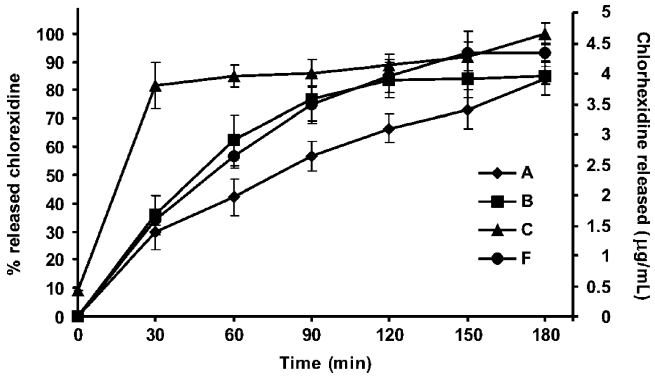

Fig. 4. *In vitro* release profiles (USP phosphate buffer, pH 7.0) of chlorhexidine from mono-layered buccal films (n=6)

films (formulation C) exhibited the lowest swelling index, while formulation containing alginate–chitosan (F) showed intermediate, regularly increasing values.

The swelling behavior of double-layered films varied dramatically depending on the side exposed to the buffer. Indeed, their hydration was quite poor and comparable to that one of HPMC films when they were exposed to swelling medium with their HPMC surfaces; conversely, they swelled at a remarkably greater extent when their alginate surfaces were exposed to the buffer, although their swelling indexed did not reach the values of the alginate-based mono-layered formulations.

Film Thickness

As far as thickness is concerned, films of formulation F were very homogeneous, their thickness values being 19.8± 1.1 µm.

Mucoadhesive Properties

Films made of alginate–chitosan (formulation F) demonstrated to have a good *in vitro* adhesion property, the measured detachment force needed for the separation of the two surfaces (alginate film/cellulose membrane) being 359.22± 31.81 mN/cm^2 compared to the detachment force between the films and the buffer (blanks; 233.31±7.41 mN/cm^2).

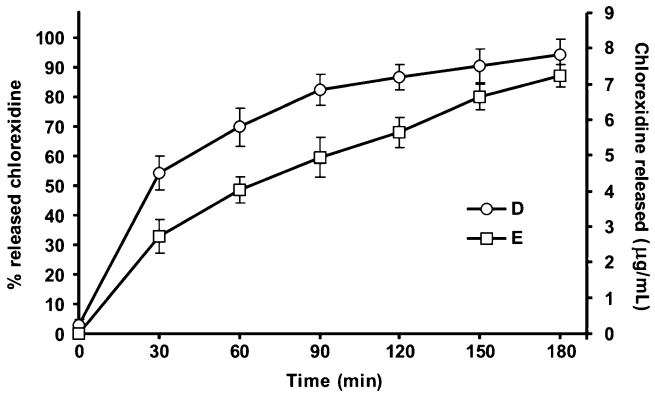

Fig. 5. *In vitro* release profiles (USP phosphate buffer, pH 7.0) of chlorhexidine from double-layered buccal films (n=6)

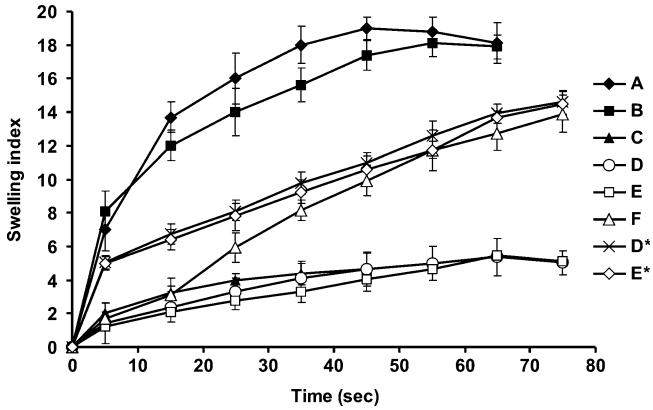

Fig. 6. Swelling index of film formulations. *D* and *E* Double-layered film samples exposed to the buffer with their HPMC surface; *D** and *E** double-layered film samples exposed to the buffer with their alginate surface

In Vivo Preliminary Evaluation of Buccal Films

For *in vivo* assays, the F formulation was chosen on the basis of its favorable *in vitro* drug release profile and its swelling ratio, which shows a good but not excessive hydration. The salivary levels of chlorhexidine diacetate obtained after application of formulation F on the cheek mucosa of a healthy volunteer are shown in Fig. 7. Chlorhexidine concentrations rose in a regular way, reaching its highest level (33.18 µg/mL) 120 min after the application and starting to decrease after 150 min. It is remarkable that the application of film F maintained chlorhexidine levels above the minimum inhibitory concentration against *C. albicans* (7.8 µg/mL) (20) from 15 min onwards. Moreover, the films showed comfortability and tolerability in the oral cavity because they did not cause irritation or pain on the cheek mucosa and did not produce appreciable saliva level variations.

CONCLUSIONS

Two kinds of polymeric films, mono- and double-layered, were designed and prepared to produce an intraoral controlled delivery of chlorhexidine diacetate. Mono-layered

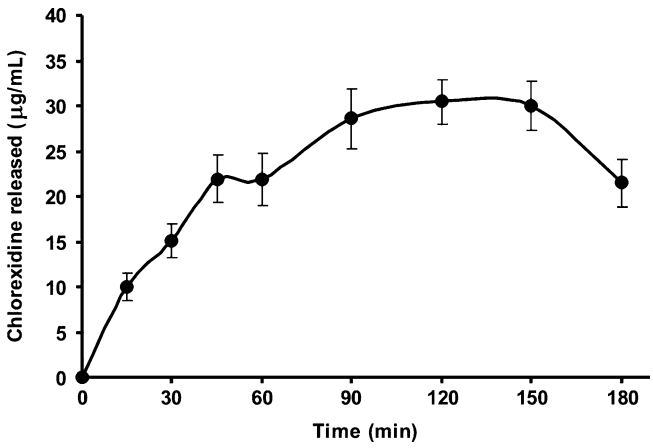

Fig. 7. *In vivo* concentrations (µg/mL) of chlorhexidine after application of formulation F to buccal mucosa of healthy volunteers. Each point represents the mean±SD (n=3)

films, loaded with 5 mg of chlorhexidine diacetate, were obtained with a casting-solvent evaporation technique by using as polymers sodium alginate or sodium alginate/chitosan because of their mucoadhesive properties; they aimed to give a sustained release of antiseptic drug, at active concentrations, over a reasonable period of time. Double-layered films were prepared by sticking together a HPMC film, containing 5 mg of chlorhexidine diacetate, and an alginate film, containing the same amount of drug; when applied to the oral mucosa with their mucoadhesive side, these devices were supposed to release quickly high chlorhexidine concentrations from HPMC layer and, subsequently, to deliver the residual drug in a sustained way from the alginate surface.

B and F mono-layered and both double-layered formulations showed favorable *in vitro* release profiles because they delivered therapeutically significant concentrations of chlorhexidine for 3 and 4 h, respectively. In dependence on the higher amount of loaded drug, double-layered films allowed to reach higher chlorhexidine concentrations and for a more prolonged time.

Since B and F formulations exhibit very similar drug delivery patterns, the presence of a small amount of chitosan in F films seems to have no influence on the profile of their chlorhexidine release. However, a remarkable difference can be observed in the swelling behavior of these formulations; indeed, the alginate-based formulations are characterized by a very fast swelling, whereas an addition of a small amount of chitosan results in a decrease of the speed and of the extent of swelling of the films. The swelling is directly related to the rate of hydration, and if some degree of hydration is required for a satisfying bioadhesion, we might suppose that the buccal mucoadhesive performance and the residence of a film are negatively affected by an excessive water absorption by the polymeric network.

Considering our *in vitro* data about dissolution and swelling, F formulation was judged the best candidate for an *in vivo* investigation in a healthy volunteer. These films, placed on the buccal mucosa, showed a good performance, with a prolonged residence time at the application site in agreement with the good results obtained in the *in vitro* mucoadhesion experiments and useful chlorhexidine saliva levels for an appropriate period of time.

On the whole, our results demonstrated that our polymeric films are promising candidates for the sustained release of chlorhexidine diacetate in the oral cavity: the polymers used guarantee effective mucoadhesion, good comfortability, and satisfactory drug release profiles. Moreover, the presence of chitosan in some of our formulations can also contribute to prevent the development of mycoses because it inhibits *Candida* adhesion to buccal mucosa (21).

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