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International Journal of Pharmaceutics 311 (2006) 196–202
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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# In vivo human buccal permeability of nicotine

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Received 24 November 2005; accepted 19 December 2005
Available online 7 February 2006

**Abstract**

The aim was to examine the in vivo buccal pH-dependent permeability of nicotine in humans and furthermore compare the in vivo permeability of nicotine to previous in vitro permeability data. The buccal permeability of nicotine was examined in a three-way cross-over study in eight healthy non-smokers using a buccal perfusion cell. The disappearance of nicotine from perfusion solutions with pH 6.0, 7.4, and 8.1 was studied for 3 h. The apparent permeability of nicotine ($P_{app}$) was determined at each pH value. Parotid saliva was collected in an attempt to assess systemic levels of nicotine. The disappearance rate of nicotine increased significantly as the pH increased, which resulted in $P_{app}$ values of $0.57 \pm 0.55 \times 10^{-4}$, $2.10 \pm 0.23 \times 10^{-4}$, and $3.96 \pm 0.54 \times 10^{-4}$ cm s$^{-1}$ (mean ± S.D.) at pH 6.0, 7.4, and 8.1, respectively. A linear relationship ($R^2 = 0.993$) was obtained between the $P_{app}$ values and non-ionised nicotine, which indicates that the nicotine transfer occurred by means of passive diffusion. $P_{app}$ values of $0.60 \times 10^{-4}$ and $6.18 \times 10^{-4}$ cm s$^{-1}$ were obtained for the mono-protonated and non-ionised species of nicotine, respectively. The analysis of the parotid saliva samples indicated that these samples might be useful in the assessment of systemic absorption of nicotine. Previous buccal in vitro models underestimated the in vivo human permeability of nicotine. However, the in vitro models were capable of predicting the effect of pH on the nicotine permeability.


*Keywords:* Human buccal permeability; Perfusion cell; Nicotine; pH; In vivo; Saliva

## 1. Introduction

The buccal route is an attractive route of administration for drugs since the drug can reach the systemic circulation without the risk of being degraded by gastrointestinal and hepatic first-pass metabolism (Rathbone and Hadgraft, 1991). The rate-limiting step in the buccal drug absorption process is often the permeability across the buccal epithelium. Therefore, permeability studies of various drugs have been performed using buccal in vitro models (Nielsen and Rassing, 2000). However, comparisons of in vitro and in vivo human buccal permeability are sparse mainly due to the lack of human in vivo data.

Perfusion cells applied on the buccal mucosa allow for in vivo studies of buccal drug permeability. A drug solution is perfused through the cell, and the drug permeability is calculated from the amount of drug disappeared from the solution over a certain period of time. A major disadvantage of using this method is that the disappearance of drug from the perfusion solution is not necessarily equal to the drug appearance in the systemic circulation (Rathbone and Hadgraft, 1991). To verify whether the disappeared drug is absorbed to the systemic circulation, the drug absorption should be determined on the basis of both the disappearance of drug from the perfusion solution as well as the appearance in the systemic circulation. In previous studies with human perfusion cells, the oromucosal absorption of different drugs was estimated solely on the basis of the disappearance (Barsuhn et al., 1988; Rathbone, 1991a,b; Kurosaki et al., 1997, 1998).

A pilot study of in vivo human buccal permeability of nicotine revealed the difficulty of determining the systemic levels of nicotine during a buccal perfusion study (unpublished data). The amount of drug absorbed during a perfusion study is usually low, making it difficult to detect the drug in the systemic circulation. In the pilot study, approximately 5 mg nicotine disappeared from the perfusion solution during the 3 h of perfusion. However, the concentration of nicotine in plasma was below the quantitation

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doi:10.1016/j.ijpharm.2005.12.039

limit of 1 ng ml$^{-1}$. The levels of nicotine in whole saliva have been reported to be approximately 8 times higher than those in plasma (Rose et al., 1993). This makes it possible to detect small amounts of absorbed nicotine, which would otherwise be undetectable. A disadvantage of collecting whole saliva during a buccal perfusion study is that these samples could contain leaked nicotine from the perfusion cell. To circumvent this uncertainty, saliva samples can be collected directly from the parotid gland with a modified Carlson–Crittenden cup (Bardow et al., 2000). The opening of the duct of the parotid gland is placed in the buccal mucosa above the upper teeth (Kidwell et al., 1998). In the present study, parotid saliva samples were collected to examine the utility of this sampling procedure to assess systemic levels of nicotine during a buccal perfusion study.

Nicotine is a base with p$K_a$ values of 3.26 and 7.90 at 37 °C (Nielsen and Rassing, 2002), and different fractions of ionised and non-ionised nicotine are hence present at physiological pH values. The buccal permeability of nicotine has been examined in various in vitro studies and has shown to increase as pH and the amount of non-ionised nicotine increases (Squier, 1986). The comparability of the results from those in vitro studies with the results from the present study will be examined.

Thus, the aim of the present study was to examine the in vivo buccal pH-dependent permeability of nicotine in humans using perfusion cells and, furthermore, to compare the in vivo permeability of nicotine to previous in vitro permeability data.

## 2. Materials and methods

### 2.1. Materials

Nicotine (USP/Ph.Eur.) from Siegfried Ltd., Zofingen, Switzerland. The perfusion solutions contained potassium dihydrogen phosphate (Ph.Eur.), disodium hydrogen phosphate (Ph.Eur.), dipotassium hydrogen phosphate (Ph.Eur.) and sodium chloride (Ph.Eur.) from Unikem A/S, Copenhagen, Denmark. The mobile phase for the RP-HPLC analysis consisted of acetonitrile, triethylamine and sodium dihydrogen phosphate, all of analytical grade from Merck, Darmstadt, Germany.

### 2.2. Methods

#### 2.2.1. Study design

The study was a three-way cross-over study including eight healthy non-smokers (four female and four male) with an average age of 25 years ± 4 years (mean ± S.D.). The buccal permeability of nicotine from perfusion solutions with pH 6.0, 7.4, and 8.1 was examined using either the right or the left inner side of the cheek. There was a minimum of 1 day between the experiments, and when the same cheek was used there was a wash-out period of 2 weeks between the experiments. The study protocol was approved by The Regional Ethics Committee (KF 02-043/99) and The Danish Medicines Agency (2612-961). Written consent was obtained from the subjects before initiating the study.

#### 2.2.2. In vivo human buccal perfusion

The perfusion set-up was a modified version of a previously described set-up (Barsuhn et al., 1988). The perfusion cell was designed and created at the Danish University of Pharmaceutical Sciences, Department of Pharmaceutics. The cell was made of Teflon® with an internal volume of 1.0 ml and a perfusion area of 2.0 cm$^2$ (Fig. 1a). A mesh (squares: 0.42 mm × 0.42 mm) prevented the cheek from being sucked into the cell. Silicone tubing with an internal diameter of 1 mm was used to combine the cell to the pump (Minipuls 3, Gilson, Villiers Le Bel, France) and the perfusion solution.

The study was initiated by perfusing the cell with the buffer to be examined, i.e. pH 6.0, 7.4 or 8.1. Initially, the subject kept the cell in place onto the buccal mucosa with the fingers, and as the perfusion elapsed, the natural suction pressure of the circuit caused the cell to be attached to the mucosa. Perfusion of the nicotine solution was then initiated and continued for 3 h. The perfusion solution (10.0 ml) was maintained at 37 °C and recirculated at 10 ml min$^{-1}$. Samples (50 μl) were taken from the recirculating perfusion solution at 0, 15, 30, 45, 60, 90, 120, 150, and 180 min. The samples were collected in polyethylene tubes and stored at −20 °C until RP-HPLC analysis. The well-being of the subjects was monitored during the study, and the blood pressure and pulse were measured with an automatic digital blood pressure monitor (model UA-767, A&D Instruments Ltd., Oxfordshire, United Kingdom) to verify if the absorbed nicotine affected the cardiovascular system. The buccal mucosa was visually inspected before and after the study.

During the perfusion period, the saliva was collected directly from the parotid gland. The collection was performed with a modified Carlson–Crittenden cup made out of Plexiglas® (Fig. 1b). The cup was placed over the opening of the parotid duct on the cheek opposite to the perfusion cell. The cup was connected to a vacuum pump (Heto Master Jet, Heto Holten Allerød, Denmark) by means of polyethylene tubing and held in place by applying suction to its outer compartment. The saliva was collected from the inner compartment of the cup into a syringe via a 5 cm long polyethylene tube. The saliva was drawn into the syringe by gentle suction applied through the plunger and was collected at three intervals: 0–1, 1–2, and 2–3 h. A sample of whole saliva was collected just before initiating the perfusion study. The saliva samples were collected in polyethylene tubes and stored at −20 °C until the LC/MS/MS analysis.

The volume of the perfusion solution was monitored during the perfusion period to verify if any solution leaked from the perfusion cell. The nicotine content in samples of whole saliva taken during the perfusion period could not be used to verify if any nicotine solution leaked to the oral cavity. Nicotine was assumed to be present in whole saliva during the perfusion period since nicotine could reach the oral cavity via the parotid saliva excreted from the “uncupped” parotic duct on the cheek with the perfusion cell. Therefore, an additional check of leak from the perfusion cell during the study was whether the taste in the mouth changed during the perfusion period. The taste of the examined nicotine solutions was extremely bitter and it was therefore expected that the subjects would immediately taste any leaked nicotine solution.

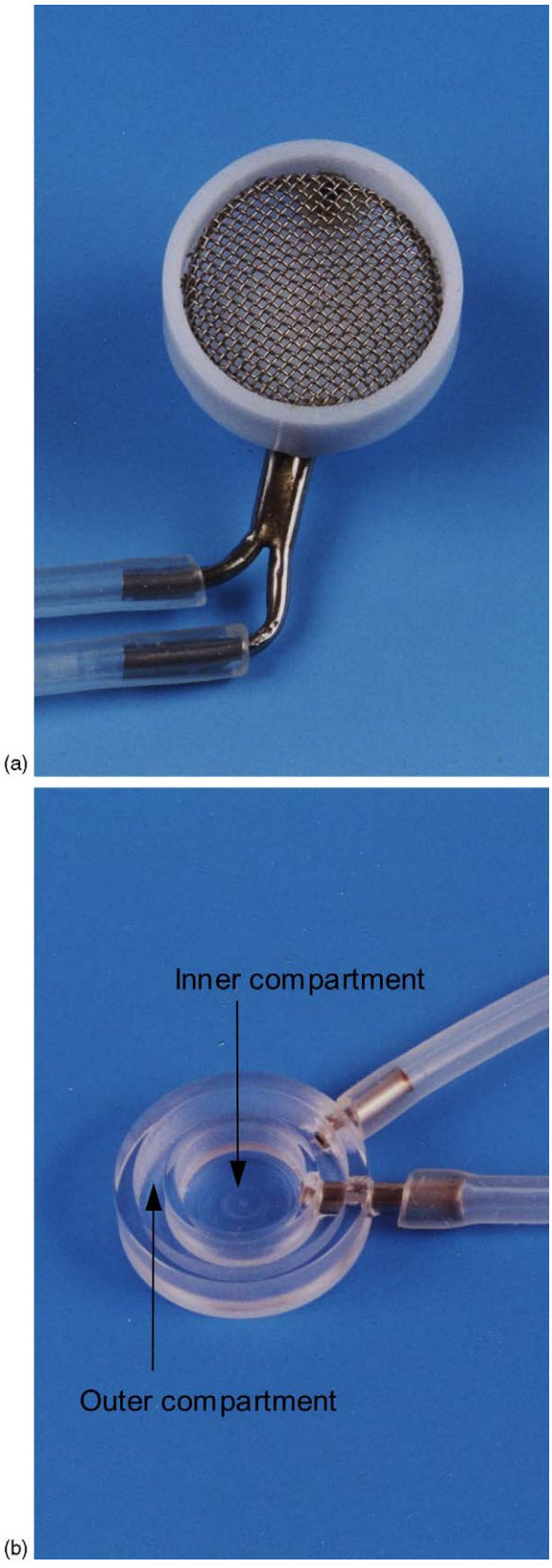


Fig. 1. (a) The buccal perfusion cell. (b) The modified Carlson–Crittenden cup used for collecting parotid saliva.

*2.2.3. Perfusion solutions*

The perfusion solutions consisted of 1.2 mg ml$^{-1}$ nicotine in 0.1 M phosphate buffer at a pH of 6.0, 7.4, and 8.1. Where otherwise not specified, the term "nicotine" in this case refers to the total amount of nicotine, i.e. the sum of ionised and non-ionised species. The tonicity of the perfusion solutions was adjusted with sodium chloride to approximately 300 mosmol kg$^{-1}$. The osmolarity of the solutions was measured with an Osmomat 030-D (Gonotec GmbH, Berlin, Germany). The solutions were made at the H:S Hospital Pharmacy, Copenhagen Hospital Corporation. Prior to this study, it was shown that no detectable amount of nicotine adsorbed to the perfusion circuit materials or the perfusion cell (data not shown).

*2.2.4. Analysis of nicotine in the perfusion solutions*

RP-HPLC UV-analysis of nicotine was performed with a Merck Hitachi system (Merck, Darmstadt, Germany), as previously described (Nielsen and Rassing, 2002). Briefly, the mobile phase consisted of 12% acetonitrile (v/v) in 0.01 M phosphate buffer with 0.005 M triethylamine, the pH was adjusted to 6.7. The flow rate was 1 ml min$^{-1}$ and a wavelength of 261 nm was used. The limit of quantification was $1 \times 10^{-4}$ mg ml$^{-1}$, the accuracy >99% and the repeatability expressed as the relative standard deviation was 0.6% ($n = 6$). The applied concentration range was $1 \times 10^{-1}$ to $1 \times 10^{-2}$ mg ml$^{-1}$.

*2.2.5. Analysis of nicotine in saliva*

The quantitative analysis of nicotine in saliva was performed at the Institut für Klinische Pharmakologie Bobenheim, Grünstadt, Germany. The method was LC/MS/MS (triple quadrupole mass spectrometry) with electrospray ionisation (ESI) with a limit of quantification of 5 ng ml$^{-1}$ nicotine.

*2.2.6. Data analysis*

The buccal permeability of nicotine was determined on the basis of the remaining concentration of nicotine in the perfusion solution versus time. The data was fitted to first order kinetics, i.e. the natural logarithm of the concentration of nicotine was plotted against time for each subject. The slope obtained with linear regression was the first order rate constant $k$ expressed as the mean ± S.D.

The apparent permeability coefficient ($P_{app}$, cm s$^{-1}$) was calculated using Eq. (1)

$$P_{app} = \frac{k}{A/V} \quad (1)$$

where $k$ is the first order rate constant (s$^{-1}$), $A$ is the perfusion area (cm$^2$), and $V$ is the volume of the recirculating perfusion solution (cm$^3$).

Statistical differences between the $k$ values were evaluated by ANOVA and the Tukey–Kramer multiple comparison test and considered significant at $P < 0.05$. The correlation coefficient ($R^2$) from linear regression was used to test the relationship between $P_{app}$ and the amount of non-ionised nicotine. The percentage of non-ionised nicotine at each pH value was calculated by means of the Henderson–Hasselbach equation using p$K_a$ values obtained at conditions similar to the ones in the present study.

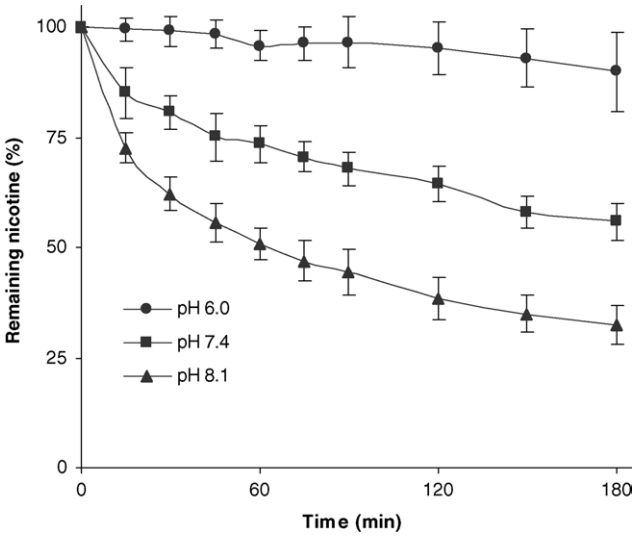


Fig. 2. Time course of percentage nicotine remaining in the perfusion solution at pH 6.0, 7.4, and 8.1. Values are mean ± S.D.

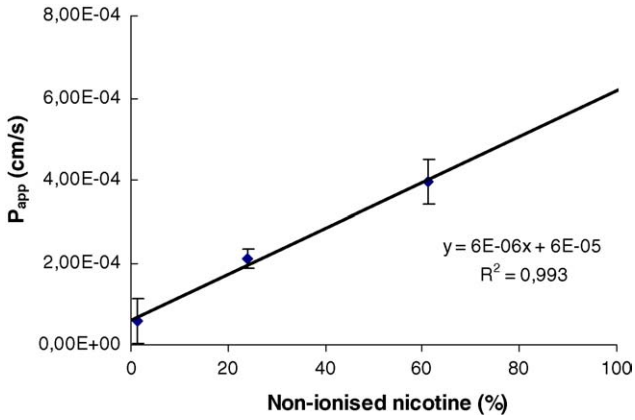


Fig. 3. The apparent permeability ($P_{app,}$ mean ± S.D.) of nicotine as a function of percentage non-ionised nicotine. The solid line represents the linear least square regression line, $R^2 = 0.993$.

## 3. Results

The remaining nicotine in the perfusion solution versus time is presented in Fig. 2. An increase in pH of 1.4 and 2.1 units caused an increase of approximately four and six times, respectively, in the amount of disappeared nicotine during 180 min of perfusion. The percentage of remaining nicotine at 180 min was 90.0 ± 9.2% at pH 6.0, 56.1 ± 4.2% at pH 7.4, and 32.5 ± 4.4% at pH 8.1 (mean ± S.D.). These values correspond to 1.2, 5.3, and 7.5 mg disappeared nicotine. The amount of disappeared nicotine from the three different perfusion solutions varied significantly at all time points ($P < 0.001$).

During the study, no signs of any leak were observed. These findings are consistent with the small standard deviations shown in Fig. 2. The deviations ranged from 2.6 to 9.2%, being greatest when pH 6.0 was examined. Of the 24 experiments, one experiment was discarded because the perfusion cell fell off after one hour of perfusion. The subjects did not feel any discomfort during the study and no increase in their blood pressure and pulse was measured during the study (data not shown). An impression from the perfusion cell was observed on the buccal mucosa after removal of the cell.

The first order rate constant, $k$, for the disappearance of nicotine was calculated for each pH value, (Table 1). At pH 6.0, the S.D. was in the same range as the mean $k$ value, whereas the S.D. was 11 and 14% at pH 7.4 and 8.1, respectively. The $k$ values and hence the $P_{app}$ values were significantly different ($P < 0.001$). Table 1 also lists the relative amount of non-ionised nicotine calculated at each pH value. It is seen that the values for $k$ and $P_{app}$ increased with increasing amounts of non-ionised nicotine. Fig. 3 shows a plot of the $P_{app}$ values versus the percentage of non-ionised nicotine, indicating a linear relationship ($R^2 = 0.993$).

Collecting parotid saliva was only a successful procedure for three to four subjects in each of the experiments with the same pH in the perfusion solution. The concentration of nicotine in saliva increased as the pH increased (Table 2). At pH 8.1, the nicotine concentrations in saliva were significantly higher than the concentrations at pH 6.0 and 7.4, whereas the concentrations at pH 7.4 and 6.0 were not significantly different. The S.D. was of the same size as the mean of the nicotine concentration at almost all time points. On average, 0.5 ± 0.2 ml parotid saliva was collected at each time interval which corresponds to a parotid flow rate of approximately 0.01 ml $min^{-1}$ per gland. Although the volumes of parotid saliva collected at each time point varied from 0.3 to 1.3 ml, the calculated amounts of nicotine in the parotid saliva samples showed the same relative changes over time as the nicotine concentrations (data not shown).

Table 1
The rate constant ($k$) and the apparent permeability ($P_{app}$) obtained at the tested pH values

| pH | $n$ | $k$ ($s^{-1}$) | $P_{app}$ ($cm\,s^{-1}$) | Percentage of non-ionised nicotine |
|---|---|---|---|---|
| 6.0 | 8 | $1.14 \pm 1.09 \times 10^{-5}$ | $0.57 \pm 0.55 \times 10^{-4}$ | 1.2 |
| 7.4 | 7 | $4.19 \pm 0.47 \times 10^{-5}$ | $2.10 \pm 0.23 \times 10^{-4}$ | 24.0 |
| 8.1 | 8 | $7.92 \pm 1.08 \times 10^{-5}$ | $3.96 \pm 0.54 \times 10^{-4}$ | 61.3 |

Values are mean ± S.D. The calculated percentages of non-ionised nicotine at the each pH value are also shown. "$n$" signifies the number of replicates.

Table 2
The concentration of nicotine in whole and parotid saliva collected during the perfusion studies

| Time (h) | Concentration of nicotine in saliva (ng $ml^{-1}$) | | | |
|---|---|---|---|---|
| | Saliva | pH 6.0 ($n = 3$) | pH 7.4 ($n = 3$) | pH 8.1 ($n = 4$) |
| 0 | Whole | 31.75 ± 33.06 | 7.44 ± 8.60 | n.a.[a] |
| 0–1 | Parotid | 17.44 ± 22.85 | 45.57 ± 54.62 | 5982.83 ± 6092.08 |
| 1–2 | Parotid | 16.60 ± 2.21 | 80.54 ± 92.60 | 5662.02 ± 4898.60 |
| 2–3 | Parotid | 14.94 ± 8.52 | 96.00 ± 80.35 | 2974.77 ± 1648.61 |

The saliva was collected at the time intervals 0–1, 1–2, and 2–3 h. Values are mean ± S.D.

[a] Below limit of quantitation.

## 4. Discussion

The designed perfusion cell was applicable for studying human buccal permeability of nicotine for 180 min. Disappearance profiles with small intersubject variation were obtained, and during the study, no leakage from the cell was observed. Apart from one experiment, the cell adhered to the epithelium during the entire perfusion period. Thus, the applied perfusion cell performed in a similar manner as the ones previously described (Rathbone, 1991a; Kurosaki et al., 1997).

The disappearance rate of nicotine from the perfusion solutions could be described by first order kinetics. This indicates that tissue accumulation of nicotine does not affect its systemic absorption. Consequently, the permeability across the buccal epithelium is considered to be the rate-limiting step in the absorption process. The first order rate constant for the disappearance increased significantly with increasing pH in the perfusion solution; thus, the nicotine permeability was highly influenced by the pH. An aqueous boundary layer is believed to be present on the surface of the buccal membrane (Ho, 1993). In the present study, it was assumed that the aqueous boundary layer did not affect the apparent permeability of nicotine, since nicotine has high water solubility.

Different fractions of ionised and non-ionised species of nicotine were present at the examined pH values since the nicotine base has p$K_a$ values of 3.26 and 7.90 at 37 °C (Nielsen and Rassing, 2002). The obtained $P_{app}$ values therefore reflected the permeability of both the ionised and non-ionised species of nicotine. The fraction of di-protonated nicotine at the tested pH values was insignificant ranging from 0.001 to 0.2%. Thus, the $P_{app}$ values only reflected the permeability of the mono-protonated species and the non-ionised species. A plot of $P_{app}$ versus percentage of non-ionised nicotine demonstrated a linear relationship (Fig. 3). This linear relationship indicates that the permeability of nicotine across the buccal mucosa occurred by means of passive diffusion at the tested species concentrations. This result is in accordance with previous findings. Rathbone et al. (1996) have shown that the pH partition hypothesis and Fick's first law of diffusion is applicable to buccal perfusion studies when the drug disappearance data can be described by first order kinetics. Furthermore, Chen et al. (1999) examined nicotine permeability in vitro using porcine buccal mucosa and also concluded that the nicotine transfer occurred by means of passive diffusion.

The intercept of the line in Fig. 3 corresponds to $P_{app}$ of mono-protonated nicotine. When the line is extrapolated to 100% $P_{app}$ of non-ionised nicotine is obtained. This results in $P_{app}$ values of $0.60 \times 10^{-4}$ cm s$^{-1}$ for the mono-protonated species and $6.18 \times 10^{-4}$ cm s$^{-1}$ for the non-ionised species. The permeability of non-ionised nicotine was hence approximately 10 times higher than mono-protonated nicotine permeability. It has also previously been reported that the non-ionised species of nicotine had the highest permeability compared to the ionised species (Nair et al., 1997; Chen et al., 1999). This finding shows the great importance of controlling pH when formulating a buccal drug delivery system with an ionisable compound. In the case of nicotine, the pH in the delivery system or the oral environment should be as high as possible since the higher the pH, the more non-ionised nicotine will be available for systemic absorption. Optimising the pH therefore allows for a reduction of the loaded dose in the delivery system.

Nielsen and Rassing (2002) examined the permeability of nicotine across two in vitro models of the human buccal epithelium. $P_{app}$ values of nicotine were obtained at pH 5.5, 7.4, and 8.1 using the TR146 cell culture model and a porcine diffusion chamber model. The $P_{app}$ values from the in vitro studies were notably lower than the $P_{app}$ values obtained in the present study. On average, the TR146 model showed a tendency to underestimate the $P_{app}$ values from the present study approximately nine times whereas the in vitro porcine model underestimated the present $P_{app}$ values on an average of 17,000 times. Thus, the experiments with the TR146 model resulted in $P_{app}$ values closest to the values obtained in the present study. The relative increase in $P_{app}$ with increasing pH was the same for both in vitro models and the present in vivo model, which indicates a strong relationship between the $P_{app}$ values. Overall, the in vitro models were able to predict the effect of pH on the nicotine buccal permeability but not able to predict the actual in vivo human buccal permeability of nicotine. $P_{app}$ values of nicotine and mono-protonated and non-ionised nicotine across in vitro porcine buccal mucosa have previously been reported (Squier, 1986; Nair et al., 1997; Chen et al., 1999). The $P_{app}$ values from these studies also underestimated the $P_{app}$ values obtained in the present study.

As the $P_{app}$ values from the present study were higher than the reported in vitro values, it is possible that the $P_{app}$ values from the present study overestimate the permeability of nicotine. It is possible that the buccal mucosa was "stressed" when the perfusion cell was sucked onto the mucosa, which could in turn have resulted in an increased blood flow in the submucosa. The increased blood flow could then have initiated a more rapid depletion of a drug from the "stressed" mucosa as compared to the normal buccal mucosa. Consequently, this could have resulted in a more rapid transport of drug across the "stressed" mucosa.

In the present study, there were no signs of damage to the buccal mucosa caused by the perfusion cell or the perfusion solution. It would have been expected that the $P_{app}$ values had been almost the same at the tested pH values if the mucosa was damaged. Consequently, a linear relationship between $P_{app}$ and the percentage of non-ionised nicotine would not have been obtained. Furthermore, greater intersubject variations than the ones obtained would have been expected if the mucosa was damaged. It could be speculated whether the human buccal epithelium is more permeable than in vitro porcine buccal mucosa and the TR146 cell culture model. Nielsen and Rassing (2000) did not find significantly different permeabilities of mannitol and testosterone using in vitro porcine and in vitro human buccal mucosa, while the results of Van Eyk and Van der Bijl (2004) showed that the human buccal mucosa was more permeable in vitro than porcine buccal mucosa to water, 17β-estradiol, arecoline, and vasopressin.

To the authors' knowledge, there are no previous reports of comparisons of data from in vivo human buccal permeability

studies using perfusion cells and studies using various buccal in vitro models. Therefore, more studies need to be performed to verify the utility of the buccal perfusion cells to predict in vivo human buccal drug permeability.

If the amount of disappeared nicotine was equal to the absorbed amount, the subjects in the present study received a high dose of nicotine (1.2, 5.3, and 7.5 mg). In comparison, the nicotine intake is approximately 1 mg when smoking a cigarette or chewing a piece of gum with 2 mg nicotine (Benowitz et al., 1987). After administration of a subcutaneous nicotine dose of 1 mg, non-smokers have been reported to experience mild side effects such as light-headedness and slight dizziness, probably caused by an increased heart rate (Russell et al., 1990). In the present study, the nicotine did not affect the subjects, probably because the nicotine dose was administered gradually during the 3 h of perfusion. Furthermore, the half-life of nicotine is 2 h (Rosenberg et al., 1980); the nicotine concentration in plasma was thus probably relatively constant.

The collection of parotid saliva with a modified Carlson–Crittenden cup during a buccal perfusion study was possible although not successful in all experiments. The analysis of the parotid saliva samples showed that the concentration of nicotine increased as pH and the amount of non-ionised nicotine increased. However, the increase of nicotine in saliva with increasing pH was not of the same magnitude as the increase in the disappearance of nicotine from the perfusion solution. However, the fact that the concentration of nicotine in the parotid saliva increased as the pH increased indicates that the disappeared nicotine was absorbed to the systemic circulation.

In the unstimulated state, the two parotid saliva glands contribute approximately 30% of the whole saliva, i.e. 15% pr. parotid gland (Sreebny, 1999). As mentioned previously, the levels of nicotine in whole saliva are roughly 10 times higher than those in plasma. Thus, to obtain a rough estimate of the plasma concentrations of nicotine during the perfusion period, the parotid saliva concentrations can be multiplied by 0.15 and divided by 10, assuming that an equal amount of nicotine was secreted from each parotid gland. At pH 8.1, this estimation results in plasma concentrations of nicotine of 45–90 ng $ml^{-1}$ while at pH 5.5 and 7.4, the estimated plasma concentrations are less than 2 ng $ml^{-1}$. In comparison, the plasma concentration of nicotine during smoking is approximately 30 ng $ml^{-1}$ (Benowitz et al., 1987). Since none of the subjects experienced any discomfort during the study, it seems unlikely that the plasma concentration of nicotine has been as high as 45–90 ng $ml^{-1}$. The present results indicate that the concentration of nicotine in parotid saliva is higher than in whole saliva. A study of nicotine would consequently have to be performed to clarify if a correlation between the nicotine levels in plasma, whole saliva and parotid saliva exists and further to verify whether parotid saliva levels can be used to assess systemic absorption of nicotine.

The average flow rate of parotid saliva in the present study was lower than the reported flow rate of unstimulated parotid saliva. The obtained flow rate was approximately 0.01 ml $min^{-1}$ per gland while the flow rate of unstimulated parotid saliva has been reported to be 0.04 ml $min^{-1}$ per gland (Rathbone et al., 1994). The pH in unstimulated saliva is lower than pH in stimulated saliva, which has a high flow rate (Dawes, 1969). The pH of saliva can be as low as 5.5 and increase to over pH 7 at high flow rates (Rathbone et al., 1994). The low flow rate of the parotid saliva in the present study therefore indicates that pH in the parotid saliva was low. This may have influenced the parotid saliva concentration of nicotine since the entrapment of nicotine in saliva is dependent on pH. More nicotine is entrapped in the saliva when the pH is low because more nicotine is ionised at a low pH. The non-ionised nicotine can easily return to the plasma while the ionised nicotine will to a higher extent be entrapped in the saliva (Kidwell et al., 1998). In the present study, a plausible explanation for the high levels of nicotine in the parotid saliva may therefore be that more nicotine was entrapped in this saliva because pH in the parotid saliva was low.

The high concentration of nicotine in the parotid saliva indicates that sampling of parotid saliva may be useful in buccal perfusion studies in which only small amounts of the examined compound are absorbed to the systemic circulation. However, a correlation between the levels of the examined compound in plasma and in parotid saliva has to be established to verify whether the parotid samples may be used to assess the systemic absorption.

## 5. Conclusion

In vivo human buccal permeability of nicotine can be obtained using a buccal perfusion cell. The disappearance of nicotine from the perfusion solution increased significantly as the pH was increased. This finding could be attributed to a higher amount of non-ionised species of nicotine at the higher pH values and the fact that the apparent permeability of the non-ionised species of nicotine was approximately 10 times higher than that of the mono-protonated species. This result is in accordance with previous in vitro studies of the relationship between buccal permeability and the ionised and non-ionised species of nicotine. A linear relationship between the apparent permeability of nicotine and the amount of non-ionised nicotine was obtained, indicating that the nicotine transfer occurred by means of passive diffusion. The analysis of the parotid saliva samples indicated that the parotid sampling procedure might be useful to assess systemic absorption of nicotine. The concentration of nicotine in the parotid saliva samples increased with increasing pH, indicating that the disappeared nicotine from the perfusion solutions was absorbed to the systemic circulation. Previous buccal in vitro models were capable of predicting the effect of pH on the nicotine buccal permeability. However, the models underestimated the in vivo human buccal permeability of nicotine.

## Acknowledgements

Fertin Pharma A/S (Vejle, Denmark) is acknowledged for supplying nicotine and financing the analysis of nicotine in saliva. The following persons at The Danish University of Pharmaceutical Sciences are acknowledged: Niels Erik Nielsen for making the perfusion cell, Bente Steffansen and Carsten Uhd Nielsen for assistance with data analysis and for fruitful

discussions, and Ida Boldsen and Lotte Jespersen for skilful technical assistance.

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