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Needle-free buccal anesthesia using iontophoresis and amino amide salts combined in a mucoadhesive formulation

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ABSTRACT

Iontophoresis is a strategy to increase the penetration of drugs through biological membranes; however, its use has been underexplored in mucosa. The aim of this work was to investigate the influence of iontophoresis in the mucosal penetration of prilocaine hydrochloride (PCL) and lidocaine hydrochloride (LCL), which are largely used in dentistry as local anesthetics, when combined in the same formulation. Semisolid hydrogels containing these drugs either alone or in combination were developed at two different pHs (7.0 and 5.8) and presented adequate mechanical and mucoadhesive properties for buccal administration. The distribution coefficients between the mucosa and the formulations ($D_{m/f}$) and the *in vitro* mucosa permeation and retention rates were evaluated for both PCL and LCL. At pH 7.0, the combination of the drugs decreased the $D_{m/f}$ of PCL by approximately 3-fold but did not change the $D_{m/f}$ of LCL; iontophoresis increased the permeation rate of PCL by 12-fold and did not significantly change LCL flux compared with the passive permeation rate of the combined drugs. Combining the drugs also resulted in an increase in both PCL (86-fold) and LCL (12-fold) accumulation in the mucosa after iontophoresis at pH 7.0 compared with iontophoresis of the isolated drugs. Therefore, applying iontophoresis to a semisolid formulation of this drug combination at pH 7.0 can serve as a needle-free strategy to speed the onset and prolong the duration of buccal anesthesia.

1. Introduction

The development of injectable, efficient and safe local anesthetics is considered a breakthrough of 20th century dentistry [1]. Their use has allowed the improvement of routine and surgical procedures and has increased access to dental care for thousands of patients. However, the use of injectable anesthetics is a main source of fear and phobia in a significant portion of dental patients [2], making them delay or even avoid visits to the dentist [3–5].

To minimize patient discomfort at initial needle perforation, topical anesthetics are used in the clinic prior to local anesthetic injection [6,7]. Topical anesthetics can be found in a number of different preparations, such as in hydrogels, ointments and sprays; the most commonly used are hydrogels containing 20% benzocaine [7–9]. However, the use of amino esters such as benzocaine has been replaced by the use of amino amides such as lidocaine and prilocaine. When compared to amino esters, amino amides present higher lipophilicity, easier tissue penetration, faster onset and longer anesthesia duration due to their stability when in contact with tissue enzymes [10–12].

Formulations that combine lidocaine and prilocaine at 2.5% each, such as EMLA® (AstraZeneca, King's Langley, Hertfordshire, UK) and ORAQIX® (Dentsply Pharmaceutical, York, PA, USA), are an interesting approach toward improving the anesthetic performance of each of these drugs, taking advantage of their slightly different partition coefficients and pharmacological effects: the more lipophilic lidocaine, which also has greater vasodilatory properties confers a rapid anesthetic onset, whereas prilocaine confers a smaller vasodilator effect, leading to a longer duration of action [7].

Although capable of inducing superficial anesthesia, the use of topical anesthetics does not substitute the use of local anesthetic injections, especially due to the low concentration of drugs able to penetrate the epithelium after topical application [6]. Therefore, topical anesthetics are only recommended for superficial procedures, for cases where a patient presents a high level of anxiety and

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pain sensibility, or for procedures that requires multiple anesthetic injections [13].

Non-invasive administration of local anesthetics in the buccal cavity to replace painful injections would enable the improvement of routine and surgical procedures. Among other advantages, non-invasive administration could save costs, improve patient compliance, facilitate application and decrease risk of contamination. Moreover, in areas such as the hard palate, injected anesthetics cannot completely reduce the pain due to the displacement of the firmly attached mucoperiosteum by needle and liquid insertion [14,15]. Therefore, an effective topical anesthetic formulation that could totally reduce pain without the need for an additional injection would be a contribution to dentistry.

However, there are some challenges to overcome when developing an effective drug delivery system for topical deep anesthesia: the system should deliver the drug quickly; keep the drug in intimate contact with a restricted area; target the release and the permeation of the drug through the mucosa; and induce anesthesia, maintain it for a suitable period of time, and enable it to be easily removed after the procedure. These challenges can be overcome by using a mucoadhesive delivery system that employs iontophoresis to enhance and target anesthetic permeation.

Iontophoresis is a non-invasive technique that is based on the application of a low-density electric current to facilitate the release of drugs, regardless of whether they are charged, through biological membranes. The electrical current is provided by a battery or power source and is distributed *via* positive (anode) and negative (cathode) electrodes through an electrolytic solution. When the current is applied, the cations that are in contact with the anode move in the direction of the cathode, whereas the anions at the cathode move in the opposite direction [16].

Iontophoresis has been studied since 1900, and it is typically used in medicine for transdermal drug delivery [17,18]. Although buccal mucosa represents an attractive site for iontophoretic release of drugs because it remains moistened for long periods of time and offers lower resistance to the passage of current than the stratum corneum [19,20], it has not been deeply explored in dentistry. Iontophoresis of lidocaine, for instance, which has been widely explored for transdermal application in surgical treatments [21–25] has mostly been studied for transbuccal delivery in combination with penetration enhancers [26,27]. Therefore, the aim of this work was to evaluate the influence of iontophoresis on the mucosal penetration of prilocaine and lidocaine salts when combined in a mucoadhesive semisolid formulation and used for deep buccal anesthesia.

2. Material and methods

2.1. Reagents

Hydroxypropyl methylcellulose (HPMC) 2208 (15,000 mPas at 2.0%), prilocaine hydrochloride (PCL) and lidocaine hydrochloride (LCL) were obtained from Henrifarma (São Paulo, SP, Brazil); glycerin was purchased from Merck (Merck KGaA, Darmstadt, Germany); methanol and acetonitrile were obtained from J.T. Backer (Phillipsburg, New Jersey, USA); anhydrous Na_2HPO_4 and anhydrous NaH_2PO_4 were purchased from Tedia (Tedia Brazil, Rio de Janeiro, RJ, Brazil); NaCl, anhydrous KH_2PO_4 and NaOH were obtained from Labsynth (Diadema, SP, Brazil); and diethylamine was purchased from Sigma–Aldrich (St. Louis, Missouri, USA). Ag/AgCl electrodes were prepared according to Ref. [28]; Ag-wire (99.99%, 1.5 mm diameter), AgCl (99.99%) and Pt-wire were purchased from Sigma–Aldrich (St. Louis, Missouri, USA). Briefly, AgCl electrodes (cathode) were prepared by dipping the Ag-wire into molten AgCl. Ag electrodes (anode) were prepared by reducing AgCl electrodes, in contact with a NaCl electrolyte solution, under an electric current of 0.3 mA for 12 h using Pt-wire as the anode. Deionised water (18.2 MΩ cm^{-1}) from a Milli-Q system (Millipore, Bedford, MA, USA) was employed to prepare all solutions.

2.2. Porcine mucosa

Porcine esophagus epithelium was used as a model of mucosa tissue for permeation studies [29–32]. Porcine esophagus was obtained from a local slaughterhouse (Frigorífico Olhos D'agua, Ipuã, Brazil). It was collected immediately after the slaughter of the animal, kept at 4 °C while being transported to the laboratory and used within 24 h of animal sacrifice. Then, the esophagus was cut longitudinally and rinsed with water. The mucosa was then separated from the muscular layer by cutting any loose connective fibers with a scalpel. To isolate the epithelia, the excised esophageal mucosae were immersed in distillated water at 60–65 °C for 3 min, after which the membrane was carefully teased away from the underlying tissue using a gingival peeler, model Freer Quinelato QX 54618 (Surya Dental, Maringá, PR, Brazil). It has been previously demonstrated that the use of thermal treatment to separate the epithelium from the connective tissue does not affect epithelium's integrity and permeability properties when done as in this experiment [31,33]. To ascertain the integrity of the mucosa, its electrical resistivity was measured prior to the permeation studies [34]. Resistivity values were considered adequate for the permeation studies when they were equal to or higher than 3.0 KΩ/cm^2 (Supplementary material, Section S3).

2.3. Buffer solution

The buffer solution that was used in the studies was prepared according to Mashru et al. [35] by adding 2.38 g/L of Na_2HPO4, 0.19 g/L of KH_2HPO_4 and 8.0 g/L of NaCl to deionized water and then adjusting its pH 7.0 with 1 M NaOH.

2.4. Hydrogel preparation

Hydrogels were prepared at room temperature by dispersion of HPMC (5% w/w) in 0.01 M phosphate buffer at pH 7.0 (0.47 g/L of NaH_2PO_4 and 0.25 g/L of Na_2HPO_4) or pH 5.8 (1.1 g/L of NaH_2PO_4 and 0.03 g/L of Na_2HPO_4), depending on the experiment and glycerin (0.5% w/w). PCL and LCL were added at a concentration of 2.5% (w/w) each, alone or in combination.

2.5. Hydrogel characterization

2.5.1. Mechanical properties

A texture profile analysis (TPA) of the formulations was made by a texture analyzer (TA-XT texture analyzer, Stable Micro Systems Ltd., Surrey, UK) as described in Refs. [36,37]. Samples of 10 g of each formulation were placed in vessels, avoiding the introduction of air bubbles, and were kept in a water bath (37 °C) for 24 h before the test. In TPA mode, a polycarbonate probe (10 mm diameter) was compressed twice in the interiors of the samples, with a test speed of 2 mm/s, a depth of 5 mm and a delay period of 15 s between the first and the second compressions. Analyses were performed seven times for each sample at 25 °C and 37 °C. From graphs of force *vs.* distance (Scheme S.1, Supplementary material), the hardness, compressibility, elasticity, cohesiveness and adhesiveness of the samples were calculated by *ExponentStable Micro Systems* software (Stable Micro Systems Ltd., Surrey, UK).

2.5.2. Determination of mucoadhesive properties

The mucoadhesive properties were evaluated by a texture analyzer in tension mode. Porcine buccal mucosa from the cheek was

used in these experiments instead of esophageal mucosa. Briefly, the mucosa was washed with water, and its conjunctive tissue was removed, preserving its basal membrane, and kept at −80 °C. Before use, the defrosted mucosa was kept in buffer solution for 1 h. Samples of the mucosa were placed on the inferior accessory of the equipment, and 0.5 g of the formulation was placed on the probe (mucoadhesion rig). The surface of the mucosa was humidified with 50 μL of buffer solution for 5 min. The probe was lowered until it entered into contact with the mucosa, maintaining a compression of 0.5 N [36]. The contact times between the mucosa and the formulation that were evaluated during the test were 30, 60, 120, 180 and 300 s. Then, the probe was raised at a speed of 1 mm/s until the hydrogel detached from the buccal mucosa. Analyses were performed five times for each sample. The force necessary to detach the formulation from the mucosa (detachment force) and the work of mucoadhesion (area under the curve of force *vs.* distance) were calculated using *Exponent* software (Stable Micro Systems, UK).

2.5.3. Differential scanning calorimetry (DSC)

Hydrogels (0.45 mg) containing PCL and LCL at 2.5% w/w each, both isolated and in combination at pH 7.0 and a hydrogel (0.45 mg) containing no drug (blank) were sealed in aluminum pans. Calorimetric data were obtained with a differential scanning calorimeter thermal analyzer (Shimadzu DSC-50, Shimadzu, Kyoto, Japan) with a heat rate of 10 °C/min and a heat interval of 25°–300 °C under air atmosphere.

2.5.4. Determination of mucosa/formulation distribution coefficient ($D_{m/f}$)

The PCL and LCL $D_{m/f}$ values were determined according to Lopez et al. [38]. Samples of the hydrogels (1.0 g) were diluted with 25 mL of 0.01 M phosphate buffer at pH 7.0 or 5.8. Samples of porcine esophageal epithelium (0.3 g) were cut into small pieces and placed in contact with 5 mL samples of the diluted hydrogels, at pH 7.0 or 5.8, containing PCL, LCL or PCL and LCL. The dispersions were maintained under magnetic stirring overnight (600 rpm), mixed in a vortex (400 rpm) for 2 min, centrifuged (10,000 × g, 10 min), filtered and analyzed by HPLC (Section 2.7). The distribution coefficients were determined by Eq. (1):

$$D_{m/f} = \frac{(C1 - C2)}{C2} \quad (1)$$

where C_1 is the concentration of drug in the water phase before homogenization, and C_2 is the concentration of the drug in the water phase after homogenization.

2.6. In vitro *permeation studies*

For *in vitro* passive permeation studies, Franz glass diffusion cells were used [37]. The donor was filled with 1 g of the formulations containing 2.5% of PCL or LCL. Porcine esophageal epithelium was used as a diffusion membrane. The diffusion area was 0.64 cm^2. The receptor was filled with buffer solution and kept at 35 °C under magnetic stirring (600 rpm). The experiment was conducted for 1 h, and a formulation with no drug (blank) was used as a control.

For the iontophoretic studies, modified vertical diffusion cells [33] were used, which were assembled as described above for the passive experiments. The positive electrode (Ag) (anodal iontophoresis) was put into contact with the formulations in the donor, whereas the negative counter-electrode (AgCl) was put into contact with the buffer solution to complete the circuit. A constant electrical current of 1 mA/cm^2, generated by a power supply (Futron data ACC 200.2.20, São Paulo, Brazil), was applied for 1 h. Samples of 1 mL were collected from the receiving solution at 10, 20, 30, 40, 50 and 60 min while the receptor was replaced with 1 mL of fresh buffer solution after each collection time respectively. During the iontophoresis, the voltage of the complete circuit was measured with the voltmeter of the power supply aforementioned, to ensure both the intactness of the epithelium and that the Ag/AgCl electrode reactions were occurring as expected. At the end of the experiments, the mucosa was removed, gently washed with distillated water and carefully dried with absorbent paper. The mucosa was cut into small pieces, put into contact with 5 mL of the mobile phase and left in an ultrasound bath for 30 min to extract the drugs. The samples were filtered through 0.45 μm Millex® membranes (Millipore, São Paulo, SP, Brazil), and the PCL and LCL that had permeated through or were retained in the mucosa were quantified by HPLC (Section 2.7).

2.7. Analytical method

Analyses were performed using a Liquid Chromatographer UFLC Prominence (Shimadzu®, Kyoto, Japão) with a binary pump (LC-20AD), degasser (DGU-20A3 Prominence), auto-sampler (SIL-20AHT Prominence) and diode array detector (SPD-M20A Prominence) [39]. PCL and LCL were simultaneously quantified using a reverse-phase Gemini RP-C18 column (250 mm × 4.6 mm, 3 μm, 110 Å) inserted into an oven (CTO-20A Prominence). Data acquisition and analysis were made by a controller (CBM-20A Prominence) coupled to a computer with Shimadzu® LC Solution software. The analytical conditions included a mobile phase composed of a mixture of acetonitrile, 0.01 M phosphate buffer at pH 7.0 (1:1 v/v) and 0.05% (v/v) of diethylamine and were configured to have an isocratic flow of 1.0 mL/min, a detection wavelength of 203 nm, an oven temperature of 30 °C, an injection volume of 20 μL and a total analysis time of 20 min.

PCL and LCL exhibited retention times of 8.9 and 12.9 min, respectively. The method was validated based on parameters for selectivity, sensitivity, linearity, accuracy and precision [39]. Linear calibration curves for PCL ($94197.9x + 947$; $r = 0.997$) and LCL ($110104x - 1586$; $r = 0.999$) over a concentration range of 0.25–10.0 μg/mL were achieved. The intra- and inter-day precision and accuracy of the method presented a coefficient of variation (%CV) and a relative error (%*E*) of no greater than 4.8% and 1.1% for PCL and 1.5% and 1.6% for LCL, respectively. The limit of quantification of the method, calculated as three times the standard deviation of the linear regression of three analytical curves, was 0.25 μg/mL for PCL and 0.13 μg/mL for LCL. The selectivity of the method was investigated using the hydrogel without drugs (blank) and porcine esophageal mucosa. No interference was observed in PCL or LCL retention times, indicating the specificity of the method. The efficiency of drugs recovery from the epithelium ranged from 95.3 ± 2.77% to 104.98 ± 3.57 % for PCL and from 96.51 ± 4.45% to 102.84 ± 0.33% for LCL [39].

2.8. Data analysis

The data are presented as the mean (standard deviation) (SD). The data were evaluated using Student's *t*-test or an analysis of variance (ANOVA) with a subsequent parametric Tukey test. In all of the tests that were performed, p values <0.05 were considered significantly different. Analyses were made using GraphPad Instat 3.0 (GraphPad Software Inc., CA, EUA).

3. Results

3.1. Characterization of the formulations

Table 1 shows the mechanical properties of the formulation containing both PCL and LCL at pH 7.0, at both 25 and 37 °C. At 37 °C, the hardness and the compressibility were significantly smaller than at

Table 1
Mechanical properties of formulation containing PCL and LCL at 25 °C and 37 °C.

Mechanical properties	Temperature (°C)*	
	25 °C	37 °C
Hardness (N)	0.13 (0.00)[a]	0.08 (0.01)
Compressibility (N.mm)	0.06 (0.00)[a]	0.04 (0.01)
Elasticity	1.08 (0.01)	1.07 (0.01)
Cohesiveness	1.17(0.00)	1.18 (0.00)
Adhesiveness (N.mm)	0.32 (0.01)[a]	0.24 (0.00)

* (SD, $n = 5$).
[a] $p < 0.05$ (Student's t-test) between 25 °C and 37 °C.

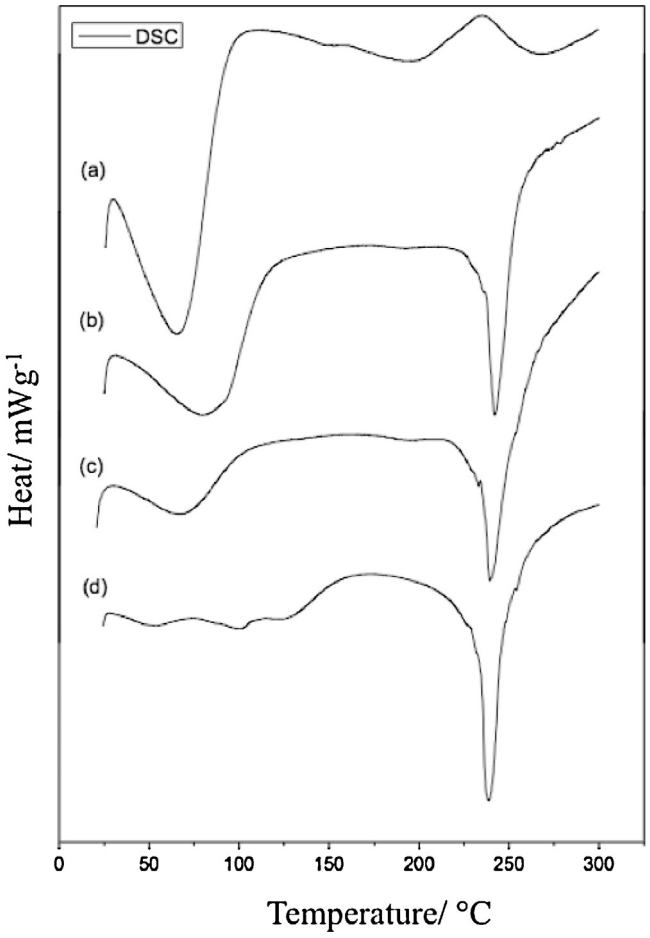

Fig. 1. DSC curves of (a) HPMC hydrogel, (b) HPMC hydrogel with PCL, (c) HPMC hydrogel with LCL and (d) HPMC hydrogel with PCL and LCL at pH 7.0.

25 °C. The cohesiveness, the elasticity of hydrogel and the adhesiveness showed values similar to those shown by other formulations based on HPMC for buccal administration [40]. The detachment force and the work of mucoadhesion (Table S2.1, Supplementary material) showed no significant difference as a function of the time that the formulation remained in contact with the mucosa, suggesting that HPMC might be capable of interacting quickly with the mucosa surface; therefore, 30 s would be enough time to enable the formation of these interactions. The detachment force was approximately 0.1 N and the work of the mucoadhesion was about 0.1 mJ/cm^2, which was similar to that found by karavana et al. [40]. The rupture or failure of mucoadhesive bonds occurred inside of the mucoadhesive system (Fig. S2.1, Supplementary material).

DSC curves of the blank hydrogel at pH 7.0 and of the hydrogels containing PCL and LCL either isolated or combined at the same pH are presented in Fig. 1.

Table 2
DSC parameters of HPMC hydrogels at pH 7.0 containing PCL and LCL isolated or in combination.

Formulation	Peak of the events (°C)		Enthalpy change (ΔH)	(mJ g^{-1})
	1	2	1	2
Blank	65.2	–	−228	–
PCL	79.5	242.0	−234	−129
LCL	67.5	239.4	−80	−76
PCL and LCL	–	239.2	–	−214

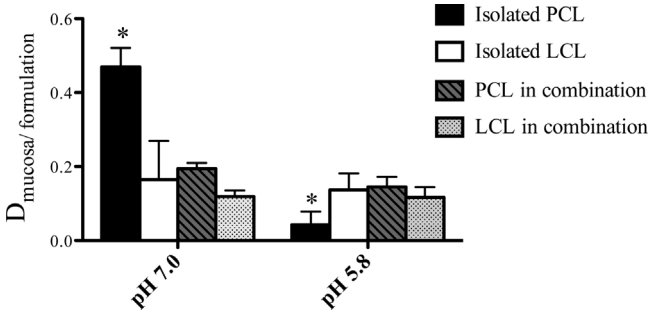

Fig. 2. Distribution coefficients of PCL and LCL when isolated or combined in the formulation at pH 7.0 and 5.8. (SD; $n = 3$). ANOVA followed by Tukey's multiple comparison test ($p < 0.05$), where (*) represents significant differences between the formulations at the same pH.

Table 2 shows the events that were found in each of these curves, including their temperature peaks and enthalpy changes (ΔH). The hydrogel formulation (Fig. 1, curve (a)) shows only one endothermic peak at 65.2 °C, which is characteristic of solvent evaporation. A solvent evaporation peak at a temperature of approximately 70 °C can also be observed in the thermograms corresponding to the hydrogels that contained PCL or LCL individually (Table 2). An endothermic peak corresponding to PCL and LCL fusion occurs at almost the same temperature, close to 240 °C (Fig. 1, curves (b) and (c)). The combination of the two drugs in the same formulation did not change the endothermic peak that was characteristic of PCL and LCL fusion (Fig. 1, curve (d)), but there was an increase in enthalpy change (Table 2) due to their combination in the same formulation. Fig. 2 shows the mucosa/formulation distribution coefficients ($D_{m/f}$) of PCL and LCL when isolated or combined in the same formulation at pH 7.0 and pH 5.8.

At pH 7.0, the $D_{m/f}$ of the PCL when it was isolated in the formulation was approximately 3-fold higher than that of isolated LCL. At pH 5.8, on the other hand, the $D_{m/f}$ of PCL was approximately 3-fold smaller than that of LCL. When combined in the same formulation, both at pH 7.0 and at pH 5.8, the $D_{m/f}$ of PCL is similar to that of LCL.

3.2. In vitro *permeation studies*

Fig. 3 shows the passive and iontophoretic permeation profiles of PCL and LCL when isolated and combined in the formulation at pH 7.0.

It can be observed in Fig. 3 that the permeation profiles of the drugs, isolated or in combination are linear after passive or iontophoretic treatment. Changing the pH of the formulation from 7 to 5.8 also did not result in modifications of the drug permeation profiles (results not shown). In all cases, a linear relationship was obtained when the amount of permeated drug was plotted against time, suggesting zero-order permeation kinetics. However, the total amounts of permeated drug varied depending on the drug, the presence of an electrical current and the formulation pH.

Table 3 shows the PCL and the LCL permeation rates (J) that were calculated from the slopes of the linear curves that represented amount of drug permeated (μg/cm^2) *vs.* time (minutes). Passive

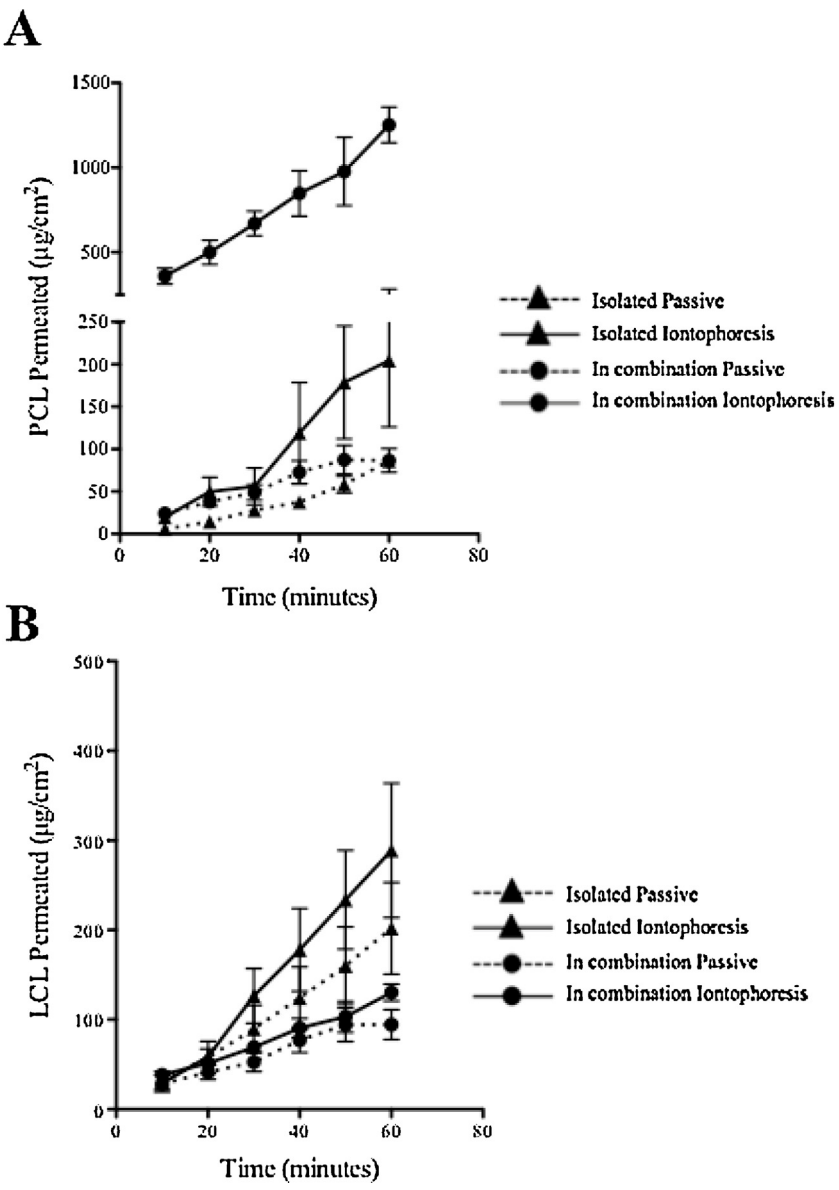

Fig. 3. Passive and iontophoretic permeation profiles of (A) PCL isolated and combined with LCL and (B) LCL isolated and combined with PCL from the HPMC hydrogel at pH 7.0 that contained each of the drugs at 2.5% (SD; $n = 5$).

Table 3
PCL and LCL permeation rates (J) from the formulations at pH 7.0 and 5.8.

Drug in the Formulation	Passive (μg/cm² min)*		Iontophoresis (μg/cm² min)*	
	pH 7.0	pH 5.8	pH 7.0	pH 5.8
PCL isolated	1.55 (0.15)	1.00 (0.33)	6.94 (1.87)[b]	8.52 (2.62)[a,b]
LCL isolated	4.05 (0.72)[a]	0.60 (0.09)	5.37 (1.26)	2.53 (0.09)[b]
PCL combined	1.56 (0.29)	0.60 (0.09)	18.78 (2.20)[a,b]	0.60 (0.09)
LCL combined	1.61 (0.32)	0.60 (0.10)	1.93 (0.22)	0.65 (0.09)

* (SD, $n = 5$).
[a] $p < 0.05$ (One-way ANOVA, Tukey's multiple comparison test) showing difference for formulations at same pH.
[b] $p < 0.05$ (Student's t test) showing difference between passive and iontophoresis permeation.

experiments showed that LCL permeated the skin with a permeation rate 3-fold higher than that of PCL when the drugs were used as individual formulations at pH 7.0. However, when the drugs were combined into the same formulation, the flux of LCL decreased, becoming similar to that of PCL (Table 3). At pH 5.8, the permeation of the drugs was reduced relative to the results obtained at pH 7.0, but PLC and LCL fluxes were again similar when the drugs were combined into the same formulation.

Iontophoresis significantly increased drug permeation in the formulations containing the isolated drugs at both pH 7.0 and 5.8, except for LCL at pH 7.0 (Table 3). PCL had the most noticeable increase, which was 4.5- and 8.5-fold higher than the passive flux at pH 7.0 and 5.8, respectively; for LCL, a 4-fold increase was observed

Table 4
PCL and LCL recovered from the mucosa after 60 min of experiment.

Drug in the Formulation	Passive	(μg/cm²)*	Iontophoresis	(μg/cm²)*
	pH 7.0	pH 5.8	pH 7.0	pH 5.8
PCL isolated	407 (97)	655 (160)	655 (72)	2138 (790)
LCL isolated	302 (45)	191 (24)	529 (149)	557 (118)[b]
PCL combined	6463 (710)[a]	2346 (389)[a]	56534(8804)[a,b]	1993 (533)
LCL combined	412 (56)	2282 (369)[a]	6393 (285)[b]	1889 (561)

* (SD, $n = 5$).
[a] $p < 0.05$ (One-way ANOVA, Tukey's multiple comparison test) showing difference for formulations at same pH.
[b] $p < 0.05$ (Student's t test) showing difference between passive and iontophoresis permeation.

at pH 5.8, but at pH 7.0 the permeation rate was similar for both passive and iontophoretic delivery. When the drugs were combined into the same formulation, iontophoresis only increased the permeation of PCL when it was in the pH 7.0 formulation; the permeation was increased by approximately 12-fold (Table 3). LCL did not benefit from iontophoresis when it was associated with PCL at any of the tested pHs.

Table 4 shows the total amounts of PCL and LCL that were recovered from the mucosa after 60 min of passive or iontophoretic delivery. Passively, the combination of the drugs resulted in increased PCL retention in the mucosa at both pHs (16-fold at pH 7.0 and 4-fold at pH 5.8) and of LCL at pH 5.8 (12-fold). At pH 7.0, the increase in LCL retention caused by the drug combination was not significantly different ($p > 0.05$) from the formulation containing only LCL. Iontophoresis only increased the amount of drug that accumulated in the mucosa compared to passive delivery in the formulation that contained LCL alone at pH 5.8; the increase was 3-fold, and no significant differences were found for PCL at pH 5.8 or for PCL and LCL at pH 7.0. For the combined formulation, however, the increases were significant for both drugs, but only at pH 7.0 (15-fold for LCL and 9-fold for PCL); at pH 5.8, no differences were observed in the amounts of LCL or PCL that were retained by passive and iontophoretic delivery.

When analyzing the effects of the drug combination on the individual and combined retention rates when iontophoresis was applied at pH 7.0, a remarkable 86-fold increase was observed for PCL, followed by a 12-fold increase for LCL. On the other hand, at pH 5.8, there was no significant increase ($p > 0.05$) in PCL or LCL accumulation in the mucosa in the formulations containing the single drugs. Therefore, at pH 5.8, neither iontophoresis nor the drug combination increased the amount of PCL that was retained. At pH 7.0, however, the effects of both iontophoresis and the drug combination on PCL retention were remarkable.

4. Discussion

Mucoadhesive formulations demonstrate advantages such as sustained drug release, targeting to specific sites and intimate contact with a substrate [41]. The principal aspects to be considered during the development of a mucoadhesive formulation are the ease of its removal from packaging, the ease of its administration and its retention at a desired site without disintegration [40]. Texture profile analysis has been used to provide information about these characteristics, which are related to the mechanical properties of pharmaceutical semisolids, such as hydrogels. Information about the hardness, adhesiveness, compressibility, cohesiveness and elasticity of the formulations (Table 1) provided by the texture profile analysis suggested that the formulations are adequate for buccal administration [40]. Decreases in the hardness and compressibility of hydrogels in response to increasing temperature were also observed by Jones et al. [42] that correlated this effect to the rheological characteristics of the formulation at different temperatures. At higher temperatures, the higher kinetic energy of the molecules decreases the intermolecular forces, thus reducing the viscosity, consistency, hardness and compressibility of the hydrogels.

The HPMC had been described as bioadhesive [43], so it was expected that the formulation presented good mucoadhesive properties [44]. The work of mucoadhesion that was obtained in our study, approximately 0.1 mJ/cm², was similar to results found by Karavana et al. [40] and Amasya et al. [45] for buccal HPMC hydrogels. Mortazavi and Smart [44] showed that HPMC, despite its being a polymer with a lower mucoadhesive force, enables a longer duration of adhesion, which is facilitated by its rapid interaction with the mucosa surface. A flow-through evaluation of the mucoadhesion, performed to complement the texture profile and mucoadhesive tests (Supplementary material, Section S2), showed it was still possible to observe the formulation along the mucosa after 1 h of exposition to a high saliva flux (1 mL/min). Therefore, the quick interaction of our formulation with the mucosa, the relatively long period of permanence in its time of contact with the mucosa and its adequate texture profile suggest that the HPMC formulation that contained the mixture of LCL and PCL can be conveniently used for buccal administration. In clinical use, the administration site can still be isolated in some procedures, thus preventing any influence of saliva flux.

An eutectic mixture of prilocaine and lidocaine is known to be formed when equal proportions of these bases are put together [46]. Thermal analyses were performed to evaluate whether an eutectic mixture is also formed when the salts PCL and LCL are mixed. However, the recorded temperature demonstrated that there was no decrease in the melting temperature of the PCL–LCL mixture with respect to the melting points of the individual drugs (Fig. 1, Table 2). Thus, it was suggested that PCL and LCL do not form an eutectic mixture, despite what occurs with their base forms.

The esophageal epithelium of pigs was used in *in vitro* permeation and partition experiments. The substitution of the buccal epithelium for the esophageal one in *in vitro* drug permeation studies was proposed by Diaz Del Consuelo et al. [29–31]. These authors demonstrated that the epithelium of buccal and esophageal mucosa presented similar lipid composition, both qualitatively and quantitatively, and showed similar permeability properties *in vitro*. The epithelium of the buccal mucosa of pigs is firmly attached to the subjacent muscular tissue, rendering the dissection process difficult. Also, because of the chewing, it is difficult to obtain large intact areas of the epithelium. Therefore, we choose to work with the esophageal epithelium because of the easiness of dissection, higher efficiency and less variability in terms of *in vitro* permeability [32].

Partition and distribution coefficients are important lipophilicity descriptors that are associated with drug release, permeation, absorption, distribution, biotransformation and excretion. For ionizable compounds, the distribution of a drug in distinct mediums is pH-dependent and should be determined based on its distribution coefficient (D) [47]. Lidocaine and prilocaine are weak bases, with pKas of 7.86 and 7.89, respectively [48]. In this way, to complete the characterization of the formulation and to obtain further

understanding of the permeation of the drugs through the mucosa, the distribution of PCL and LCL between the mucosal epithelium and the formulation was assessed at two different pHs, 7.0 and 5.8, by determining the $D_{m/f}$ values of each of the drugs, both alone and in combination.

We decided to determine the $D_{m/f}$ values of LCL and PCL between the mucosal epithelium and the hydrogel. The epithelium was chosen because it is the site where our formulation is applied; it is considered a lipophilic medium, whereas the hydrogel is considered a hydrophilic medium because 90% of its composition is water (Item 2.4). The choice of pH 7.0 was based on the pH of saliva; pH 5.8 was chosen based on iontophoretic studies showing that this more acidic pH can increase the electromigration of drugs due to the high percentage of ionized species in this condition (approximately 99% of the molecules of both drugs should be cationic at this pH). Buffered hydrogels were used so as to guarantee no significant changes in the pH of the formulations during the permeation experiments. The pH of the gel and receptor buffer was measured before and after the experiments. Both formulations at pH 7 and 5.8 showed a less than 2% decrease at the end of the experiment. This slight variation was considered not to affect the electrotransport and the control of the transport of the drugs

The results showed that the $D_{m/f}$ of the PCL-only formulation was significantly higher at pH 7.0, than that of the LCL-only formulation (Fig. 2). When both drugs were combined in the same formulation, the $D_{m/f}$ of PCL decreased, suggesting an enhancement of the hydrophilic character of PCL in the presence of LCL. At pH 5.8, however, PCL showed a smaller distribution in the mucosa than did LCL; PCL in combination with LCL increased this distribution, indicating an increase in PCL hydrophobicity in the presence of LCL.

In summary, the $D_{m/f}$ results showed that the combination of PCL and LCL is capable of changing the $D_{m/f}$ of PCL at both pH values. The calculated log D of prilocaine and lidocaine at pH 7 and 5.8 shows that lidocaine is more lipophilic than prilocaine, especially at pH 7 (lidocaine $\log D_{5.8} = 0.9$, lidocaine $\log D_7 = 2.02$; prolocaine $\log D_{5.8} = -0.56$, prilocaine $\log D_7 = 0.49$; calculated using the software MarvinSketch 6.2.0, ChemAxon Ltd.). These differences in drugs log$D$ at different pHs may be responsible for the different affinities of them for the epithelium and for the hydrogel. We hypothesize that changes in $D_{m/f}$ of PCL when associated with LCL in the same formulation could be related to the rate of ion pair formation [52] of each drug's cation with anions present in the buffer solution used in making the gel formulation.

To evaluate the impact of $D_{m/f}$ on the abilities of the drugs to permeate the mucosal epithelium, passive and iontophoretic permeation studies were performed. Iontophoresis was performed by applying an electric current of 1 mA/cm^2. A range of 0.25–1.0 mA/cm^2 is typically used for iontophoretic drug delivery through the mucosa *in vitro* [19,26,27,49–51]. We choose to apply 1 mA/cm^2 because the period of our experiments was shorter (only 1 h) when compared to others (typically 6–8 h). The influence of iontophoresis in mucosa properties has been previously studied [19,49]. We standardized our iontophoretic experiments conditions by using only fresh epithelium with an initial electrical resistivity greater than 3 KΩ/cm^2 and monitored the voltage of the complete circuit during the experiment (Supplementary material, S3). With these careful procedures, we believed that the influence of iontophoresis on the mucosal penetration of PCL and LCL when combined in a mucoadhesive semisolid formulation could be properly evaluated.

Passive permeation studies showed that the combination of PCL and LCL in the same formulation changed the amount of drug that was delivered (Tables 3 and 4) as a function of pH and $D_{m/f}$ (Fig. 2). At pH 5.8, the passive permeation of the drugs was reduced in relation to the experiments performed at pH 7.0 (Table 3). This result was expected due to the increased ionization of weak bases at more acid pH values and the favored permeation of non-ionized substances over ionized species. A decrease in the $D_{m/f}$ values of the isolated drugs was also noticed at pH 5.8 compared to pH 7.0 (Fig. 2), indicating a greater distribution of the drugs in the hydrophilic formulation rather than in the mucosa.

Analyzing the combination of the drugs with respect to their passive permeation in the isolated formulations at the same pH showed that the combination of PCL and LCL decreased the passive flux of LCL to a value similar to that of PCL's flux at pH 7.0. The distribution coefficient studies showed that PCL was better distributed in the mucosa at pH 7.0 than was LCL. This higher distribution of PCL in the mucosa may have increased PCL accumulation therein, thus hindering LCL permeation through competition. Indeed, the total amount of PCL that was recovered from the mucosa at pH 7.0 was higher when it was associated with LCL in the same formulation (Table 4). At pH 5.8, the combination of the drugs resulted in similar $D_{m/f}$ values for both PCL and LCL (Fig. 2), leading to similar permeation rates and retention amounts in the mucosa (Tables 3 and 4).

In general, iontophoresis increased drug permeation in the formulations containing the isolated drugs at both pH studied (Table 3). When compared to passive delivery, iontophoresis at pH 5.8 resulted in a higher improvement of drugs delivered than at pH 7. The ionic strength of the buffer solution (at 0.01 M) used to prepare the gels was 1.3-fold higher at pH 5.8 than at pH 7 (0.0444 at pH 7 and 0.0568 at pH 5.8). Addition of LCL or PCL in the gels increased the ionic strength to about 0.20 at pH 7 and 0.23 at pH 5.8. Therefore, the ionic strength was higher at pH 5.8 and PCL and LCL ions could transport a higher fraction of the electric current at pH 5.8 than at pH 7 when they were alone in the gels. However, iontophoresis did not significantly increase PCL and LCL fluxes at pH 5.8 when the drugs were combined in the same formulation (ionic strength of 0.41), but substantially increased PCL permeation at pH 7 (ionic strength of 0.35) (Table 3). In Fig. 2, it can be observed that the combination of the drugs significantly enhanced the distribution of PCL in the formulation at pH 7.0 (the PCL $D_{m/f}$ was decreased by 3-fold), suggesting an improvement in the ionization of PCL when it is associated with LCL. The higher hydrophilicity of PCL could be confirmed by HPLC analysis, in which PCL is eluted from a C18 column first than LCL (PCL and LCL exhibited retention times of 8.9 and 12.9 min respectively) [39]. Thus, it is possible that PCL possesses more ions to carry electric current and therefore presents higher iontophoretic flux than does LCL when they are associated in the same formulation at this pH. Moreover, the electromigratory flux of each ion is related to its electric mobility and concentration [53]. The concentrations of PCL and LCL in the formulation are the same and thus the determining factor of iontophoretic permeation rate should be ion electric mobility. In general, the electric mobility of a molecule is inversely proportional to its size [53]. Lai and Roberts [54] observed in iontophoresis studies conducted across the skin that PCL presented a higher electric mobility than LCL, which could be related to its molecular volume, facilitating PCL permeation.

When the drugs were combined at pH 5.8, iontophoresis did not significantly increase their fluxes. This result could also be related to the physicochemical changes that occurred in both substances when they were incorporated together at this pH. At pH 5.8, the $D_{m/f}$ of PCL was 14-fold lower than at pH 7.0 (0.57 at pH 7.0 *vs.* 0.04 at pH 5.8), corroborating the higher ionization of the molecule at this pH however, it increased by 3.5-fold when associated with LCL (from 0.04 to 0.14). This increase in the $D_{m/f}$ of PCL when in combination with LCL resulted in a $D_{m/f}$ value that was very similar to LCL, which did not undergo any alteration when in the combination. It is possible that at pH 5.8, this combination lead to the formation of ion pairs between the drugs and the ions present in the buffered formulation [44] such as the $H_2PO_4^-$, decreasing the efficiency of the electric current and consequently preventing iontophoresis from improving drug flux with respect to passive per-

meation. The total amount of drug recovered from the mucosa after 1 h of passive or iontophoretic permeation from the hydrogel containing the combination of PCL and LCL at pH 5.8 was also similar (Table 4), therefore corroborating the $D_{m/f}$ results of both drugs in this condition.

In summary, the formulation that included the combination of the drugs at pH 7.0 significantly enhanced the total permeated amount and flux of PCL, in addition to increasing the amounts of both drugs that were recovered from the mucosa. It is interesting to use the combination of the drugs at pH 7.0 because, besides combining a higher onset (LCL) and duration of action (PCL) [7], it is possible to achieve higher amounts of both anesthetics permeating through and recovered from the mucosa.

5. Conclusion

The developed formulation presented adequate mechanical and mucoadhesive properties for buccal administration. The combination of PCL and LCL does not cause the formation of an eutectic mixture, as verified by DSC studies, but it did change the distribution coefficients of PCL into the epithelium. The combination of PCL and LCL at pH 7.0, when subjected to iontophoresis, resulted in fast permeation of high concentrations of PCL through the mucosa together with high amounts of both PCL and LCL being retained in the mucosa. Therefore, this can be considered a valuable strategy for the administration of needle-free anesthesia during dental procedures, likely offering a fast onset and prolonged duration of anesthesia.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2015.11.005.

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