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Research paper

Release of naltrexone on buccal mucosa: Permeation studies, histological aspects and matrix system design

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Abstract

Transbuccal drug delivery has got several well-known advantages especially with respect to peroral way. Since a major limitation in buccal drug delivery could be the low permeability of the epithelium, the aptitude of NLX to penetrate the mucosal barrier was assessed. *Ex vivo* permeation across porcine buccal mucosa 800 μm thick was investigated using Franz type diffusion cells and compared with in vitro data previously obtained by reconstituted human oral epithelium 100 μm thick. Both fluxes (Js) and permeability coefficients (K_p) are in accordance, using either buffer solution simulating saliva or natural human saliva. Permeation was evaluated also in presence of chemical enhancers or iontophoresis. No significant differences in penetration rate were observed using chemical enhancers; in contrast, Js and K_p were extensively affected by application of electric fields. Tablets, designed for Naltrexone hydrochloride (NLX) administration on buccal mucosa, were developed and prepared by direct compression of drug loaded (56%) poly-octylcyanocrylate (poly-OCA) matrices. NLX is slowly discharged from buccal tablets following Higuchian kinetic. Histologically, no signs of flogosis ascribable to NLX and/or poly-OCA were observed, while cytoarchitectural changes due to iontophoresis were detected. Buccal tablets containing NLX may represent a potential alternative dosage form in addiction management.

Keywords: Transbuccal permeation; Porcine buccal mucosa; Iontophoresis; Naltrexone hydrochloride; Poly-octylcyanoacrylate matrices; Buccal tablets

1. Introduction

Buccal administration of drugs is a valid alternative to the peroral one since drugs directly diffuse into the systemic circulation. In particular, it is advantageous for those drugs that encounter degradation in the gastrointestinal tract or severe hepatic first-pass metabolism and require the administration of large doses to reach effective therapeutic levels in the target site [1,2]. Among the epithelial tissues, the buccal mucosa offers good performance for local/systemic pharmacological actions because of its permeability. Buccal drug delivery specifically refers to the delivery of drugs within/through buccal mucosa [3]. Buccal administration could be an alternative, non-invasive delivery route also for Naltrexone hydrochloride (NLX).

In formerly opioid dependent patients who have undergone detoxification, NLX is often used to assist relapse prevention also called ''Narcotic Antagonist Treatment Using Naltrexone'' [4]. Clinical pharmacology studies demonstrated that oral NLX at 50, 100 and 150 mg effectively blocks the physiological and subjective effects of parenterally administered heroin, hydromorphone or morphine for 24, 48 and 72 h, respectively [5]. It has been reported that the maintenance of blood NLX concentrations of at least 1 ng mL^{-1} provides prevention of overdoses for

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approximately 3 months [4,5]. A reduced number of opioid overdoses were also observed 7–12 months post treatment [6,7]. Recently, NLX has been approved also for alcoholism treatment, to stop the alcohol cravings during early days of abstinence from alcohol [8].

Usually, NLX is taken *per os* as conventional capsules or tablets either daily or three times a week for a sustained period of time and, as it is not addicting, itself has no subjective effects or potential for abuse. Following peroral administration, NLX is rapidly and quite completely absorbed (about 96%) from the gastrointestinal tract but the drug undergoes a significant first-pass effect [9]. Thus hepatic metabolism (>98% metabolized) will result in having a very low drug concentration in the brain [10].

Although peroral NLX is effective in treating alcohol and opiate dependencies, fluctuating plasma levels and a variety of adverse reactions to the medication limit its efficacy [11–13]. By delivering efficiently the drug through buccal mucosa the hepatic first-pass metabolism should be reduced; as a consequence, low drug doses could be administered and side effects minimized.

In this study we developed a new formulation of tablets suitable for the administration of NLX on buccal mucosa. Since a major limitation in the development of a buccal drug delivery device could be the low permeability of the buccal mucosa, the aptitude of NLX to penetrate the barrier was preliminarily evaluated.

To assess drug permeability, buccal mucosa from various animals (rabbits, dogs, monkeys, hamsters and pigs) as well as cultured tissues like reconstituted human oral epithelium have been used as models for human mucosa. However, when compared to the other animal models, porcine buccal mucosa has been considered the most representative model for human tissue as it is non-keratinised like human buccal mucosa [14].

Similar to other mucosal membrane, the buccal mucosa has disadvantages as well. Low drug bioavailability due to low mucosal membrane permeability, relatively small surface area available for absorption and poor retention of the drug and/or drug formulation at the site of absorption are the major limitations. These restrictions could be successfully altered using chemical penetration enhancers [15]. As NLX is a hydrophilic molecule, electrically charged at physiological pH ($pK_a = 8.1$ at 37 °C) also iontophoresis could be used to promote movement through the mucosal membrane [16,17].

Using reconstituted human oral epithelium, as *in vitro* model, we demonstrated that NLX well permeates the membrane [18]. Nevertheless, the cultured tissue is about 100 μm thick, whereas the epithelium of the human buccal mucosa is 500–800 μm thick [19,20]. Accordingly, our previous data on NLX permeation through reconstituted human oral epithelium [18] need further validation on a more thick tissue.

In this paper the aptitude of NLX to penetrate porcine buccal mucosa and reach therapeutical steady-state plasma concentrations following buccal administration is reported. The NLX diffusional behaviour through porcine mucosa is compared with that observed through the cultured tissue model [18].

Since the drug, the formulation components and the electric field could damage the structure of the biological tissue, we studied also the effects of their application on histology of porcine buccal mucosa.

2. Materials and methods

2.1. Materials

Naltrexone hydrochloride (NLX), USP grade, was purchased from Sun Pharmaceutical Industries LTD (Cujart, India) and 2-octylcyanoacrylate (2-OCA) from GluStich Inc. (Delta, Canada). Sodium dehydrocholate (NaDHC), EDTA disodium salt (NaEDTA) and trisodium citrate dihydrate (TNaC) were from Polichimica s.r.l. (Bologna, Italy). As simulated non-enzymatic plasma a phosphate buffered saline (PBS) Ca^{2+} and Mg^{2+} free solution, pH 7.4, was used. It was prepared by dissolving KH_2PO_4 (0.144 g), anhydrous Na_2HPO_4 (0.795 g) and NaCl (9.0 g) in 1 L of distilled water [18]. Buffer solution simulating saliva (pH 6.8) was prepared by dissolving NaCl (0.126 g), KCl (0.964 g), KSCN (0.189 g), KH_2PO_4 (0.655 g), and urea (0.200 g) in 1 L of distilled water [21]. All components of buffer solutions were from Sigma–Aldrich (Milano, Italy). Natural human saliva (pH 6.8) was obtained from a healthy donor without any conditioning habits i.e. smoking, alcohol, coffee drinking or any other further habit able to alter its composition.

Unstimulated mixed saliva was collected from one of the authors who, after overnight fasting, first brushed his teeth and thoroughly rinsed the mouth using only deionized water, then sat in a relaxed position with the head in a slightly-inclined forward position, allowing saliva to accumulate on the floor of the mouth. The first few millilitres of saliva were discarded. The accumulated saliva was then withdrawn using disposable sterile plastic pipettes until about 1.5 mL had been collected. The samples of saliva were not further handled to evaluate the drug behaviour in environmental conditions similar to those of the administration site.

All chemicals and solvents were of analytical grade and were used without further purification. All other reagents for cell culture were obtained from Sigma and solutions were prepared in endotoxin-free water.

2.2. Methods

2.2.1. Ex vivo permeation of NLX throughout porcine buccal mucosa

Mucosal specimens were obtained from tissue removed from the vestibular area of retromolar trigone of freshly slaughtered domestic pigs. After sampling, all specimens were immediately placed in a refrigerated transport box and transferred to laboratory within 1 h. Excesses of

connective and adipose tissue were trimmed away until slides 0.8 ± 0.1 mm thick were obtained. Some specimens were used fresh; the remaining specimens were stored at −40 °C for periods up to six months. It has been reported that frozen tissue specimens do not change their diffusional behaviour when used in permeation studies and that the mucosae are not adversely affected by freezing in liquid nitrogen [22]. The frozen specimens were equilibrated in PBS for 3 h at room temperature to thaw completely prior to the start of experiments. The equilibration medium was replaced with fresh PBS every 15 min.

Appropriate sections of mucosa were mounted in Franz type diffusion cells. Tissue disks were equilibrated for 1 h at 37 °C [Polimix EH 2 bath equipped with a constant-rate adjustable stirrer RECO® S5 (Kinematica, Switzerland)] adding PBS in both the donor and the acceptor compartment. This step was followed by the removal of PBS from the compartments. In the donor compartment was then placed a NLX solution (30 mg of NLX in 1.0 mL of buffer solution simulating saliva or natural human saliva), in the acceptor compartment was placed PBS. At regular time intervals (30 min), samples were withdrawn from the acceptor compartment and the sample volume taken out was replaced by fresh fluid. Each experiment was carried out for 6 h and repeated using six different fractions of the same portion of tissue. The integrity of the mucosal tissue was monitored before and after each permeability study.

Permeation of NLX in presence of chemical enhancers was investigated as above. To the donor phase increasing amounts of chemical enhancer (NaDHC, NaEDTA or TNaC) were added (enhancer/drug ratio 0.1/100, 0.5/100 and 1/100). In all experiments the drug transferred from the donor to the acceptor compartment was monitored spectrophotometrically (see Section 2.2.6.1).

2.2.2. Iontophoretic permeation

In the donor chamber of the equipment described in Section 2.2.1 a silver electrode (active electrode) was placed for anode; in the acceptor chamber a silver chloride–coated silver electrode (reference electrode) was applied for cathode. Prior to chloridation, the silver electrode was dipped in distilled water, ethanol, fuming nitric acid and finally washed with distilled water. The electrode was then treated with 0.1 N HCl and a current of 1 mA was applied for 24 h using silver as cathode [23]. The distance between the electrode and the tissue was 5 mm. Current densities of 1 or 2 mA/cm^2 expressed per unit of crossing area of tissue were applied to observe the effects of iontophoresis on permeation rate. The effect of initial donor fluid (buffer solution simulating saliva or natural human saliva) was evaluated using NLX solutions prepared as described in Section 2.2.1. Withdrawals of samples were timed as described above. The drug transferred from the donor to the acceptor compartment was monitored spectrophotometrically (see Section 2.2.6.1).

2.2.3. Preparation of drug loaded matrices

Solid dispersions (matrices) were prepared by the solvent evaporation method. NLX (0.60 g) was powdered, passed through a 100 μm standard mesh wire stainless steel sieve and put in a porcelain mortar. To the powder the appropriate amount of 2-OCA monomer (0.40 g) was added by drops. To the blend was rapidly added acetone (5 mL/g of mixture) to obtain a workable combination. The whole mixture was stirred with the pestle to obtain a uniform creamy mass. Grinding/mixing was continued until complete solvent evaporation has occurred. The residual mass was set aside overnight to allow complete polymerization to occur. At this point, the typical smell of the octyl-derivatives disappeared and a solid hard material was formed. The solid drug loaded matrix formed was then crushed, powdered, air-dried at room temperature for 24 h, and stored as free flowing material. The amount of NLX entrapped into the matrix was determined spectrophotometrically as described in Section 2.2.6.2. The average drug content in the matrix was 56% w/w.

2.2.4. Preparation of tablets

Tablets (13 mm diameter, 1.33 cm^2 surface and 1.20 mm thickness, weighing 200 mg) were obtained by direct compression (Perkin-Elmer IR Accessory, hydraulic, single die, Tabletting Machine) (10 tons) of drug loaded matrix using two flat-faced punches and a die. Before compression the crushed matrix was passed through a 100 μm standard stainless steel sieve.

2.2.5. In vitro drug release from tablets

The drug release from tablets was assessed using a flow through system (HSG-IMIT, Villingen-Schwenningen, Germany) [24]. Briefly, the system consists of a buffer solution simulating saliva container (100 mL) from which liquid is forced to a release cell. The flow rate of saliva was controlled by a peristaltic pump (Biorad econo pump, USA) and maintained constant (1.2 mL/h) during the experiments. In the cell, the salivary film wetting the pill is about 0.1 mm thick. During the flow through, saliva wets the drug loaded tablet embedded inside the cell. The wetted tablet releases the drug enriching saliva in NLX content. The temperature was controlled by submerging the cell and the saliva container in a thermostatted bath. The drug amount in the taken out saliva solution was quantitatively determined spectrophotometrically (see Section 2.2.6.3). Experiments were performed on six different batches and mean results were reported. The residual drug content in the tablets after release studies was determined for selected samples. The amount of drug released and the residual drug content matched the original drug content within 2–8%.

2.2.6. Drug assay

2.2.6.1. Drug assay in permeation studies. The amount of NLX permeated was monitored by measuring spectrophotometrically (UV/VIS Shimadzu mod. 1700 Pharmaspec

instrument) the drug that reached the acceptor fluid using the appropriate calibration curve and blank ($\lambda_{max} = 281.0$ nm, $E_{1\%} = 0.0350$ in PBS). In these conditions the UV absorption peak was highly reproducible and linearly related to concentration over a range 0.004–0.4 mg/mL. At testing concentrations, buffer components and used chemical enhancers do not interfere significantly with the UV absorption of NLX. Nevertheless, NaDHC at 281 nm could interfere but enhancer/drug ratio was very low and the NaDHC contribution to absorption was negligible. In our hand, no significant differences in drug concentrations were observed in presence or in absence of the enhancer. The sensibility was less than 0.0004 mg/mL. Intraday and interday variations, observed during collection of experimental data, were lower than sensibility.

At the end of all experiments portions of the used tissue were treated with a freshly prepared $FeCl_3$ water solution (20% w/v) to underscore the drug eventually entrapped into the tissue.

2.2.6.2. Drug content into the matrix. NLX incorporated into the matrix was determined spectrophotometrically at $\lambda = 284.0$ nm using the appropriate blank and calibration curve ($E_{1\%} = 0.0474$ in acetonitrile). Aliquots of randomly selected material of each batch were accurately weighed (10.00 ± 2.00 mg), transferred into 50 mL flask, sonicated and brought to volume with acetonitrile to solubilize both the drug and the polymer. The UV absorption peak was highly reproducible and linearly related to concentration over a range 0.004–0.4 mg/mL. At the concentrations used, dissolved poly-OCA does not interfere with the UV absorption of NLX.

2.2.6.3. Drug assay in release studies. The drug released from the tablets in buffer solution simulating saliva was quantitatively determined by UV spectrophotometric analysis at $\lambda = 281.0$ nm using the appropriate blank and calibration curve ($E_{1\%} = 0.0270$ in simulated saliva). The UV absorption peak was highly reproducible and linearly related to concentration over a range 0.004–0.4 mg/mL. At testing concentrations, buffer components do not interfere significantly with the UV absorption of NLX.

2.2.7. Data analysis

The flux values (Js) across the membranes and the permeability coefficients (K_p) were calculated at the steady state per unit area. Lag times were obtained by linear regression analysis of permeation data. In Tables 1 and 2 fluxes and lag times, obtained as average value of six replicated experiments, were reported with the standard deviations. All differences were statistically evaluated by the Student's *t*-test with the minimum levels of significance with $P \leqslant 0.05$.

All release data were elaborated according to Vergnaud [25], Korsmeyer et al. [26] and Peppas et al. [27] equations using Curve Expert program version 1.3. Linear or non-linear least squares fitting methods were used to determine the optimum values for the parameters present in each equation. Fittings were validated using χ^2 test and analysis of residuals.

2.2.8. Histological methods

Histomorphological analyses were performed to evaluate the pathological changes occurring in cell morphology and tissue organization. For analysis the epithelial tissues were fixed in 10% neutral-buffered formalin for 2 h, washed in water for 1 h, dehydrated in graded ethanol (60%, 80%, 90%, 95%, and 100%) and, after permeation in xylene, embedded in paraffin using the standard procedures. Formalin-fixed, paraffin-embedded samples were cut in 4-μm-thick sections on a microtome with a disposable blade and conventionally stained with hematoxylin-eosin.

Table 2
Lag time values calculated as the intercept with the time axis of the slope of the linear portion of permeation profiles shown in Fig. 2

Donor medium	Diffusion	Lag time (min)
Simulated saliva	Simple	107.26 ± 0.04
	Iontophoretic (1 mA/cm^2)	99.12 ± 0.03
	Iontophoretic (2 mA/cm^2)	81.36 ± 0.05
Natural human saliva	Simple	105.68 ± 0.03
	Iontophoretic (1 mA/cm^2)	89.84 ± 0.03
	Iontophoretic (2 mA/cm^2)	79.38 ± 0.06

Values are presented as means ± SD ($n = 6$).

Table 1
Calculated steady-state flux values, permeability coefficients and enhancement ratios, for NLX permeation through porcine buccal mucosa and RHO with and without application of electric fields

Donor medium	Diffusion	Porcine buccal mucosa			Reconstituted human oral epithelium		
		Js (mg cm^{-2} h^{-1})	ER	K_p (cm/h)	Js (mg cm^{-2} h^{-1})	ER	K_p (cm/h)
Simulated saliva	Simple	1.89 ± 0.03		0.063	1.19 ± 0.02		0.040
	Iontophoretic (1 mA/cm^2)	2.29 ± 0.05	1.2	0.076	1.81 ± 0.03	1.5	0.060
	Iontophoretic (2 mA/cm^2)	3.38 ± 0.05	1.8	0.113	3.74 ± 0.06	3.1	0.125
Natural human saliva	Simple	1.50 ± 0.02		0.050	1.13 ± 0.02		0.038
	Iontophoretic (1 mA/cm^2)	2.71 ± 0.04	1.8	0.090	3.21 ± 0.05	2.8	0.107
	Iontophoretic (2 mA/cm^2)	4.52 ± 0.07	3.0	0.151	5.60 ± 0.09	4.9	0.187

Values are presented as means ± SD ($n = 6$). Donor phase was buffer solution simulating saliva or natural human saliva, receptor phase was PBS simulating plasma.

Three different types of negative controls were included in the histomorphological analysis. Blank controls were considered those samples not subjected to the experimental phase; permeation controls were those samples subjected to the experimental phase in the absence of drug; iontophoresis controls were those subjected to iontophoresis in the absence of drug. Slides were evaluated at optical microscopy by two of the authors in a blind and independent fashion. Microphotographs of relevant fields were taken at 40× magnification. Digital images were captured using a Leica DC 300F camera (Leica, Nidau, Switzerland) mounted on a Leitz DMRB microscope with the Leica IM50 Image Manager program version 1.20.

3. Results and discussion

The most important limitation in the development of a buccal drug delivery device could be the low drug passage through the buccal mucosa, so we preliminarily had assessed the aptitude of NLX to penetrate the barrier using reconstituted human oral epithelium, as an *in vitro* model [18]. Nevertheless, at 12th day of culture, stratified cultured cells used to obtain the mucosal membrane has about 4–6 cell layers and the tissue is about 100 μm thick [19]. On the other hand, the epithelium of the human buccal mucosa has about 40–50 cell layers and thickness measures 500–800 μm (about 12.5–20.0 μm for each layer) [20]. In view of a possible experimental phase on animal models, our data on NLX permeation through the cultured tissue needed further validation using a more thick tissue. Given that porcine buccal mucosa is one of the most useful models to simulate human buccal epithelium [14], we measured drug fluxes and permeability coefficients throughout domestic pig mucosa 800 μm thick. In order to avoid possible uncertainties attributable to the saliva composition, comparative experiments were carried out using both buffer solution simulating saliva and natural human saliva. A limitation, however, in the present work could be the use of saliva collected from a single person.

The permeation kinetic throughout the barrier was evaluated *in vitro* using the culture inserts as Transwell diffusion cell system [18] and in *ex vivo* using Franz type diffusion cells which could represent two compartment open models.

In Fig. 1 there is shown drug movement expressed as cumulative amount of permeated NLX from the mucosal to the serosal side of porcine tissue versus time. Fluxes (Js) were obtained at the steady state per unit area from the slope of the linear portion of the plot. Flux values obtained for porcine buccal mucosa were in agreement with those observed for reconstituted human oral epithelium using either buffer solution simulating saliva or natural human saliva (Table 1). The permeability coefficients were processed by dividing the flux values by the drug concentration in the donor compartment (Table 1).

A major limitation in the development of a buccal device could be the low permeability of the buccal mucosa. This might be altered by the use of penetration enhancers even if not much is known about the mechanism involved in buccal penetration enhancement [28]. In this study, the enhancing effects of co-administration of chemical penetration enhancers from different classes on buccal penetration of NLX were investigated. We chose sodium dehydrocholate (NaDHC), EDTA disodium salt (NaEDTA) and trisodium citrate dihydrate (TNaC) as NLX penetration promoters. In both buffer solution simulating saliva and natural human saliva no significant differences were observed in the permeation rate through the porcine mucosa. This result was probably due to the physico-chemical properties of naltrexone hydrochloride. In fact, at pH = 6.8 the ionized form of NLX prevails and the paracellular penetration route could be preferred. Since the enhancing effect is dependent on the concentration of enhancer especially for bile salts, three different concentrations were used for each promoter. At the used concentrations, the chosen enhancers of the three classes gave the same results; on the other hand, the increase of

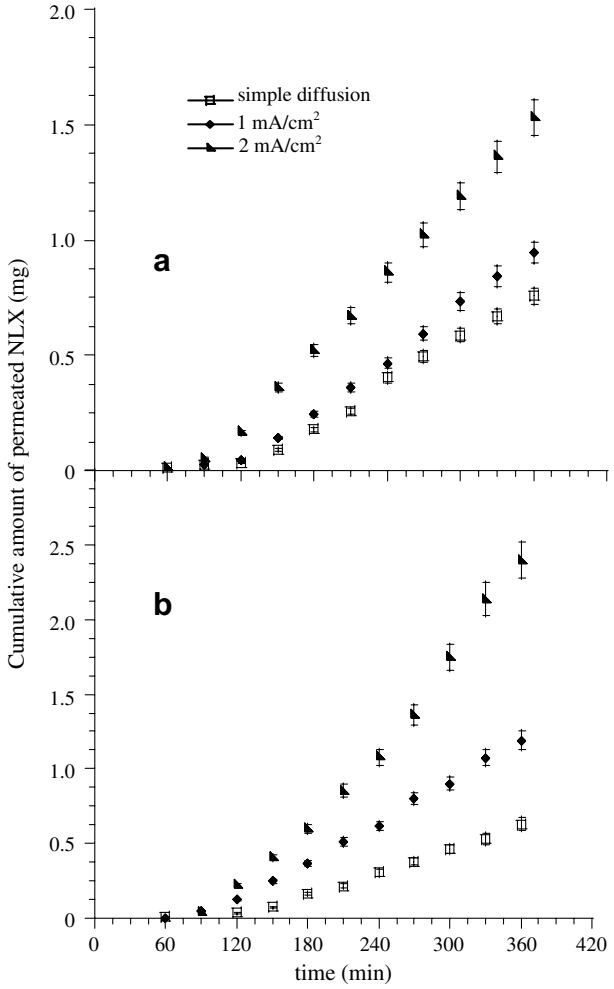

Fig. 1. Plot of cumulative amount of NLX permeated across porcine buccal mucosa on simple diffusion and on iontophoretic diffusion versus time, using: buffer solution simulating saliva (a) or natural human saliva (b) as donor medium and PBS simulating plasma as receptor phase. Values are presented as means ± SD ($n = 6$).

concentration of the penetration promoter could cause damage on buccal tissue [28].

We have previously demonstrated that the physicochemical properties of NLX allow permeation through reconstituted human oral epithelium in presence of electric field. In particular, the application of a current density of 1 mA/cm^2 or more determines a good improvement [18]. Also through porcine buccal mucosa the iontophoretic enhancement is proportional to the intensity of the electric field as shown in Fig. 1. As iontophoresis enhances only the aqueous pathway, we attributed the improvement of permeability to the increase of NLX movements in the membrane aqueous domain.

The ratio of the permeability coefficient of NLX under iontophoretic conditions to the values under passive diffusion is the enhancement ratio (ER) and it is listed in Table 1. Worthy of note, in presence of electric fields the flux in natural human saliva is greater than that observed in buffer solution simulating saliva using both cultured tissue and porcine buccal mucosa. We attributed this result to the composition of natural saliva which in addition to inorganic salts contains also several protein components that might alter the movement of solutes in electric field.

In all experiments, in the first period of drug permeation was observed a lag time which decreased when the current density was raised (Fig. 2). The lag time values, calculated as the intercept with the time axis of the slope of the linear portion of the permeated NLX/time plot, are reported in Table 2. No great differences were observed using buffer solution simulating saliva or natural human saliva. Flux and lag time values were significantly affected by application of electric fields (t-test).

To verify accumulation of NLX into the membrane, at the end-time of all experiments mucosal portions were treated with a $FeCl_3$ solution to underscore the drug eventually entrapped into the tissue. A green-blue colour developed as a consequence of complexation of the phenolic functional group of NLX with $FeCl_3$, thus indicating that tissue contained effective drug concentration. The colour intensity in the tissue became deeper when iontophoresis was applied and was improved by the current density increase.

The drug itself and the application of current on buccal mucosa could cause some changes in its structure, so we investigated the potential histomorphological changes and tissue organization. As previously observed using reconstituted human oral epithelium, also in the present study no significant cytological or architectural changes were highlighted [18]. In Fig. 3a it is shown a microphotograph of untreated sample which reveals the appearance of the superficial cells of the epithelium. Samples submitted only to NLX simple diffusion (Fig. 3b) showed a uniform cellular swelling in the absence of other significant changes in cell morphology or tissue structure. No sign of flogosis was found in any of mucosal specimens treated. In detail, cells appeared vacuolated due to the presence of intracytoplasmic material, which is likely ascribable to NLX accumulated, as confirmed by the $FeCl_3$ reaction.

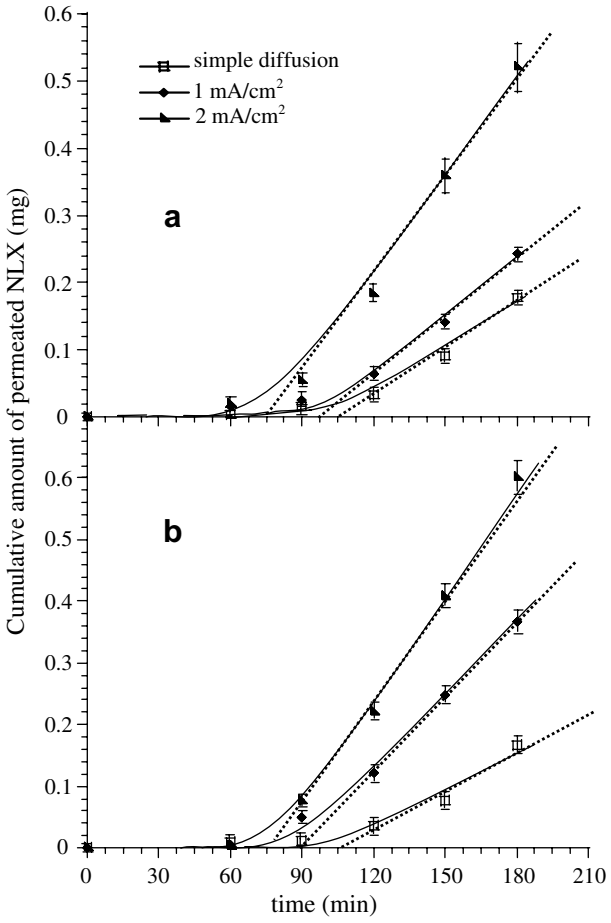

Fig. 2. Estimated lag times by the plot of cumulative amount of NLX permeated across porcine buccal mucosa on simple and iontophoretic diffusion versus time, using: buffer solution simulating saliva (a) or natural human saliva (b) as donor medium and PBS simulating plasma as receptor phase. Values are presented as means ± SD ($n = 6$).

However, the effects observed in samples treated with NLX alone were modest and should not constitute a key obstacle to the drug administration on buccal mucosa.

The application of iontophoresis was found to cause cytoarchitectural changes consisting in cellular disarray. No severe cytopathic effects were found in any mucosal specimen treated with the lowest applied current density. Increasing the current density, superficial cellular disarray, we defined "wave effect", was observed (Fig. 3c). Cytopathic effects represented by nuclear pycnosis, diffuse signs of abrupt keratinisation and loss of cellular alignment were considered in association with an increase in current density over 2 mA/cm^2.

In the present study, we report also the development of tablets suitable for the administration of NLX on buccal mucosa. The desired tablets were prepared by direct compression of drug loaded matrices. Firstly, as matrix components we considered different polymers generally used as photosensitive (light-cured) bonding agents or adhesive agents for restoration techniques in dentistry (mixtures of Bisphenol A diglycidyl ether dimethacrylate and triethylene glycol dimethacrylate; mixtures of Bisphenol A polyathyl-

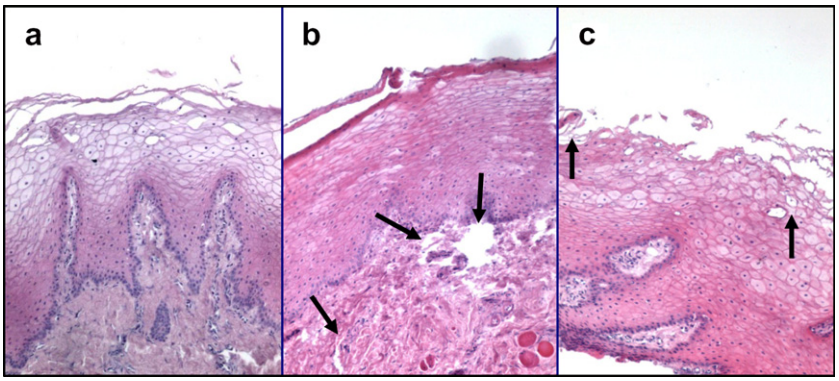

Fig. 3. Microphotographs of formalin-fixed, paraffin embedded cross-sections of porcine buccal mucosa: (a) control untreated, (b) sample subjected to simple diffusion of NLX in natural human saliva; vacuolization and NLX accumulation are pointed by black arrows, (c) sample subjected to enhanced permeation of NLX in natural human saliva on application of 2 mA/cm^2 current density; superficial cellular disarray caused by electric field is pointed by black arrows. (Magnification 40×).

ene glycol diether dimethacrylate and urethanedimethacrylate; mixtures of methacrylates and urethanedimethacrylate; Eudragit® RL100 and/or Eudragit® RS100). Unfortunately, NLX forms polymeric complexes with the well established Eudragit [29]. On the other hand, the bonding and adhesive agents generally used in dentistry contain large amounts of inert materials and low drug quantities may be charged in the final matrix.

The best results were obtained using cyanoacrylates. These types of polymers may be very simplistically defined as solvent free, synthetic adhesives. They are reactive monomer liquids that polymerize when initiated by moisture, forming high quality and very tenacious polymers. Cyanoacrylates typically polymerize in a few minutes and achieve full bond strength in 24 h [30]. Nevertheless, short-chained cyanoacrylates may cause histologic toxicity related to the by-products of degradation. The local concentrations of these breakdown products are proportional to the rate of degradation.

The longer-chain compounds degrade much more slowly than the shorter-chain compounds. As 2-octylcyanoacrylate (2-OCA) is an 8-carbon alkyl derivative, it should be less reactive than the shorter-chain derivatives, and was chosen to avoid by-products in surrounding tissues, resulting in less inflammation [31].

In the present study, loaded poly-OCA matrices containing 56% (w/w) of NLX were successfully prepared, powdered and compressed to obtain the desired tablets suitable for buccal administration.

Drug release tests are currently carried out according to the official pharmacopoeias. They require a large volume of dissolution medium and are operated under sink or pseudo-sink conditions. However, the initial fast release of some buccal dosage forms cannot be measured with the existing methods. These methods do not simulate the conditions prevailing for buccal administration where low environmental liquid exists and a non-sink condition is more appropriate for poorly permeable drugs. Hence, *in vitro* dissolution tests for buccal delivery systems should be performed in small volumes of dissolution medium. In this study, the NLX release from the tablets was evaluated using the apparatus previously described [24] which complies with the saliva turnover in the buccal environment. Experimental results, reported in Fig. 4a, showed that about 20% (22.4 mg) of the loaded NLX was released in 5 h. The fraction of drug released from the formulated tablets was calculated and the most common models used in dissolution analysis were curve fitted to our experimental data. The best fit was obtained using the equation $M_t/M_\infty = kt^n$ (where M_t/M_∞ is the fraction of drug released at time t and k is the kinetic constant) setting $n = 0.5$ (Higuchian behaviour) [32]. This kinetic behaviour suggested that diffusion through the inert polymer matrix was the primary mechanism of dissolution: a linear trend was observed (correlation coefficient 0.9993; standard error 0.0020) plotting M_t/M_∞ against $t^{1/2}$ (Fig. 4b).

The formulation of the matrix tablet was aimed to continuously release the drug in adequate amount to replace exactly the same eliminated by the body. Constant blood levels can be achieved when the rate of drug entry in the systemic circulation is equal to the rate of drug disappearance from the blood. As the drug declining is a first order process, it is equal to the product of the steady state concentration (maintenance dose) with the first order rate constant of elimination (K_e) which can be calculated by the biological half-life of the drug. In other words:

$$\text{Drug input rate} = \text{Drug output rate} = \text{Steady state concentration} \times K_e$$

where $K_e = 0.693/t_{1/2}$.

Taking into account the pharmacokinetic parameters of NLX ($t_{1/2}$ about 4 h, clearance 3.4 L min^{-1}, volume of distribution about 1200 L and the steady state levels at least 1 ng mL^{-1}) [4,33–35] we predicted that at least 0.17 ng mL^{-1} h^{-1} of NLX should permeate throughout the buccal mucosa to maintain the steady state levels. As the steady state flux was experienced as 1.89 mg cm^{-2} h^{-1} and considering the distribution volume, 1 cm^2 of mucosal tissue is able to absorb 1.5 ng mL^{-1} h^{-1}. This means that

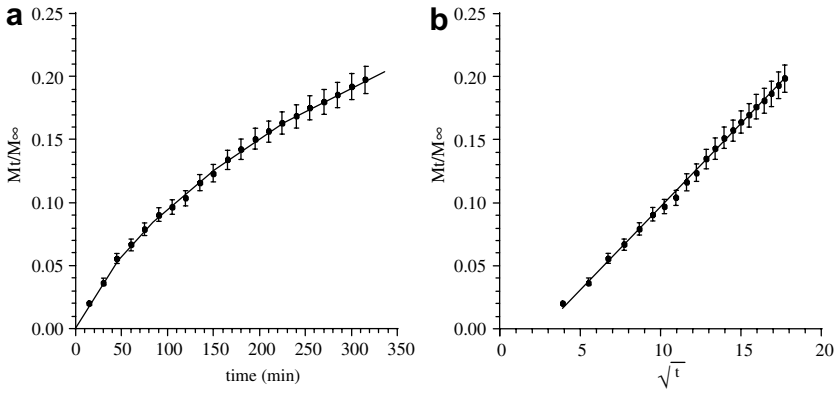

Fig. 4. *In vitro* profile of drug release from tablets prepared with poly-OCA matrices loaded with NLX. (a) Data are reported as dose fraction discharged against time. (b) Data are reported as dose fraction discharged against the root square of time. Values are presented as means ± SD ($n = 6$).

the minimum mucosal absorptive area should be about 0.2 cm^2. Since the matrix tablet we used had reached active surface of 1.33 cm^2 and the average drug amount released was 4.5 mg h^{-1}, the NLX release rate appeared adequate to achieve and maintain therapeutic blood levels. This prediction takes into account drug losses due to facial movements, dilution, swallowing, intraindividual variations or other similar factors might occur as well.

As the application of formulation components on buccal mucosa could cause some consequences on its structure, we performed histological analyses after application of dispersions of empty poly-OCA matrices upon the tissue. As above, lack of significant changes in cell morphology or tissue structure was observed and no sign of flogosis or vacuolization was found in any of mucosal specimens, either in simulated or in natural human saliva. Poly-OCA should not constitute a key obstacle to the drug administration on buccal mucosa.

The data suggested that buccal mucosa does not block diffusion of NLX. The drug passively crosses the membrane whereas the application of electric fields promotes drug diffusion. Both fluxes and permeability coefficients, in buffer solution simulating saliva or in natural human saliva, increase significantly when the applied current density grows.

On the basis of these considerations we could suppose that buccal mucosa could represent an alternative way of administration for NLX. We can propose that the drug loaded poly-OCA matrix tablets could be allocated in the vestibular area of retromolar trigone in intimate contact with the mucosa. An impermeable and adhesive material could be applied as backing external layer to hinder drug losses into mouth and provide unidirectional release and penetration only *via* the buccal mucosa. Anyhow, these assumptions should be verified on animal models.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejpb.2007.02.020.

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