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Research Article

A Comprehensive Development Strategy in Buccal Drug Delivery

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Abstract. This work combines several methods in an integrated strategy to develop a matrix for buccal administration. For this purpose, tablets containing selected mucoadhesive polymers loaded with a model drug (omeprazole), free or in a complexed form with cyclodextrins, and in the absence and presence of alkali agents were subjected to a battery of tests. Mucoadhesion studies, including simple factorial analysis, *in vitro* release studies with both model-dependent and model-independent analysis, and permeation studies were performed. Mucoadhesive profiles indicated that the presence of the drug decreases the mucoadhesion profile, probably due its hydrophobic character. In tablets loaded with the drug complexed with β-cyclodextrin or methyl-β-cyclodextrin, better results were obtained with the methylated derivative. This effect was attributed to the fact that in the case of β-cyclodextrin, more hydroxyl groups are available to interact with the mucoadhesive polymers, thus decreasing the mucoadhesion performance. The same result was observed in presence of the alkali agent (L-arginine), in this case due to the excessive hydrophilic character of L-arginine. Drug release from tablets was also evaluated, and results suggested that the dissolution profile with best characteristics was observed in the matrix loaded with omeprazole complexed with methyl-β-cyclodextrin in the presence of L-arginine. Several mathematical models were applied to the dissolution curves, indicating that the release of the drug, in free or in complexed state, from the mucoadhesive matrices followed a super case II transport, as established on the basis of the Korsmeyer–Peppas function. The feasibility of drug buccal administration was assessed by permeation experiments on porcine buccal mucosa. The amount of drug permeated from mucoadhesive tablets presented a maximum value for the system containing drug complexed with the methylated cyclodextrin derivative in presence of L-arginine. According to these results, the system containing the selected polymer mixture and the drug complexed with methyl-β-cyclodextrin in presence of L-arginine showed a great potential as a buccal drug delivery formulation, in which a good compromise among mucoadhesion, dissolution, and permeation properties was achieved.

KEY WORDS: buccal delivery; cyclodextrins; mucoadhesion; permeation; release.

INTRODUCTION

Drug delivery through the mucosa that line the oral cavity offers the possibility of circumventing the hepatic 'first-pass' elimination that follows gastrointestinal absorption. In addition, gastric acid or digestive enzyme-mediated degradation in the gastrointestinal tract is also avoided (1,2). Moreover, absorption following oramucosal administration is not influenced by the potential variation in the gastric-emptying rate or the presence of food. These advantages are of value in the systemic delivery of drugs that are subject to extensive hepatic clearance (3). However, to administrate a pharmaceutical dosage form in the mucosa of the oral cavity, it is necessary take into account two important parameters. First, it is necessary to prolong the time of contact between the drug formulation and the mucosal route of administration. Second, the oral mucosal shows lower permeability to large molecules, which can be problematic for achieving therapeutic levels of such molecules.

For this purpose, mucoadhesion studies are used to select polymers to prolong the contact time in the various mucosal routes of drug administration (4). The ability to maintain a delivery system at a particular location for an extended period of time has great appeal for both local disease treatment, as well as systemic drug bioavailability (5). When the objective is drug delivery, the term mucoadhesion implies a connection of the drug transport system to a specific biological substrate covered by a mucus tissue surface (6). Considering the mucus that covers the oral cavity, from a technological point of view, as a biological substrate, it can be inferred that the presence of a mucin film (saliva) covering the oral mucosal surface will allow the delivery system to remain in contact with the oral mucosa for a long time, made longer in the presence of mucoadhesive compounds (7). This

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is a practical method to immobilize the drug at the oral mucosal surface and is an important parameter in extended drug delivery systems (8,9).

Recently, cyclodextrins have been classified as a new class of penetration enhancers (10–12). Cyclodextrins can enhance drug permeation by increasing drug availability and stability at the surface of the biological barriers (13). This kind of penetration enhancers can keep the hydrophobic molecules in solution by complexation and delivering them to the surface of the target mucosa. However, some more hydrophobic cyclodextrins act by different pathways. The cyclodextrins can permeate the buccal mucosa, forming inclusion complexes with hydrophobic molecules, namely lipids from the cellular membrane, interacting with these lipids and are consequently able to modify the buccal mucosa permeability (14,15).

The aim of this study is to develop a buccal tablet for the delivery of a poorly water-soluble drug. The chosen model drug is omeprazole (OME), a proton pump inhibitor in gastric parietal cells (16,17). This drug is poorly soluble in water and sensitive to heat, moisture, organic solvents, and, to some degree, light (18,19). OME shows low physicochemical stability at acidic conditions, degrades very rapidly in the stomach and undergoes hepatic first-pass metabolism (20–22), with a bioavailability not exceeding 35%. All these drawbacks give rise to difficulties in obtaining an oral pharmaceutical formulation with an acceptable bioavailability. This has prompted previous studies on the evaluation of buccal adhesive tablets, published some years ago. These have focused on stability/absorption (23), and on *in vivo* studies using hamsters as animal model (24), which makes it difficult to extrapolate to human use. There has been, to our knowledge, no attempt to introduce permeation enhancers in the formulation.

In this work, a matrix was designed using two polymers as a mucoadhesive sustained release platform, and cyclodextrins as modulators of drug release and permeation enhancers. The mucoadhesive profiles of the formulated matrices were evaluated to determine the influence of cyclodextrins in the mucoadhesion. The effect of cyclodextrins in the drug release features from the loaded matrices was also studied, and mathematical models were applied to determine the mechanism of drug release from the matrices. Finally, the potential of complexed OME-loaded matrices to obtain a buccal delivery system was assessed by permeation studies in the porcine buccal mucosa.

MATERIALS AND METHODS

Materials

β-cyclodextrin (βCD, KLEPTOSE®, M_w=1,135) and methyl-β-cyclodextrin, (MβCD, CRYSMEB®, M_w~1,190, with an average degree of substitution of 0.5) were kindly donated by Roquette (Lestrem, France) and omeprazole (OME, M_w=345.42) was gently donated by Belmac Laboratory, S.A. (Madrid, Spain). L-arginine (ARG) was purchased from Panreac (Santiago de Compostela, Spain). Sodium carboxymethylcellulose (NaCMC) was purchased from Akucell AHX 0.701, Netherlands. Poly(ethylene oxide) (Sentry, Polyox WSR N80) and Eudragit RS were donated by The Dow Chemical Company. All other reagents (chemicals and solvents) were of analytical grade.

Preparation of Inclusion Complexes

Solid inclusion complexes were prepared by the freeze-drying method. Systems were prepared in a 1:1 stoichiometry (drug/cyclodextrin) according to previous phase solubility studies (25) and ARG was added in a 6:1 molar proportion, relative to OME (26). The same systems were prepared in the absence of ARG to observe the influence of the alkali agent on the mucoadhesion, release, and permeation profiles. All the clear solutions were frozen by immersion in an ethanol bath at −50°C (Shell Freezer, Labconco, Freezone® model 79,490) and the frozen solution was lyophilized in a freeze dryer (Lyph-lock 6 apparatus, Labconco) for 72 h (27).

Preparation of Tablets

Tablets were prepared by direct compression with a mixture of mucoadhesive polymers, NaCMC, and polyox, in order to development a mucoadhesive formulation for buccal delivery. Eudragit RS was used as impermeable backing layer. All these polymers were chosen with the objective to maintain drug stability in aqueous saliva environment (28,29). Each component was previously screened and powders were mixed during 10 min and then compressed in a single-punch hydraulic press (Speca Press, UK) at 1 ton during 5 s. The composition of the studied formulations is described in Table I.

The surface area of the tablet exposed on the buccal mucosa was 1.327 cm^2 and the average thickness 2.3 mm.

The use of omeprazole would require the presence of a taste-masking agent. This would be, naturally, added in minute quantities and was, for simplicity, omitted.

Mucoadhesive Studies

TA.XTplus texture analyzer equipped with a computer-integrated data acquisition system was used to determine mucoadhesive parameters, force, and integrated work of mucoadhesion. While the former corresponds to the maximum measured force, the latter results from the numerical integration along the process. During the experiment, the tablet was fixed to the probe, while the porcine buccal mucosa was fixed to the bottom support by means of a cyanoacrylate adhesive. The surface of buccal mucosa was wetted with 0.1 mL of simulated saliva (30), also used in the subsequent studies, by using a micropipette. The composition

Table I. Composition of the Studied Formulations

Formulations	1 (mg)	2 (mg)	3 (mg)	4 (mg)	5 (mg)
OME	12.00				
OME βCD		51.43			
OME MβCD			53.34		
OME βCD ARG				87.74	
OME MβCD ARG					89.65
Polyox 80 N	26.25	26.25	26.25	26.25	26.25
NaCMC	26.25	26.25	26.25	26.25	26.25
Lactose	85.50	46.07	44.16	9.76	7.85
Eudragit RS[a]	50.00	50.00	50.00	50.00	50.00

[a] Eudragit RS was the polymer used for the backing layer

of the saliva is 5 mM sodium bicarbonate, 7.36 mM sodium chloride, 20 mM potassium chloride, 6.6 mM sodium dihydrogen phosphate monohydrate, 1.5 mM calcium chloride dehydrate in water (high-performance liquid chromatography grade). Then, the probe was moved down at 0.1 mm/s and stopped when the force between the tablet and the mucosa was 1 N. After 3 min of contact time, the crosshead moved upward at the same speed and with an acquisition rate of 25 points/s. Results are the mean of five force elongation experiments.

In vitro Release Studies

The release profiles of OME, in free and complexed state, from tablets were evaluated by dissolution studies. The tablet was placed in the basket and immersed in 500 ml of artificial saliva (pH=7.0±0.5) at 37±0.5°C, in order to maintain *sink conditions.* Basket rotation speed was kept at 50 rpm. The medium was previously filtered and degassed, according with Pharmacopoeia (31). At predetermined times, 5 ml of the dissolution sample was withdrawn and replaced with an equal volume of fresh medium. Samples were filtered through membrane filters of 0.45 μm pore size (La-Pha-PackR, Langerwehe, Germany) and analyzed by UV absorption (UV-1,603, Shimadzu, Kyoto, Japan) at 301 nm. Three replicates have been made for each experiment. The cumulative percentage of drug released was calculated according to the calibration curve in artificial saliva (r^2=0.9999) and a correction was applied for the cumulative dilution caused by replacement of the sample with an equal volume of fresh media. This procedure, comprising sink conditions and agitation, does not aim at mimicking conditions for buccal delivery, but allows comparing the different formulations while avoiding artifacts induced by drug accumulation.

Model-Independent Methods

To evaluate the drug release curve as a single measured response, the following parameters were used: time to release 50% of OME ($t_{50\%}$), percent of OME released at 4 and 8 h (PD_{4h} and PD_{8h}), the dissolution efficiency parameter at 8 h (DE_{8h}) and the mean dissolution time (MDT). The first three parameters were extracted directly from the dissolution data and DE_{8h} was calculated from the area under the dissolution curve (32). All values are expressed as means from separate experiments. MDT and DE_{8h} were calculated using

$$\mathrm{MDT} = \frac{\sum_{j=1}^{n} t_j \Delta M_i}{\sum_{j=1}^{n} \Delta M_i} \tag{1}$$

where j is the sample number, n is the number of dissolution sample times, t_j is the time at midpoint between t_j and t_{j-1} and ΔM_i is the amount of drug dissolved between t_i and t_{i-1}, and

$$\mathrm{D.E.} = \frac{\int_0^t y \times \mathrm{dt}}{y_{100} \times t} \times 100\% \tag{2}$$

where the integral is given by the area under the curve up to dissolution time t and y_{100} represents 100% of drug dissolution.

Simple model independent approaches, such as the difference factor (f_1) and the similarity factor (f_2) are proposed in Food and Drug Administration (FDA)'s guidelines to compare dissolution profiles (33). While f_1 calculates the percent difference between the two curves at each time point and is a measure of the relative error between the two curves, f_2 is a logarithmic reciprocal square root transformation of the sum of squared error. It is a measurement of the similarity in the percent (%) dissolution between the curves. According to the FDA guidelines, for profiles to be considered similar f_1 values up to 15 (0–15) and f_2 values greater than 50 (50–100) should be found (34–36). In this study, these two fit factors were applied to the dissolution data. The fit factors f_1 and f_2 are defined by

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} (R_t - T_t)}{\sum_{t=1}^{n} R_t} \right\} \times 100 \tag{3}$$

and

$$f_2 = 50 \times \log\left\{ \left[\frac{1}{\left(1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2\right)^{\frac{1}{2}}} \right] \times 100 \right\} \tag{4}$$

being n is the number of dissolution sample times, and R_t and T_t the individual or mean percent dissolved at each time point, t, for the reference and the test dissolution profiles, respectively.

Model-Dependent Methods

Mathematical models have also been used extensively for the parametric representation of the dissolution data (37–39). In this work, different models (see Table II) were employed so as to compare the various dissolution profiles and assess the nature of drug release.

In vitro Permeation Studies

The *in vitro* permeation studies using diffusion cells are routinely conducted in order to evaluate drug permeation through the buccal mucosa. These studies are a useful tool to assess the potential of a localized anatomical site as a route for drug delivery.

Table II. Mathematical Models used for the Study of the Dissolution Profiles of OME Tablets

Mathematical model	Equation
Zero order	$c_1 t$
Higuchi	$c_1 t^{0.5}$
Korsmeyer–Peppas	$c_1 t^{c2}$
Hixson–Crowell	$c_2(1-(1-c_1 t))^3$
First order	$c_2(1-\exp(-c_1 t))$
Weibull	$c_3\left(1-\exp\left(-\frac{t}{c_1}\right)c^2\right)$
Logistic	$c_3 \times \frac{\exp(c_1+c_2 \log t)}{1+\exp(c_1+c_2 \log t)}$

Tissue Preparation

In these studies, porcine buccal mucosa was used due to its high similarity to the human buccal mucosa in certain important characteristics such as permeability, barrier lipid composition, histology, and ultrastructural organization (40). Buccal mucosa from pigs weighing 70–100 Kg was obtained freshly from a local slaughterhouse and used not later than 2 h after slaughtering. Most of the underlying tissue was removed from the mucosa with surgical scissors. The buccal tissue was dermatomed with a thickness of 500 μm (41), resorting to a manual dermatome (Aesculap® Am Aesculap-Platz, Germany).

Permeation Studies

Dermatomed buccal mucosa was mounted in the receiver chamber of Franz diffusion cells, with a diffusional area of 1.327 cm^2. As receptor fluid, bis-tris buffer at pH=7.0±0.5 was used. It was continuously stirred and maintained at 37±0.5°C during the time of the study.

The buffer was previously filtered under vacuum through a 0.45 μm Millipore filter, followed by 15 min at 40°C in ultrasounds in order to prevent the formation of air bubbles between the buccal mucosa and the receptor medium during the permeation experiments.

OME permeation from tablets was measured by applying the tablet wetted with 1 mL of bis-tris buffer to the mucosa in the donor side. Aliquots from samples containing OME or its equivalent in complexed form were withdrawn at each hour, and analyzed by HPLC. For this purpose, a LC-2010C_{HT} (Shimadzu, Japan) system consisting of a quaternary pump with a programmable multiple wavelength detector set at 300 nm and an autosampler was used. The separation was carried out at room temperature and the column used was a reverse-phase Purospher® RP-18 endcapped (5 μm), 125 cm×4 mm. The mobile phase was a mixture of phosphate buffer (pH=7.6) and acetonitrile (75:25, *v*/*v*), filtered through 0.45 μm filters (Millipore), degassed and pumped at a constant flow rate of 1 $mL\,min^{-1}$. Chromatograms were recorded and the peak area response was measured using an automatic integrator. The injection volume was 20 μl for all samples. The cumulative amount of drug permeated per cm^2 of buccal mucosa (Q) was plotted against time (t) and the steady state flux (Jss) was calculated using

$$\mathrm{Jss} = \frac{\Delta M}{A.\Delta t} \tag{6}$$

where ΔM is the amount of drug transported across the membrane during the time interval Δt and A is the diffusional area.

Data Analysis

The statistical analysis was resorted using the GraphPad Prism® version 4.00 software. Comparison between two variables was performed using Student's t test ($p<0.05$). Comparison between more than two variables was made with one-way analysis of variance (ANOVA) with a level of statistical significance $p<0.05$. When the ANOVA analysis detected differences between samples, a post hoc test (Tukey's test) was conducted to identify those differences. All experiments were run at least in triplicate.

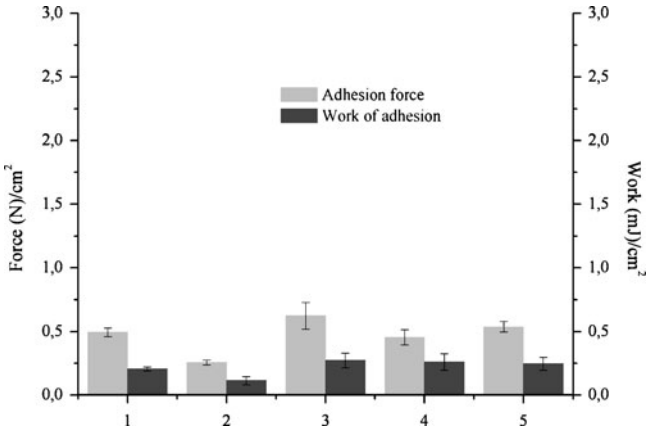

Fig. 1. Mucoadhesive profiles of formulations containing: 1) the drug; 2) and 3) the drug in complexed state with cyclodextrins (βCD and MβCD); 4) and 5) in complexed state and in the presence of the alkali agent (ARG)

RESULTS

Mucoadhesive Studies

Mucoadhesive studies were carried out in order to evaluate the effect on the mucoadhesive performance of the selected polymers after the introduction of the drug, cyclodextrins and the alkali agent in the matrix. They were selected taking into account the respective mucoadhesive properties and the fact that it is necessary to maintain drug stability in aqueous saliva environment. Based on previous results (data not shown), polyox and NaCMC were selected to develop a mucoadhesive formulation for OME buccal administration.

The values for the maximum force of mucoadhesion per area were determined for three points, corresponding to pure polyox (2.19±0.46 N/cm^2), pure NaCMC (1.61±0.55 N/cm^2), and for a 50% *w*/*w* mixture of these polymers (2.50±0.15 N/cm^2). These three points were fitted to a second-order polynomial, so as to assess the optimal composition in

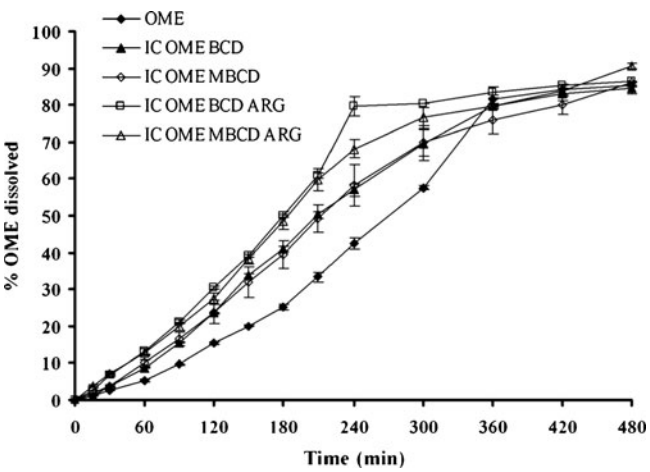

Fig. 2. Dissolution profiles formulations 1), 2), 3), 4) and 5) in artificial saliva at pH=7.0

Table III. Values of Dissolution Parameters, $t_{50\%}$, PD_{4h}, PD_{8h}, MDT, and $DE_{8h,}$ for the studied formulations

Formulation	$t_{50\%}$ (min)	PD_{4h} (%)	PD_{8h} (%)	MDT (min)	DE_{8h} (%)
1	260.43±02.88	42.48±1.47	85.15±0.11	307.12±1.11	51.89±0.13
2	209.11 ±03.90	57.03±1.73	84.68±1.48	285.64±1.17	59.86±1.55
3	213.68±15.25	58.39±5.67	85.94±0.44	283.99±3.75	58.23±3.53
4	179.91±01.66	69.54±2.66	86.53±0.11	273.98±0.15	66.82±0.23
5	185.39±09.33	68.17±2.41	90.69±0.84	275.42±2.22	65.17±1.23

terms of maximizing the mucoadhesion. For this particular case, it corresponds to a mixture with a fraction of 0.37 of NaCMC and 0.63 of polyox. However, if a corresponding procedure is carried out for the work of mucoadhesion (polyox=0.77± 0.20 mJ/cm^2; NaCMC=0.81±0.09 mJ/cm^2 and 50% *w*/*w* mixture of these polymers=0.72±0.01 mJ/cm^2), it is seen that a maximum is obtained for the pure NaCMC polymer. We have selected the value 50% *w*/*w* mixture of these polymers as a compromise between the two sets of observations. Subsequently, the effect in the mucoadhesive profile due the addition of cyclodextrins and ARG was also investigated.

Differences in the mucoadhesive profiles of tablets, namely in the force and work of mucoadhesion, after the addition of the drug, by itself or complexed with cyclodextrins, in absence and in the presence of the alkali agent are visible in Fig. 1.

When the drug was introduced in the matrix, the values of the mucoadhesive parameters decreased. Due to its hydrophobic character, the drug shows low capacity to absorb water, necessary to hydrate the mucoadhesive matrix and consequently to develop a mucoadhesive bond.

Cyclodextrins are large molecular weight oligosaccharides. These materials have the ability to form water-soluble complexes with hydrophobic drugs. They can form hydrogen bonds with some polymers, interfering in the formation of mucoadhesive bonds. For this reason, the effect of cyclodextrins, namely βCD and MβCD in the mucoadhesive performance of the selected matrix was also studied. In the presence of βCD, it was possible to observe a decrease in the work and force of mucoadhesion comparatively with the tablet containing the drug alone. βCD is a natural cyclodextrin with hydroxyl groups available to establish hydrogen bonds with the polymeric chains, consequently, when this cyclodextrin was added to the formulation, a largest reduction in the mucoadhesion was observed. This effect was not detected in the presence of MβCD. This cyclodextrin displays a larger capacity to absorb water from the mucosa, necessary for the hydration of the polymer, thus increasing the flexibility and interpenetration of the moieties available for bonding to the mucus. On the other hand, MβCD shows some additional methoxy groups available to establish interactions with the mucus layer contributing to a stronger mucoadhesive performance.

In the presence of ARG, the mucoadhesion performance increases when OME is complexed with βCD. In this case, ARG can establish hydrogen bonds with the βCD hydroxyl groups and the polymeric chains remain free for mucoadhesion. In the case of MβCD, the presence of ARG increases very much the absorbance of water, causing an exaggerated hydration of polymers and consequently reducing the mucoadhesion (6,42).

The aspect of all formulations was observed at the end of the study, and it was possible to detect the formation of a gelatinous layer around the matrix, due the presence of the mucoadhesive polymers. When the mucoadhesive matrix was loaded with drug, the gelatinous layer is present, but a smaller, and a large amount of OME not dissolved was observed in the center of the matrix. Finally, matrices loaded with OME complexed with cyclodextrins in the presence of ARG showed a larger degree of solubilization.

Release Studies

The mean dissolution profiles of formulations containing OME and corresponding inclusion complexes, with and without ARG, at pH 7.0 in artificial saliva are presented in Fig. 2.

In the early stages of the dissolution process, OME in the complexed form was rapidly released from the matrix. This behavior was observed in all formulations, except for the one containing OME in the free form. The general trend can be attributed to the polyox polymer that gradually hydrates, swells, and dissolves once in contact with the dissolution media. In the tablet loaded with OME in free form, due the insolubility at this pH values, the behavior was different.

Release studies show that the complexation of OME with cyclodextrins can enhance drug solubility, and it does facilitate the process of hydration, by allowing continuous water penetration through diffusion and dissolution (43). When ARG was added, the drug release was the largest. This is, probably, due to the conjunction of the solubilizing effect of cyclodextrins and the stabilizing effect of the basic amino-acid. After 4 h, the rate of release decreased. A possible explanation is that all polyox is dissolved, and only NaCMC is present in the formulation. After hydration, NaCMC swells and may form a gelatinous layer around the matrix. This layer acts as a barrier against fast drug release, controlling water

Table IV. Difference (*f*1) and Similarity (*f*2) Factors for Reference *versus* Test Formulations (reference/test)

	Fit factor	
Formulations	f_1	f_2
1/2	17.20	48.78
1/3	18.74	48.80
1/4	27.49	37.54
1/5	25.52	40.96
2/3	03.08	85.94
2/4	13.75	52.91
3/5	12.75	60.68
4/5	04.80	68.06

Table V. Models Parameters (c_1, c_2, and c_3) for the Logistic, Weibull, and Korsmeyer–Peppas Functions, Applied to the Different Formulations

Models	Formulation	c_1±SEM	c_2±SEM	c_3±SEM	r^2
Logistic	1	$-1.6\times10^{1}\pm2.4\times10^{2}$	$1.6\pm1.8\times10^{-1}$	$1.0\times10^{5}\pm2.4\times10^{7}$	0.9979
	2	$-9.8\pm2.8\times10^{-1}$	$1.7\pm7.1\times10^{-2}$	$1.3\times10^{2}\pm8.4$	0.9996
	3	$-9.7\pm6.9\times10^{-1}$	$1.7\pm1.7\times10^{-1}$	$1.3\times10^{2}\pm1.9\times10^{1}$	0.9972
	4	$-1.1\times10^{1}\pm2.1$	$2.0\pm4.8\times10^{-1}$	$1.1\times10^{2}\pm2.2\times10^{1}$	0.9808
	5	-9.9 ± 1.3	$1.8\pm3.1\times10^{-1}$	$1.1\times10^{2}\pm1.8\times10^{1}$	0.9918
Weibull	1	$1.7\times10^{-4}\pm3.7\times10^{-3}$	$1.6\pm1.8\times10^{-1}$	$7.5\times10^{3}\pm2.6\times10^{5}$	0.9979
	2	$3.9\times10^{-3}\pm1.7\times10^{-4}$	$1.6\pm4.7\times10^{-2}$	$9.9\times10^{1}\pm3.7$	0.9996
	3	$4.1\times10^{-3}\pm3.6\times10^{-4}$	$1.6\pm1.1\times10^{-1}$	$9.3\times10^{1}\pm6.8$	0.9980
	4	$5.3\times10^{-3}\pm5.6\times10^{-4}$	$1.8\pm2.8\times10^{-1}$	$9.1\times10^{1}\pm7.8$	0.9852
	5	$5.1\times10^{-3}\pm4.3\times10^{-4}$	$1.7\pm1.7\times10^{-1}$	$8.9\times10^{1}\pm5.9$	0.9947
Korsmeyer–Peppas	1	$1.1\times10^{-2}\pm2.5\times10^{-3}$	$1.5\pm4.0\times10^{-2}$	–	0.9982
	2	$5.0\times10^{-2}\pm1.3\times10^{-2}$	$1.3\pm4.8\times10^{-2}$	–	0.9974
	3	$5.2\times10^{-2}\pm3.9\times10^{-3}$	$1.3\pm1.4\times10^{-2}$	–	0.9997
	4	$9.0\times10^{-2}\pm1.3\times10^{-2}$	$1.2\pm2.9\times10^{-2}$	–	0.9992
	5	$7.4\times10^{-2}\pm2.2\times10^{-2}$	$1.3\pm5.8\times10^{-2}$	–	0.9970

penetration in the tablet and the rate of release of the drug (44).

Some parameters were evaluated from the dissolution profiles of the studied formulations and are presented in Table III.

Regarding the two first parameters ($t_{50\%}$ and PD_{4h}), all the formulations exceeded 50% of drug dissolution in the first 4 h, except the tablet loaded with OME in free form. The percentage of drug dissolved in the end of the assay reached 90% in the matrix loaded with OME complexed with MβCD in the presence of ARG. The MDT and DE_{8h} parameters present information about the entire curve of dissolution. The calculated values for both parameters indicate that matrices containing OME complexed with cyclodextrins in the presence of ARG present the best profiles, with lower MDT and higher DE_{8h} values. These results are in agreement with the data obtained from the mucoadhesion studies.

Model-Independent Methods

The drug, inclusion complexes OME/βCD, OME/MβCD. and OME/βCD/ARG were used as references to calculate the fit factors (f_1 and f_2) for the dissolution profiles (see Table IV). The number of points was limited to one after 85% dissolution, as recommended (45). As can be observed, when the dissolution profile of OME alone was used as reference, the profile was not similar to the dissolution profiles of OME complexed with cyclodextrins nor to OME complexed with cyclodextrins in presence of ARG.

However, when the dissolution profiles of the drug were compared between cyclodextrins, with and without ARG, or between the same cyclodextrin in the absence and presence of ARG, f_1 <15 and $f2$ >50, indicating that these profiles are similar.

The application of these model-independent methods to the dissolution profiles suggests that the complexation of the drug with cyclodextrins in the absence and in the presence of ARG can change the mechanism of drug release from the matrix.

Model-Dependent Methods

According to Table II, several mathematical models were tested in order to better understand the mechanism of drug release from tablets. Excipients (cyclodextrins, polymers, alkali agents) can have an effect that tends to vary during the dissolution profile. In these cases, information obtained essentially describes the dominant mechanism. Table V is a compilation of results obtained after application of three functions, *Logistic*, *Weibull*, and *Korsmeyer–Peppas* to the dissolution profiles of the studied formulations. These functions produced the best quality fits, among all those present in Table II.

For the general case of tablets, the interaction of disintegration and dissolution is complex and requires models which are capable of describing S-shaped dissolution profiles. This includes the Weibull (46) and the Logistic (47) models.

The Weibull model can be successfully applied to most types of dissolution curves and is commonly used in such studies (48,49), in spite of having been the subject of some criticism (50,51). According with this model, the shape parameter (c_2) characterizes the curves as either exponential (c_2=1; case 1), sigmoid, S-shaped, with upward curvature

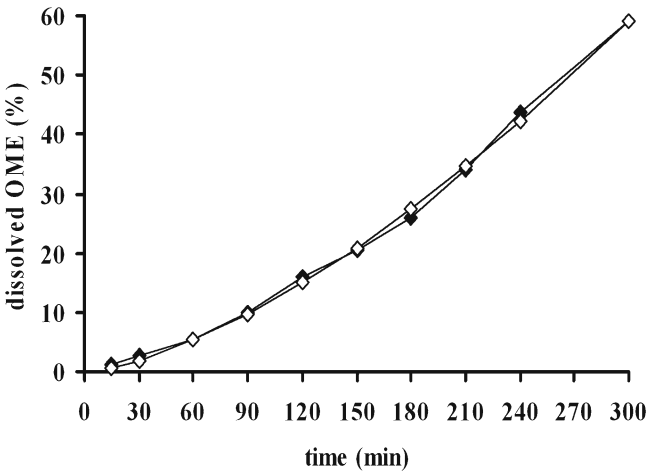

Fig. 3. Dissolution profile of formulation 1 fitted with the Korsmeyer-Peppas function (*empty symbols*)

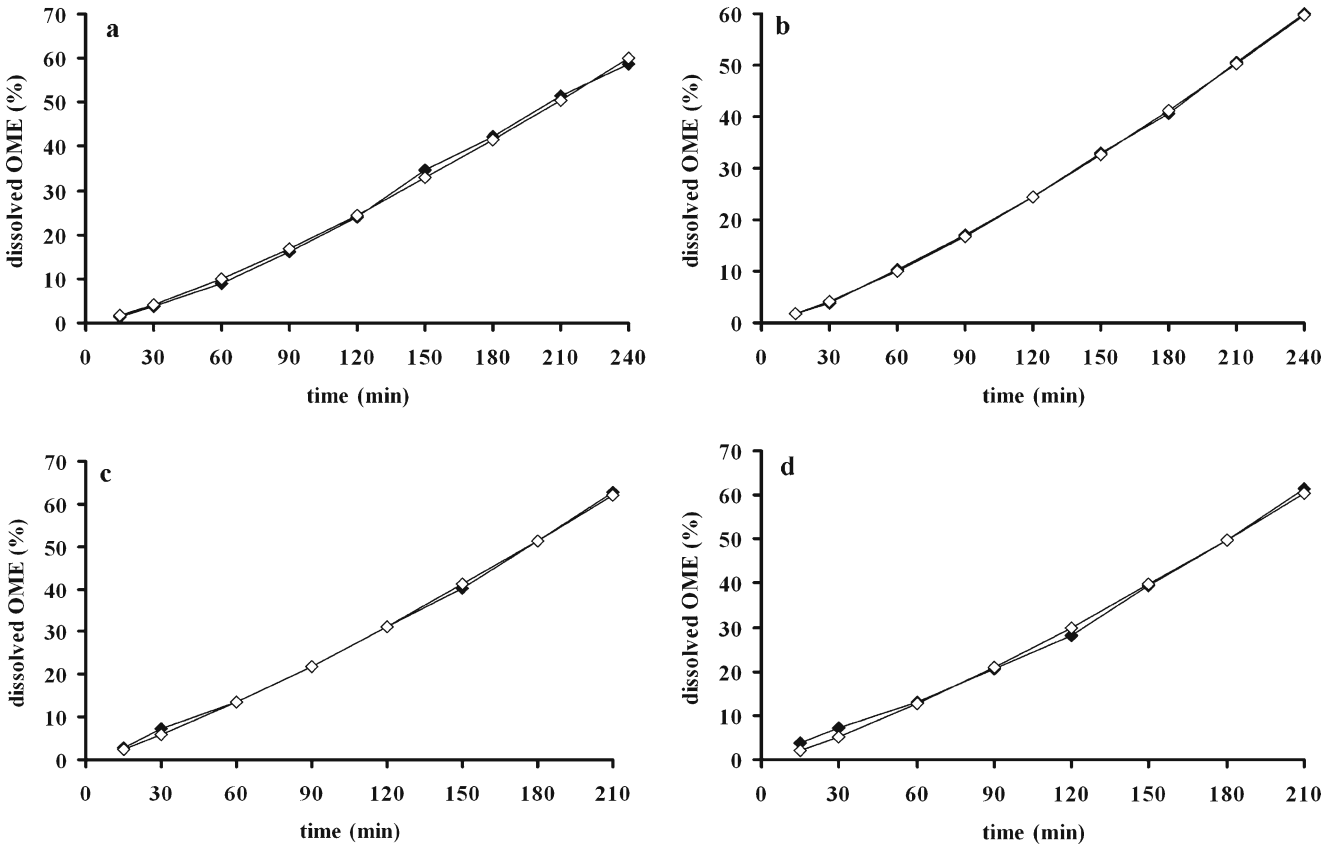

Fig. 4. Dissolution profiles of the formulations 2 **a**, 3 **b**, 4 **c** and 5 **d** fitted with the Korsmeyer-Peppas function (*empty symbols*)

followed by a turning point (c_2>1; case 2), or parabolic, with a higher initial slope and after that consistent with the exponential (c_2<1; case 3). In our case c_2>1, indicating sigmoidal dissolution profiles, and thus results consistent with those found with the Logistic and, especially, with the Korsmeyer–Peppas function. Except for the tablet loaded with the drug complexed with βCD, the best correlation coefficients (see Table V) were obtained with the latter model, and Figs. 3 and 4 represent the dissolution profiles fitted with this function up to 60% of drug release (52). The use of this limit naturally contributes to the improvement in the least-squares fit *versus* the other two models.

The Korsmeyer–Peppas c_2 parameter (release exponent) is used in order to characterize the different drug release mechanisms. It produces c_2=0.5 for Fickian diffusion and higher values between 0.5 and 1.0 for mass transfer following a non-Fickian model (53). This model is generally used to analyze the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved.

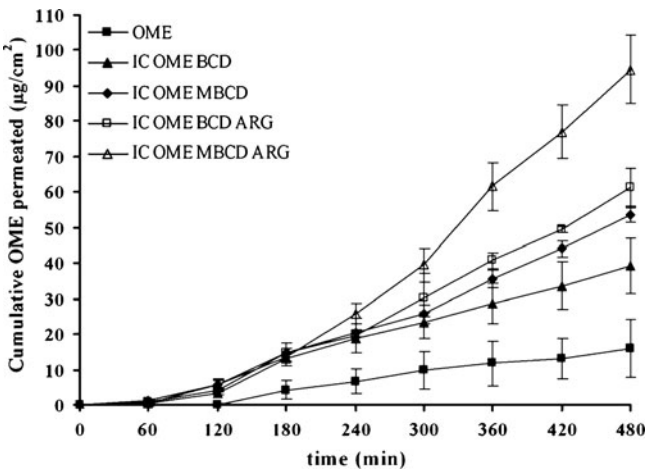

Fig. 5. The *in vitro* permeation profiles of the formulations 1), 2), 3) 4) and 5). Mean±SD (n=6)

Table VI. Flux Values, Cumulative Amount of Drug Permeated per cm^2 of Buccal Mucosa after 8 h (Q_{8h}) for the Different Systems

Systems	Flux (μg/cm².h)	Ratio[a]	Q_{8h} (μg/cm²)	Ratio[b]
1	1.905±0.884	1.0	16.098±8.132	1.0
2	4.305±0.922	2.3	39.183±7.805	2.4
3	5.876±0.273	3.1	53.750±2.283*	3.3
4	7.089±0.264*	3.7	61.196±5.427*	3.8
5	15.541±0.988*	8.2	94.616±9.745*	5.9

[a] Enhancement ratio between the flux of formulations 2, 3, 4 and 5 in comparison with formulation 1

[b] Enhancement ratio between Q8h of formulations 2), 3), 4) and 5) in comparison with formulation 1)

*p<0.05, statistically significant difference in comparison with the formulation 1

It is possible to observe from Table V that parameter c_2 always exceeds 1, indicating that the release of the drug alone, or in complexed state, from the mucoadhesive matrices followed a super case-II transport (54). In this kind of transport, there are two simultaneous fluxes. The first flux is the rate at which the diffusing material is released at an interface by relaxation of the polymer matrix (55). In the glassy state, the matrix has a finite relaxation time, associated with the length of the polymers in relation to the entanglement network (56). The second flux is the rate at which the material diffuses away from the interface. In this point, the polymer is in the rubbery state, it swells, making the relaxation time almost instantaneous (55). The parameters governing the release of the dissolved material are thus the rate at which the interface moves, the diffusivity of the dissolved material in the rubbery polymer, and the total length of the diffusional path (57). In this kind of transport, the polymer relaxation is the rate-limiting step to water transport (58).

Permeation Studies

The feasibility of a buccal delivery for OME was preliminary assessed by measuring the *in vitro* permeation of OME, alone or in complexed form and with or without ARG, through pig buccal mucosa. Results reported in Fig. 5 show that the permeation profile of OME alone through the buccal mucosa presented an initial lag time of 2 h.

When OME was in complexed state with cyclodextrins (βCD and MβCD) in the absence or in the presence of ARG the initial lag time was reduced to 1 h. This reduction is advantageous because it enables to rapidly attain the pharmacological action. Results from permeation studies clearly show that cyclodextrins, in the absence and in the presence of ARG, can increase drug permeation through the porcine buccal mucosa. This result can be explained taking into account that the formation of inclusion complexes can improve drug solubility and consequently increase the amount of drug available at the surface of the membrane for permeation.

Table VI contains flux values and the cumulative amount of drug permeated per cm^2 of buccal mucosa after 8 h, for the different systems under study.

The cumulative amount of OME permeated over 8 h through the epithelium was 16.1 μg per cm^2 of pig buccal mucosa. In the presence of βCD, the amount permeated was 2.4 higher than that of the drug alone.

In the formulation containing OME complexed with MβCD, the increase in drug being permeated was 3.3-fold compared to OME alone. In this case, the enhancement in drug permeation occurred because cyclodextrin acted as an enhancer of permeation. It is known that this cyclodextrin is more hydrophobic, can permeate the buccal mucosa and form inclusion complexes with hydrophobic molecules, such as lipids from the cellular membrane (58,59). It can also modify the buccal mucosa permeability and act as a penetration enhancer in the buccal route.

Permeation studies with OME complexed with both cyclodextrins (βCD and MβCD) in the presence of ARG showed an enhancement ratio, as calculated from Q_{8h} of 3.8- and 5.9-fold, respectively, when compared with the amount of permeated OME alone. This increase in drug permeation was statistically significant comparatively with the drug in free form ($p<0.05$). This fact resulted, probably, from the improvement of OME dissolution features caused by complexation with cyclodextrins in the presence of ARG. In the case of OME complexed with MβCD in the presence of ARG, the enhancement in drug permeation was the largest.

In the presence of ARG, drug permeation was facilitated. At neutral conditions, ARG is in the cationic form, promoting ionic interactions with any negatively charged molecules situated in the mucus layer (58).

CONCLUSION

A set of studies, which can easily be adapted to similar systems, was performed showing that a mixture of polyox and NaCMC can provide a suitable platform for designing a buccal OME delivery formulation. These studies include mucoadhesive, dissolution, and permeation experiments. The incorporation of cyclodextrins, by complexation with OME in the presence of an alkali agent, ARG, was responsible for an improved dissolution of the drug inside the polymeric matrix. After application of different mathematical functions to the dissolution profiles, the best overall correlation coefficients were obtained with the Korsmeyer–Peppas model and indicate a mechanism for drug release known as super case II transport. Permeation studies suggest that the presence of MβCD and ARG can also increase drug permeation from the mucoadhesive matrix through the buccal mucosa. According to these results, this system shows a great potential as a buccal drug delivery formulation, in which a good compromise between mucoadhesion, dissolution and permeation properties is achieved

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