International Journal of Pharmaceutics 430 (2012) 104–113

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

A biorelevant *in vitro* release/permeation system for oral transmucosal dosage forms

Poonam R. Delvadia[a,1], William H. Barr[b,2], H. Thomas Karnes[a,*]

[a] Department of Pharmaceutics, School of Pharmacy, Virginia Commonwealth University, Richmond, VA 23298-0581, United States
[b] Department of Pharmacotherapy and Outcomes Science, School of Pharmacy, Virginia Commonwealth University, 410, N 12th Street, P.O. Box: 980533, Robert Blackwell Smith Bldg, Room 409, Richmond, VA 23298-0581, United States

ARTICLE INFO

Article history:
Received 22 December 2011
Received in revised form 6 March 2012
Accepted 26 March 2012
Available online 3 April 2012

Keywords:
Oral transmucosal dosage forms
Biorelevant *in vitro* release testing
Modified USP IV flow-through apparatus
Vertical diffusion cell
Bidirectional transmucosal apparatus

ABSTRACT

This research describes the development and validation of a biorelevant *in vitro* release/permeation system to predict the *in vivo* performance of oral transmucosal dosage forms. The system is a biorelevant bidirectional transmucosal apparatus which allows better simulation of oral cavity physiological variables in comparison to compendial dissolution apparatuses and therefore may be a better predictor of *in vivo* behavior. The feasibility of the bidirectional apparatus was studied using smokeless tobacco (snus) as a model oral transmucosal product. In this research, nicotine release and permeation was investigated from commercially available snus using a modified USP IV flow-through apparatus, a commercially available vertical diffusion cell and a fabricated novel bidirectional transmucosal apparatus. The percent nicotine released/permeated was utilized as an input function for the prediction of *in vivo* plasma nicotine profiles by back calculation based on the Wagner–Nelson method. The prediction errors in C_{max} and $AUC_{0-\infty}$ with the USP IV flow-through device, vertical diffusion cell and novel apparatus were 4.03, 22.85 and 1.59 and −5.85, 5.85 and −9.27% respectively. This work demonstrated the suitability of the novel bidirectional transmucosal apparatus for predicting the *in vivo* behavior of oral transmucosal products.

1. Introduction

Oral transmucosal dosage forms have provided attractive alternative for efficacious drug delivery due to the multiple advantages that it provides over conventional products like tablets and capsules. Oral transmucosal dosage forms deliver drug directly into the systemic circulation through the mucosal linings of the oral cavity (Madhav et al., 2009; Washington et al., 2001). This route is suitable for drugs with gastric incompatibility and provides benefits such as quick onset of drug action and avoidance of hepatic first pass metabolism (Madhav et al., 2009; Patel et al., 2011). Because of the above mentioned advantages, many oral transmucosal dosage forms are on the market and many more are in the pipeline (Pather et al., 2008; Pfister and Ghosh, 2005; Rathbone et al., 1996).

Predictive drug dissolution/release testing is required as an evaluation tool for cost effective and expedited pharmaceutical product development (Azarmi et al., 2007; Emami, 2006). Drug dissolution/release tests when performed under simulated *in vivo* conditions can predict the *in vivo* behavior of the drug product through *in vitro in vivo* relationships (IVIVR) (Emami, 2006; Polli, 2000; Wang et al., 2009). These drug dissolution/release tests are referred to as biorelevant and can be used as a quality control and research tool. The IVIVR can either be linear or nonlinear; the linear IVIVR is most commonly known as *in vitro in vivo* correlation (IVIVC) (Polli, 2000). Standard/compendial methods of drug release testing do not mimic the unique physiological environment desired for performance testing of novel dosage forms (Azarmi et al., 2007). Therefore, efforts are underway to develop modified *in vitro* drug release testing to accurately characterize the release of drugs from novel dosage forms (Brown et al., 2011; Chidambaram and Burgess, 1999; Crist, 2009; Iyer et al., 2007a; Kvist et al., 1999; Morjaria et al., 2004).

The USP monograph for sublingual and buccal tablets suggests the use of conventional dissolution and disintegration tests with a large volume of media (USP, 2009a,b,c,d). These compendial dissolution methods do not allow sufficient simulation of the unique physiological environment of the oral cavity to which oral transmucosal dosage forms are exposed and therefore may not be good predictors of *in vivo* behavior. In order to test oral transmucosal

* Corresponding author at: Department of Pharmaceutics, School of Pharmacy, Virginia Commonwealth University, 410, N 12th Street, P.O. Box: 980533, Robert Blackwell Smith Bldg, Room 450A, Richmond, VA 23298-0533, USA.
Tel.: +1 804 8283819; fax: +1 804 8288359.
E-mail addresses: delvadiapr@vcu.edu (P.R. Delvadia), whbarr@aol.com (W.H. Barr), htkarnes@vcu.edu (H.T. Karnes).
[1] Address: Department of Pharmaceutics, School of Pharmacy, Virginia Commonwealth University, 410, N 12th Street, Robert Blackwell Smith Bldg, Room 325, Richmond, VA 23298-0581, USA. Tel.: +1 804 8286214; fax: +1 804 8281273.
[2] Tel.: +1 804 8288334; fax: +1 804 8286902.

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2012.03.050

formulations *in vitro* in a biorelevant fashion, low liquid surroundings, saliva composition, salivary secretion and swallowing rate, mild agitation, mucosal barriers and blood flow rate are major factors that need to be considered. Efforts have been made to modify drug release testing of oral transmucosal dosage forms either by incorporating small volume dissolution or modification of the apparatus to reflect *in vivo* conditions. These include a system for drug release from bioadhesive buccal tablets using chicken buccal membrane (Mumtaz and Ch'ng, 1995), a supported liquid membrane system for nicotine release from snuff (Luque-Pérez et al., 1999), a low liquid system for drug release based on ionic current measurement (Frenning et al., 2002), a system for drug release study of dissolve in mouth dosage forms (Hughes, 2003), a flow through diffusion cell for drug permeation study using buccal mucosa (Lestari et al., 2009) and a system for sublingual tablets (Rachid et al., 2011). The above *in vitro* systems simulated either small fluid volume or the membrane barrier or both; however, these systems did not simulate the physiological variables of the oral cavity completely. In addition, these *in vitro* systems are suited to specific types of oral transmucosal products and none of them have been validated by relationship of *in vitro* with *in vivo* behavior.

This research was initiated to design and develop a biorelevant *in vitro* system for characterization of drug release and permeation from oral transmucosal dosage forms in a more physiologically realistic manner. To fulfill this objective, a novel bidirectional transmucosal apparatus was designed and evaluated that allows better simulation of *in vivo* oral cavity conditions. The device also allows adjustment of biorelevant parameters to optimize an IVIVR. Snus, a smokeless tobacco product, was selected as a model oral transmucosal product because *in vivo* nicotine pharmacokinetic data were available for comparison. Snus is put under the upper lip for nicotine delivery through the buccal-labial and gingival membranes. In the present research, the *in vivo* prediction by novel bidirectional transmucosal apparatus was compared with that of a modified USP IV flow-through apparatus and commercially available vertical diffusion cell for its evaluation. The development of this novel system will find application in the development and regulation of smokeless tobacco products in addition to that of the pharmaceutical dosage forms.

2. Materials and methods

2.1. Materials

Snus (Nicotine 8.0 mg, 1.0 g pouch) was obtained from Old Virginia Tobacco Co., Richmond, VA, USA. Hanks' Balanced Salt (H-1387), N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES, 1 M) buffer, (−)-nicotine hydrogen tartrate and sodium hydroxide solution (10 N) were purchased from Sigma, St. Louis, MO, USA. Glacial acetic acid was procured from EMD, Gibbstown, NJ, USA. HPLC grade ammonium acetate was bought from Fisher Scientific, Fair Lawn, NJ, USA. HPLC grade methanol was purchased from Honeywell Burdick and Jackson, Muskegon, MI, USA. Water was obtained in-house (the Nanopure Diamond™, Barnstead, IO, USA). Polyethersulfone, polypropylene and regenerated cellulose membranes for permeation study were obtained from Pall Life Sciences, Ann Arbor, MI, USA; Sterlitech Corporation, WA, USA; and Thermo Scientific, Rockford, IL, USA respectively. Fluorinated ethylene propylene (FEP) and Tygon® platinized silicon tubing were purchased from Cole-Parmer, Vernon Hills, IL, USA. Teflon unions and leur fittings for tubing connections were bought from Upchurch Scientific, Oak Harbor, WA, USA.

Fig. 1. The smokeless tobacco: snus.

2.2. Description of the snus

Snus is a type of moist snuff containing tobacco packed in a porous bag. The snus weighed 0.98 ± 0.03 g ($n = 6$). Each packet of snus contained 8.0 mg of nicotine. The snus was $3.2\ \text{cm} \times 1.7\ \text{cm} \times 0.43 \pm 0.03$ cm (length × width × depth, $n = 6$) in size. The other ingredients of snus are water, salt, humectant, acidity regulators, natural and artificial flavors. Fig. 1 displays a picture of the snus used in this study. The snus is placed under the upper lip and nicotine from the tobacco permeates through the buccal-labial mucosa from one side and the gingival mucosa on the other.

2.3. Medium for release and permeation study

A Hanks' Balanced Salt Solution modified by the addition of HEPES buffer was used as a biorelevant release/permeation medium for the present study. HEPES was added to simulate blood pH of 7.4 as it has a pKa of 7.5. The medium was prepared by dissolving 9.8 g of the Hanks' salt mixture in 975 mL of deionized water followed by pH adjustment to 7.4 ± 0.1 with 1 M of sodium hydroxide solution in water. The HEPES solution (25 mL of 1 M) was added into the above medium, mixed well, and vacuum filtered through 0.45 μm nylon filter. The pH of the Hanks' medium was adjusted to 7.4 ± 0.05, if required, using 1 M sodium hydroxide or 1 M hydrochloric acid solution.

2.4. Apparatuses

Nicotine release and permeation studies from a commercially available smokeless tobacco buccal pouch (Snus, 1.0 g) were performed using a vertical diffusion cell, a modified USP IV flow-through apparatus, and a novel bidirectional transmucosal apparatus. Fig. 2 presents diagrammatic representations of all three apparatuses. The vertical diffusion cell is commercially available from Hanson Research (Chatsworth, CA, USA) and is widely used for the transdermal and semisolid dosage form drug release testing (Hanson, 2010). The USP IV flow-through cell is a compendial apparatus and has received wide acceptance for both conventional and novel dosage form testing. The USP IV flow-through apparatus was modified according to Iyer et al. (2007a) except that the two polycarbonate cells were replaced with conical glass cells (Iyer et al., 2007a). The bidirectional transmucosal apparatus is a novel system designed to better simulate oral cavity conditions and study the effect of physiological variables on drug release and permeation. The vertical diffusion cell can be used for drug permeation studies, whereas; the modified USP IV flow-through apparatus can be utilized for drug release only. The bidirectional transmucosal apparatus provides the capability of studying drug release and permeation simultaneously. Physiological variables that can be simulated using the bidirectional transmucosal apparatus include

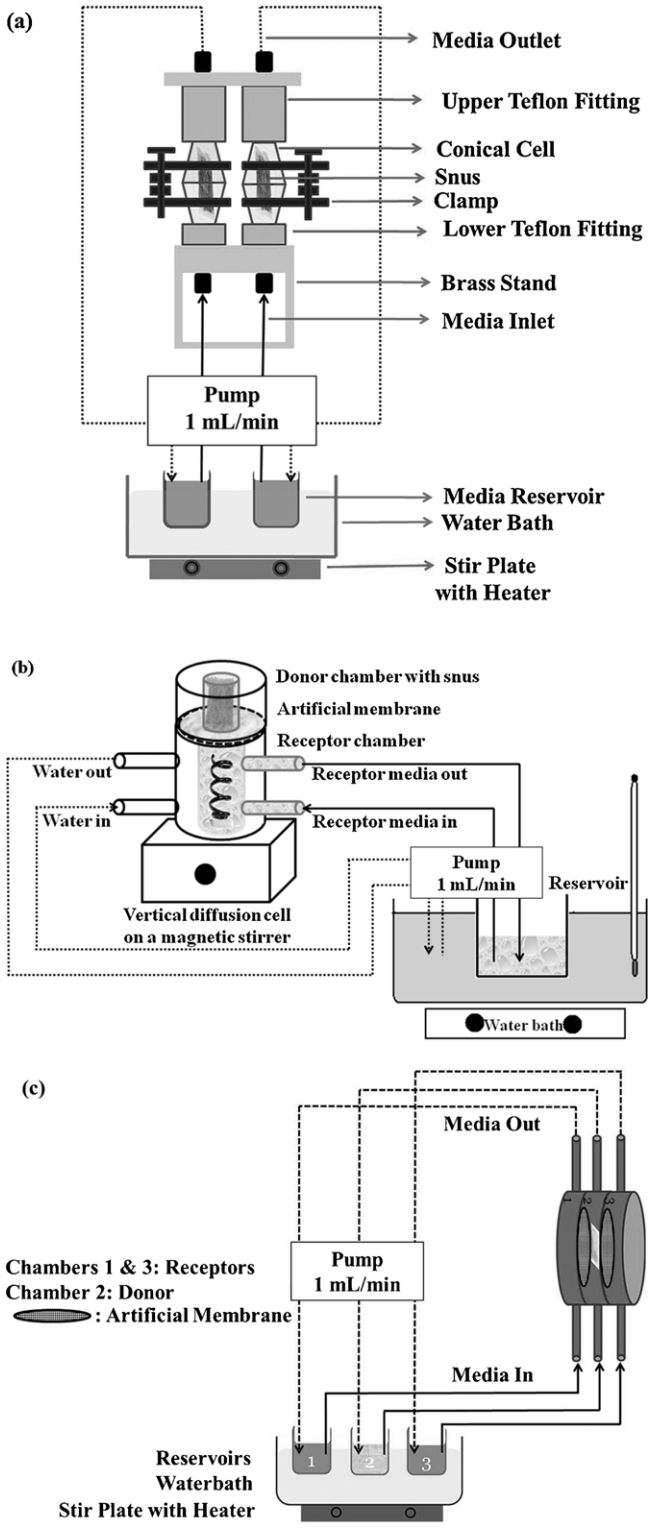

Fig. 2. Experimental set-up with three apparatuses ((a) modified USP IV flow-through apparatus; (b) vertical diffusion cell; and (c) novel bidirectional transmucosal apparatus).

low liquid surroundings, saliva secretion and swallowing rate, agitation movement, bidirectional release and permeation through biorelevant barriers and blood flow rate. In the present study, only low liquid surroundings and bidirectional permeation were simulated to test the suitability of the system.

2.4.1. Novel bidirectional transmucosal apparatus

In contrast to the vertical diffusion cell, the novel bidirectional transmucosal apparatus consisted of one donor and two receptor chambers. One receptor chamber was present on each side of the donor chamber (Fig. 2c). The presence of two receptor chambers allows study of the bidirectional permeation of nicotine from snus that occurs *in vivo*. When the snus is placed under the upper lip, nicotine from the tobacco permeates through the buccal mucosa from one side and the gingival mucosa on the other. The chambers were separated by an artificial membrane and were stacked together and secured by screws. The artificial membranes represent the buccal and gingival mucosa in the *in vitro* system. The apparatus was made of polymethyl methacrylate (PMMA). The receptor chamber volumes were 7.5 mL and that of the donor chamber was 10 mL based on the height and diameter of the chamber needed to hold the snus in its proper orientation. The dimensions of the donor chamber were chosen such that it provides low liquid surrounding and simultaneously hold the snus appropriately even after it gains height due to the media absorption. The receptor chamber s were 4 cm in diameter and 0.6 cm in height, whereas; the donor chamber was 4 cm in diameter and 0.8 cm in height. The membrane area exposed to the media in each receptor chamber was 14.5 cm^2.

2.5. In vitro release/permeation study

2.5.1. Experimental set-up/conditions

A single weighed snus was placed in the donor chamber or glass cell of the apparatuses. With the vertical diffusion cell and bidirectional transmucosal apparatuses, the donor chamber was separated from the receptor chamber by an artificial membrane. The Hanks' medium was re-circulated from the reservoir into the apparatus in a closed loop flow pattern at a flow rate of 1 mL/min using a peristaltic pump, fluorinated ethylene propylene (FEP) and Tygon® platinized silicon tubing. The donor chamber of the vertical diffusion cell contained non-circulating Hanks' medium because it was not possible to circulate donor fluid with this device. The medium reservoirs were 20 mL capacity scintillation glass vials placed in a water bath to maintain temperature at 37 °C. The Hanks' media in the reservoirs were stirred using a magnetic stir bar throughout the experiment. Three separate reservoirs were used for each chamber in the experiment with the bidirectional transmucosal apparatus. A schematic representation of the experimental set up for all apparatuses is shown in Fig. 2.

The donor chamber of the vertical diffusion cell contained 1.5 mL of Hanks' medium; whereas, the donor chamber reservoirs of the modified USP IV and bidirectional transmucosal apparatus contained 30 mL including the volumes of tubing and reservoir. The receptor chamber reservoirs of the vertical diffusion cell and bidirectional transmucosal apparatus contained 25 and 20 mL of Hanks' medium respectively which also included the volumes of tubing and reservoir. Samples (1 mL) were collected from the reservoir at an interval of 2.5 min for the initial 10 min, 5 min for the subsequent 20 min and 15 min for last 30 min (0, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 45 and 60 min) for experiments with all apparatuses. These time points were similar to the plasma sampling time points for an *in vivo* human clinical study that was to be used for IVIVR purposes. In the experiment with the vertical diffusion cell and bidirectional transmucosal apparatus, sampling was done from receptor chamber reservoirs at the above time intervals and at the last time point from the donor chamber reservoir. The samples were replaced by an equivalent volume of fresh Hanks' Balanced Salt Solution. The samples were frozen at −20 °C until analysis. The samples were analyzed for nicotine release and permeation using a validated High Performance Liquid Chromatographic (HPLC) method and cumulative nicotine amounts were calculated. Each experiment was performed in replicates. Nicotine permeated through both artificial

membranes into the receptor chambers of the bidirectional apparatus was added to represent the total permeation achieved at each time point.

2.5.2. Biorelevance

The presence of snus in the oral cavity stimulates the whole salivary secretion and swallowing rate. These changes would impact the rate and extent of nicotine release and ultimately affecting the overall extent of its permeation. Due to this, it was important to simulate the stimulated secretion rate in the donor chamber. The chewing stimulated salivary secretion rate studied in a population with an average age of 25.4 years, was 0.9 ± 0.094 mL/min (Navazesh et al., 1992). Based on this information, it was decided to use 1 mL/min flow rate in the donor chamber of the bidirectional transmucosal and modified USP IV flow-through apparatus. The flow rate in the receptor chambers of the vertical diffusion cell and bidirectional transmucosal apparatus were both maintained at 1 mL/min. Plasma composition was simulated by use of Hanks' Balanced Salt Solution in the receptor chambers as a biorelevant permeation medium (Iyer et al., 2007b). The same solution was used as a release medium in the donor chamber of the apparatuses as the major inorganic components of saliva and plasma are qualitatively similar (Krebs, 1950; Rehak et al., 2000). In addition, employing the same media in the donor and receptor chambers allows the sample analysis of both chambers utilizing the same analytical method.

2.5.3. Bidirectional transmucosal apparatus orientation

The orientation of the vertical diffusion cell and modified USP IV flow-through cell could only be vertical due to the apparatus design. However, the bidirectional transmucosal apparatus could be oriented horizontally and vertically. Therefore, it was necessary to select the orientation of the bidirectional transmucosal apparatus that provided consistent and equivalent permeation in both the receptor chambers. The effect of horizontal and vertical apparatus orientation on the nicotine permeation was studied using a polyethersulfone membrane (30 kDa MWCO, approximately 3 nm pore size) at a flow rate of 1 mL/min. The samples from the top and bottom receptor chamber's reservoir were collected at 0, 10, 20, 30, 45 and 60 min. The experiment was carried out in triplicate. It is important to mention here, that this set of experiments was done using the bidirectional transmucosal apparatus made up of PEEK which did not allow for visual examination of air entrapped inside the apparatus during the experiment. Therefore, in order to determine if equivalent permeation occurred during the study, the average of mean ratios of the cumulative amount of nicotine permeated in the bottom and top receptor chambers as a function of time was calculated. Equivalent permeation was expected as both membrane barriers are similar. The orientation that provided an average ratio of one which indicates equivalent permeation (*i.e.* the absence of air entrapment) was considered as the optimal orientation.

2.5.4. Membrane selection

Three types of membranes were studied for nicotine permeation from snus. Two of them were polymer based, polypropylene (100 nm pore size, 75–110 μm thickness) and polyethersulfone (30 and 300 kDa MWCO; 3 and 30 nm pore size respectively, 220 μm thickness) membranes, whereas; the third membrane was regenerated cellulose membrane (10 kDa MWCO, approximately 2.5 nm pore size, 23 μm thickness). The membranes were studied with both, the vertical diffusion cell and bidirectional transmucosal apparatus to confirm that the results obtained are a function of the membrane and not the apparatus. The polypropylene membrane was studied only with the bidirectional transmucosal apparatus. The effect of pore size on nicotine permeation was studied using the polyethersulfone membranes of 3 and 30 nm pore diameters using only the bidirectional transmucosal apparatus. The criteria for membrane selection were based on consistent nicotine permeation and negligible adsorption of nicotine on the membranes. The later was studied by sonication of the membrane in 5 and 10 mL of modified Hanks' Balanced Salt Solution for 10 min separately for each experiment performed with the vertical diffusion cell and bidirectional transmucosal apparatus respectively. The sonication is proved to be efficient method for cleaning membranes and hence same was used for extracting nicotine adsorbed on membranes in the present study (Kyllonen et al., 2005). The sonicated solution was analyzed for nicotine adsorption. The cumulative *in vitro* nicotine permeation from snus (1.0 g) obtained with the optimal membrane was compared to the *in vivo* nicotine absorption from the same snus product. This was done in order to examine the capability of the apparatuses for prediction of pharmacokinetic parameters for oral transmucosal dosage forms.

2.5.5. Nicotine adsorption study on acrylic bidirectional transmucosal apparatus

The study on adsorption of nicotine on the acrylic bidirectional transmucosal apparatus and assembly components was necessary due to the reported adsorption of nicotine on different materials (Caka et al., 1990; Grubner et al., 1980; Piade et al., 1999; Van Loy et al., 1997; Zahlsen et al., 1996). For this study, a single snus was put in 500 mL of Hanks' Balanced Salt Solution for 30 min under continuous stirring. The resulting nicotine solution was filtered and used for the adsorption study. Sixty eight milliliters of the above nicotine solution from a single glass reservoir at 37 °C temperature was re-circulated at 1 mL/min into the acrylic bidirectional transmucosal apparatus assembly without membranes in place. The nicotine solution in the reservoir was stirred using a magnetic stir bar throughout the experiment. The nicotine solution was sampled from the reservoir at 0, 10, 20, 30, 40, 50 and 60 min and replaced by an equivalent volume of fresh nicotine solution. The adsorption study was performed in triplicate. The samples were analyzed using the validated HPLC method. The percent deviation in the mean nicotine amount at 60 min was calculated relative to the mean nicotine amount at 0 min.

2.6. In vivo study

The *in vivo* nicotine absorption from the snus used in this research was obtained from a human clinical study designed and carried out by the Center of Research and Technology, Altria, Richmond, VA, USA. The *in vivo* nicotine pharmacokinetic study was conducted on 18 adult smokeless tobacco users and mean plasma nicotine concentration time profiles along with their standard deviations were investigated. Snus was put in the oral cavity for 30 min during the study.

2.7. Sample analysis

All *in vitro* samples were analyzed with an HPLC method which was modified from a method reported for nicotine in immediate and extended release tablets (Tambwekar et al., 2003). HPLC analysis was performed on a Waters 600E multisolvent delivery system with a Waters 717 autosampler, Shimadzu solvent degasser DGU-14A and 996 Waters photodiode array detector. A reverse phase chromatographic column, Luna C18(2) (100 Å, 250 mm × 4.6 mm, 5 μm, Phenomenex, Torrance, CA) was used for the separation. The column temperature was 45 °C. The mobile phase for isocratic mode chromatographic separation was 10 mM ammonium acetate in 0.005% acetic acid (pH 5.5): methanol (42:58%, v/v). The flow rate

of mobile phase was set to 1 mL/min. The sample volume injected was 20 μL. The UV detection of samples was performed at 260 nm, the wavelength maxima for nicotine analysis. The retention time of nicotine under the above chromatographic separation condition was 4.3 min.

2.8. Data analysis/pharmacokinetic assessment

All calculations for data analysis were performed in Microsoft® Excel 2010. Nicotine concentrations (μg/mL) were utilized for calculating the cumulative amount of nicotine released and/or permeated at each time point. The *in vitro* nicotine permeation observed with the vertical diffusion cell, modified USP IV flow-through apparatus and novel bidirectional transmucosal apparatus employing the optimal membrane was related to the *in vivo* nicotine absorption and the plasma nicotine profile was predicted.

The observed mean *in vivo* plasma nicotine concentration time profile was deconvoluted to its *in vivo* absorption time profile by Wagner–Nelson modeling assuming a one compartment open body model (Wagner and Nelson, 1964). The area under the curve, $AUC_{0-300\,min}$, was calculated by the trapezoidal rule. The elimination rate constant (K_e) was estimated from the terminal slope of the logarithmic plasma nicotine concentration time profile, which was further used for calculating $AUC_{0-\infty}$. All of the above parameters were employed for the determination of the fraction nicotine absorbed as a function of time by Wagner–Nelson modeling. The cumulative amount absorbed was further calculated by the equation: $A_t = C_t \times V_d$; where A_t = cumulative amount (ng) absorbed at time t, C_t = cumulative concentration (ng/mL) at time t obtained from Wagner–Nelson modeling and V_d = volume of distribution (mL). The volume of distribution of nicotine was obtained using $V_d = CL_{total}/K_e$; where, CL_{total} = total nicotine clearance (mL/min). The total nicotine clearance was taken to be 1307 mL/min as estimated from an intravenous (IV) pharmacokinetic study on 20 healthy adult subject of age 22–43 years (Molander et al., 2001). The percent absolute bioavailability of nicotine from snus 1.0 g was calculated. The $AUC_{0-\infty,IV}$ and $Dose_{IV}$ was 1596 ng min/mL and 1.77 mg respectively (Molander et al., 2001). The cumulative percent nicotine (% of 8 mg dose) absorption time profile obtained was employed for *In Vitro In Vivo* Relationship (IVIVR). The percent cumulative *in vitro* nicotine released and/or permeated (% of 8 mg dose) was related to the percent cumulative *in vivo* nicotine absorbed (% of 8 mg dose). The IVIVR model was used for prediction of the *in vivo* amount of nicotine absorbed. The predicted *in vivo* amount of nicotine was further applied as an input function for the prediction of *in vivo* plasma nicotine concentration time profiles by a convolution technique based on back calculation from the Wagner–Nelson equation (Gohel et al., 2005). This method of prediction for plasma drug concentration profiles has been validated by predicting pharmacokinetic parameters of diltiazem hydrochloride 300 mg extended release capsules (Bendas, 2009). The obtained IVIVR model for nicotine was established by calculating the percent prediction errors (%PE) in nicotine pharmacokinetic (PK) parameters C_{max} and $AUC_{0-\infty}$. The percent prediction errors should not exceed 15% to demonstrate the predictability of the IVIVR (Malinowski et al., 1997). The percent prediction error was calculated using the equation:

$$\%PE = \frac{[(\text{Observed PK Parameter} - \text{Predicted PK Parameter}) \times 100]}{\text{Observed PK Parameter}}$$

3. Results and discussion

3.1. Sample analysis

The *in vitro* samples were analyzed using the HPLC method described in Section 2.7. The calibration curve was linear over the range of 0.5–32 μg/mL ($r^2 > 0.99$) and the lowest standard (0.5 μg/mL) showed inaccuracy and imprecision below 4 and 3% respectively. The inaccuracy and imprecision of other standards was less than 3%. The intra run ($n = 6$) and inter run ($n = 9$) inaccuracy and imprecision of quality control (QC) samples (Lower QC = 1.5 μg/mL, Middle QC = 5 μg/mL and Higher QC = 28 μg/mL) was within 4%. The lower and higher QC samples analyzed after subjecting them to three freeze thaw cycles showed inaccuracy and imprecision less than 4%. Samples were also stable in the autosampler as evident from the insignificant difference in standard concentrations at the start and end of the unknown sample run (paired t-test: $t = 1.84$, df = 6, p-value > 0.05).

3.2. Validation of the system

3.2.1. Bidirectional transmucosal apparatus orientation

Fig. 3(a) and (b) represent the cumulative nicotine permeation in the top and bottom receptor chambers when the apparatus was oriented in horizontal and vertical positions respectively. The average of the mean ratio of the nicotine permeated in the bottom and top receptor chambers at all-time points with the horizontal and vertical orientation were 2.19 and 1.09 respectively. The ratio of 2.19 with the horizontal apparatus orientation indicates that the permeation was two times higher in the bottom receptor chamber when compared to the top receptor chamber. In the horizontal orientation, total permeation was higher in the bottom chamber due to the initial 10 min, where the nicotine permeation was approximately 2.25 times higher than in the top receptor chamber. Nicotine permeation was comparable after the initial 10 min as evident from the parallel slopes of the cumulative nicotine permeation-time profile of the horizontal apparatus orientation in Fig. 3(a). This initial difference may be due to entrapment of air in the donor chamber below the top receptor membrane or a higher degree of contact of the lower receptor membrane with the snus in the horizontal orientation. The entrapment of air creates a void space between the membrane of the top receptor chamber and the donor chamber and negatively affects the permeation process. With the vertical orientation, the ratio of 1.09 suggests equivalent permeation in both the receptor chambers of the bidirectional apparatus and the absence of air. Since, the extent of nicotine permeation was similar in both receptor chambers in the vertical apparatus orientation; it was decided to conduct future experiments positioning the apparatus vertically. Also, snus placed horizontally in the novel apparatus that is oriented vertically adds biorelevance to the novel system for the reason that the snus is placed horizontally under the upper lip. In addition, the nicotine available for permeation through the gingival and buccal membranes may be the same *in vivo* due to the absence of air at the interface between snus and mucosa. The vertical orientation mimics the availability of nicotine at both membrane-donor media interfaces as indicated by equivalent permeation in both receptors. With respect to the absence of void space and equal availability of nicotine for permeation at both membrane-donor media interfaces; the vertical orientation was considered optimal and is more physiologically relevant.

3.2.2. Membrane selection

Membrane selection was affected by the tendency of nicotine to adsorb onto various materials (Grubner et al., 1980; Piade et al., 1999; Van Loy et al., 1997; Zahlsen et al., 1996). Polypropylene (100 nm pore size, 75–110 μm thickness) was first studied for

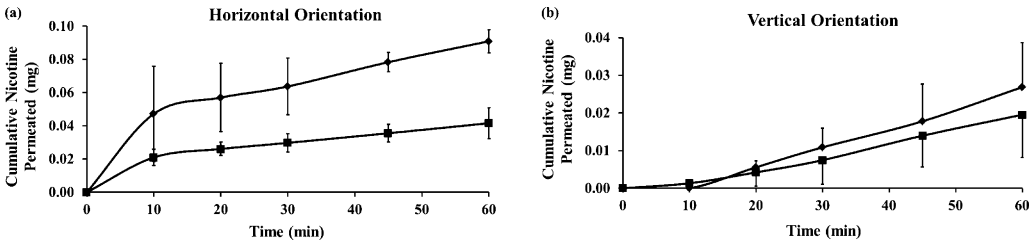

Fig. 3. Effect of apparatus orientation on nicotine permeation in receptor chambers (bottom receptor (♦) and top receptor (■)) (Error bars represents standard deviation; $n = 3$).

nicotine permeation using the bidirectional transmucosal apparatus. It was found that the nicotine permeation did not occur with this membrane within the lower limit of quantification of the HPLC method. The absence of nicotine permeation can be attributed to the lack of wettability of the polypropylene due to its hydrophobicity. The membrane observed at the end of the experiment was completely dry. Therefore, it was concluded that the polypropylene membrane was not a suitable membrane choice and was not further investigated.

Table 1 shows results of nicotine release, cumulative nicotine permeation and nicotine adsorption on membranes studied with the vertical diffusion cell and bidirectional transmucosal apparatus with both polyethersulfone and regenerated cellulose membranes. The cumulative nicotine permeation with the polyethersulfone membrane (3 nm pore size) was highly variable with both the vertical diffusion cell ($\%RSD_{60\ min} = 63.88\%$) and bidirectional transmucosal apparatus ($\%RSD_{60\,min} = 49.64\%$). The overall nicotine permeated with the vertical diffusion cell and bidirectional transmucosal apparatus at 60 min using the polyethersulfone membrane was only 0.2 and 0.6% of 8 mg nicotine in the snus respectively which is much less than the 18% nicotine absorbed *in vivo*. However, the bidirectional transmucosal apparatus provided approximately three times greater permeation than the vertical diffusion cell due to the larger membrane surface area and bidirectional permeation. In addition, an unexpected plateau in the cumulative nicotine permeation with the vertical diffusion cell was observed which could be related to saturation of the small membrane surface. At the end of the permeation study, the membranes were completely brown in color. The analysis of Hanks' media used for sonication of polyethersulfone (3 nm pore size) membranes used in both the apparatuses indicated significant adsorption of nicotine which might have been responsible for the observed low extent of permeation (two tailed t-test at $\alpha = 0.05$, vertical diffusion cell (0.114 mg/cm^2): $t = 13.30$, df = 4, p-value = 0.0002; bidirectional transmucosal apparatus (0.005 mg/cm^2): $t = 6.11$, df = 2, p-value = 0.0258). Nicotine assayed from the donor chamber accounted for more than 50% of the nicotine content in the snus (8 mg) which supports the conclusion that the nicotine release is high enough for permeation to occur.

Subsequent studies were conducted using polyethersulfone membranes with a pore size of 30 nm ($n = 3$) and the bidirectional apparatus to investigate whether or not the small pore size with the previous study might have been responsible for low permeation. This study demonstrated the complete absence of permeation. As observed previously, membranes were completely brown at the end of the study and the sonication experiment suggested adsorption of nicotine. It is possible that nicotine is adsorbed on the tar particles present in tobacco. These tar particles might have blocked membrane pores of both types of polyethersulfone membranes and thus provided low nicotine permeation. This study shows that the pore size was not limiting for nicotine permeation. We concluded that the polyethersulfone membrane was not a suitable membrane for nicotine permeation.

Cumulative nicotine permeation with the regenerated cellulose membrane (2.5 nm) was less variable as compared to the polyethersulfone membrane with both the vertical diffusion cell ($\%RSD_{60\,min} = 15.80\%$) and the bidirectional transmucosal apparatus ($\%RSD_{60\,min} = 14.77$). The overall nicotine permeated with the regenerated cellulose membrane was 12.23 and 12.30% of the nicotine content (8 mg) in snus which is close to the 18% nicotine absorbed *in vivo*. The analysis of regenerated cellulose sonicate samples with both devices again indicated adsorption onto the membrane (two tailed t-test at $\alpha = 0.05$, vertical diffusion cell (0.005 mg/cm^2): $t = 4.42$, df = 4, p-value = 0.0115; bidirectional transmucosal apparatus (0.0002 mg/cm^2): $t = 3.76$, df = 4, p-value = 0.0198) although the regenerated cellulose provided significantly less nicotine adsorption as compared to the polyethersulfone membranes (equal variance t-test at $\alpha = 0.05$, vertical diffusion cell: $t = -12.64$, df = 8, p-value < 0.0001; bidirectional transmucosal apparatus: $t = -5.86$, df = 2.02, p-value = 0.0274). Also, the regenerated cellulose membranes observed at the end of the experiment were completely clear. The regenerated cellulose membrane was selected for further study owing to consistent nicotine permeation and less nicotine adsorption relative to the polyethersulfone membrane. Nicotine, being a lipophilic molecule, is highly permeable through the oral mucosa. Therefore, the oral mucosa may not be a major barrier to the availability of nicotine in the systemic circulation. Similar nicotine permeation behavior was obtained with the use of regenerated cellulose membrane; whereas, permeation through the polyethersulfone membrane was limited due to adsorption of nicotine.

3.2.3. *Adsorption of nicotine on the acrylic bidirectional transmucosal apparatus and assembly components*

In addition to the study of nicotine adsorption onto membranes, it was also necessary to examine the adsorption of nicotine onto the acrylic bidirectional transmucosal apparatus and assembly components. The study was not performed with the glass vertical diffusion cell as the glass had shown the least adsorption of nicotine among different materials tested (Grubner et al., 1980). Besides, other assembly components used for these two set ups were similar. The nicotine adsorption study with the acrylic bidirectional transmucosal apparatus set up was conducted by re-circulation of nicotine solution of a known concentration and the nicotine time profile obtained from the study is shown in Fig. 4. The study indicated that approximately 4% of nicotine from the solution was adsorbed at 60 min. This deviation was not considered practically significant and it was concluded that the acrylic bidirectional transmucosal apparatus and assembly components were suitable for further work.

3.3. *In vitro release study*

Fig. 5 illustrates the mean cumulative nicotine release/permeation time profiles obtained with the modified USP IV flow-through apparatus, vertical diffusion cell and novel

Table 1
Nicotine release, cumulative nicotine permeation and nicotine adsorption with polyethersulfone and regenerated cellulose membranes.

Membrane (pore size)	Apparatus	n	Nicotine release in donor chamber at 60 min (mg) (%RSD)[g]	Adsorption of nicotine on membrane (mg) (%RSD)	Adsorption of nicotine per cm^2 of membrane (mg/cm^2) (%RSD)	Percent nicotine adsorption on membrane (%/cm^2)[f]	Total nicotine release in donor chamber at 60 min (mg)[a]	Percent nicotine release in donor chamber (%)[f]	Cumulative nicotine permeation at 60 min (mg) (%RSD)	Percent nicotine permeation (%)[f]
Polyethersulfone (3 nm)	Vertical diffusion cell	5	4.677 ± 0.290 (6.21)	0.090 ± 0.015[d] (16.82)	0.114 ± 0.019 (16.82)	1.425	4.767	59.588	0.017 ± 0.011 (63.88)	0.213
	Bidirectional transmucosal apparatus	3	5.960 ± 0.712 (11.95)	0.157 ± 0.044[b,c] (28.35)	0.005 ± 0.002 (28.35)	0.063	6.117	76.463	0.046 ± 0.023[e] (49.64)	0.575
Polyethersulfone (30 nm)	Bidirectional transmucosal apparatus	3	5.519 ± 0.971 (17.60)	0.241 ± 0.146[b,c] (60.67)	0.008 ± 0.005 (60.67)	0.100	5.760	72.000	not detected	–
Regenerated cellulose (2.5 nm)	Vertical diffusion cell	5	3.900 ± 0.526 (13.48)	0.004 ± 0.002[d] (50.58)	0.005 ± 0.002 (50.58)	0.063	3.904	48.800	0.978 ± 0.155 (15.80)	12.225
	Bidirectional transmucosal apparatus	5	5.860 ± 0.235 (4.02)	0.006 ± 0.004[b,c] (59.47)	0.0002 ± 0.0001 (59.47)	0.003	5.866	73.325	0.984 ± 0.145[e] (14.77)	12.300

[a] The total nicotine release in donor chamber represents the sum of nicotine release in donor chamber at 60 min and nicotine adsorbed on the membranes.
[b] Values represents the sum of nicotine adsorbed on membranes of both receptors.
[c] The total of the surface area of both membranes exposed to donor media was 29 cm^2 in the bidirectional transmucosal apparatus (14.5 cm^2 per membrane).
[d] The total surface area of membrane exposed to donor media was 0.79 cm^2 in the vertical diffusion cell.
[e] The nicotine permeation represents the sum of nicotine permeated in both the receptors at 60 min.
[f] Values represents the percent of 8 mg.
[g] Percent relative standard deviation.

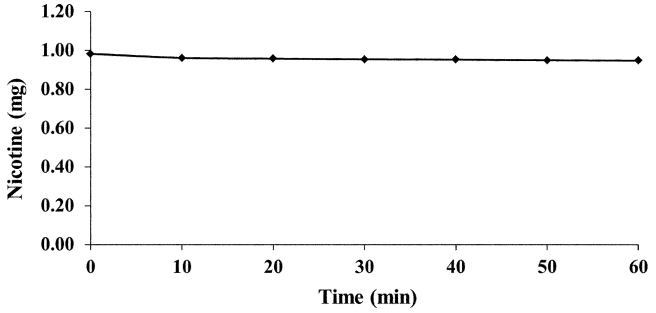

Fig. 4. Nicotine amount time profile of nicotine adsorption study on acrylic bidirectional transmucosal apparatus and assembly components ($n = 3$).

bidirectional transmucosal apparatus when regenerated cellulose membrane was used. Nicotine release using the modified UPS IV flow-through apparatus demonstrated first order release of nicotine [cumulative nicotine release (mg) = 1.7954 × Ln(time in min) − 2.0232, $r^2 = 0.957$]; whereas, nicotine permeation with the vertical diffusion cell and bidirectional transmucosal apparatus showed zero order permeation of nicotine [cumulative nicotine permeation (mg) = 0.0169 × (time in min) − 0.0342, $r^2 = 0.997$ and cumulative nicotine permeation (mg) = 0.0207 × (time in min) − 0.2392, $r^2 = 0.998$ respectively]. The first order release obtained with the modified USP IV apparatus might be due to depletion of nicotine in the snus as a function of time resulting in the decreased release rate. The cumulative nicotine amount released at 60 min accounted 71.21% (5.697 mg ± 0.341, % RSD = 5.99, $n = 5$) of nicotine content in snus (8 mg). In contrast, with the vertical diffusion cell and bidirectional transmucosal apparatus, the donor nicotine concentrations were large relative to the nicotine that permeated in the receptor chambers as shown in Table 1; consequently linear permeation was obtained during the 60 min period.

Table 2 represents the cumulative nicotine release/permeation as a function of time obtained with all apparatuses.

3.4. In vivo study

The mean plasma nicotine concentration time profile obtained from a nicotine pharmacokinetic study carried out on 18 adult smokeless tobacco users by the Center of Research and Technology, Altria, is shown in Fig. 6 and the mean pharmacokinetic parameters are summarized in Table 3. The mean plasma nicotine concentration time profile deconvoluted to its *in vivo* percent absorption-time profile by Wagner–Nelson modeling is displayed in Fig. 7.

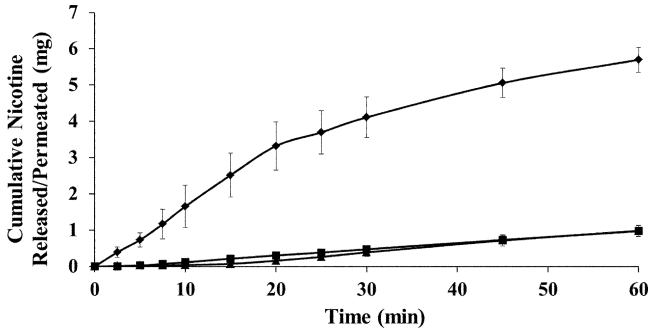

Fig. 5. The mean cumulative nicotine permeation/release time profiles with all three apparatuses (modified USP IV flow-through apparatus (♦); vertical diffusion cell (■) and bidirectional transmucosal apparatus (▲)) (error bars represents standard deviation; $n = 5$).

Table 2
Cumulative nicotine release/permeation with all apparatuses ($n = 5$).

Mean cumulative nicotine release/permeation (mg) (%RSD)[a]			
Time (min)	Modified USP IV flow-through apparatus	Vertical diffusion cell	Bidirectional transmucosal apparatus
0	0	0	0
2.5	0.396 ± 0.145 (36.73)	0.006 ± 0.007 (110.68)	0.016 ± 0.012 (73.04)
5	0.734 ± 0.196 (26.69)	0.029 ± 0.018 (64.09)	0.026 ± 0.010 (37.20)
7.5	1.171 ± 0.413 (35.28)	0.070 ± 0.030 (43.59)	0.033 ± 0.010 (29.12)
10	1.658 ± 0.586 (35.34)	0.117 ± 0.041 (35.37)	0.042 ± 0.010 (24.77)
15	2.520 ± 0.604 (23.95)	0.218 ± 0.062 (28.64)	0.076 ± 0.022 (28.81)
20	3.323 ± 0.661 (19.88)	0.307 ± 0.066 (21.64)	0.161 ± 0.071 (43.85)
25	3.699 ± 0.592 (16.02)	0.386 ± 0.085 (21.90)	0.267 ± 0.105 (39.15)
30	4.112 ± 0.559 (13.60)	0.476 ± 0.094 (19.84)	0.390 ± 0.111 (28.35)
45	5.058 ± 0.402 (7.95)	0.733 ± 0.119 (16.21)	0.719 ± 0.154 (21.42)
60	5.697 ± 0.341 (5.99)	0.978 ± 0.155 (15.80)	0.984 ± 0.145 (14.77)

[a] Percent relative standard deviation.

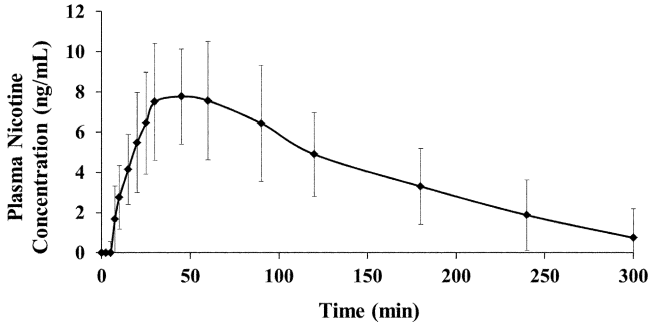

Fig. 6. Plasma nicotine concentration time profile after snus administration in 18 adult smokeless tobacco users.

Table 3
Mean pharmacokinetic parameters ($n = 18$) after administration of snus 1.0 g (8 mg nicotine).

Pharmacokinetic parameters	Snus 1.0 g
C_{max} (ng/mL)	7.80
T_{max} (min)	45.00
$AUC_{0-300\,min}$ (ng min/mL)	1203.50
$AUC_{0-\infty}$	1283.50
V_d (L)	140.54
K_e (min^{-1})	0.00932
K_a (min^{-1})[a]	0.04227
Half life ($t_{1/2}$) (h)	1.24
Absolute bioavailability (%)	18.00

[a] K_a, the rate of absorption was estimated from the slope of the nicotine amount remaining to be absorbed time profile.

3.5. In vitro–in vivo relationship and prediction of plasma profile of nicotine

The cumulative nicotine release/permeation obtained in the *in vitro* systems employing regenerated cellulose membranes is shown in Fig. 5. When related to the cumulative amount absorbed in *in vivo* (Fig. 7) an *in vitro in vivo* relationship (IVIVR) model was generated and is presented in Fig. 8(a)–(c). The IVIVR model obtained for the modified USP IV flow-through apparatus and vertical diffusion cell was linear with r^2 values of 0.99 and 0.89 respectively; whereas, the model for the bidirectional transmucosal apparatus fit a non-linear model with an r^2 of 0.98. The non-linearity of the IVIVR model with the bidirectional transmucosal apparatus might be due to the relatively rapid *in vivo* nicotine absorption when compared to *in vitro* permeation. This may be because of the lower concentration gradient that exists in the bidirectional transmucosal *in vitro* system due to the lower receptor to donor volume ratio of 1.33. In the case of the vertical diffusion cell, the *in vitro* permeation rate was slow relative to the *in vivo* absorption rate regardless of the larger concentration gradient (receptor to donor volume ratio of 16.67). This slow *in vitro* permeation rate is justified by the slope (1.6) of the IVIVR model which is greater than 1 and might be due to the lower membrane surface area available for permeation. In spite of this, the linear model was appropriate to describe the IVIVR achieved with the vertical diffusion cell. The *in vitro* release rate with the modified USP IV apparatus is relatively faster than the *in vivo* absorption as evident from the slope (0.3) of the linear IVIVR model.

The convolution of the predicted nicotine amount absorbed obtained from the respective IVIVR models provided the predicted plasma nicotine concentration time profiles as shown in Fig. 8(a)–(c). The percent prediction errors calculated for C_{max} and $AUC_{0-\infty}$ relative to that of the observed parameters are tabulated in Table 4. These established the IVIVR models for the respective apparatus since errors were below 15% (Malinowski et al., 1997). However, the prediction error in C_{max} obtained with the IVIVR model of the vertical diffusion cell was 22.85% above this acceptance limit which might be because of the less accurate IVIVR model ($r^2 = 0.89$).

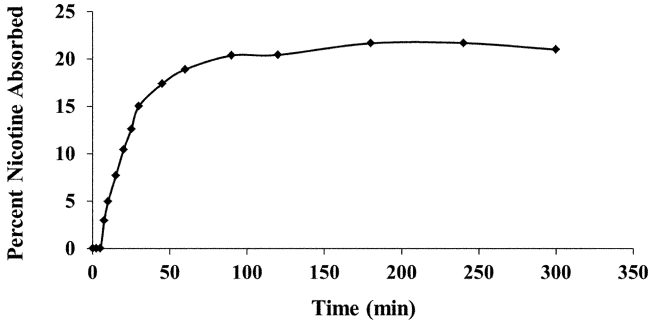

Fig. 7. The nicotine absorption time profile obtained by Wagner–Nelson modeling.

3.6. Comparison of the three apparatuses

Overall, drug release/permeation testing apparatuses used in the present research were able to provide IVIVR models that predicted the plasma nicotine time profile with less than 15% error in C_{max} and $AUC_{0-\infty}$ for smokeless tobacco although the vertical diffusion cell failed this criterion. This suggests that the modified USP IV flow-through and novel bidirectional transmucosal apparatuses are suitable for the drug release/permeation testing of oral transmucosal dosage forms. The bidirectional transmucosal apparatus allows for better simulation oral cavity conditions. Simulation and adjustment of *in vivo* conditions is very important to achieve better IVIVR for the prediction of the *in vivo* behavior of the drug product because drug dissolution and release kinetics are influenced by these conditions (Dressman et al., 1998; Wang et al., 2009).

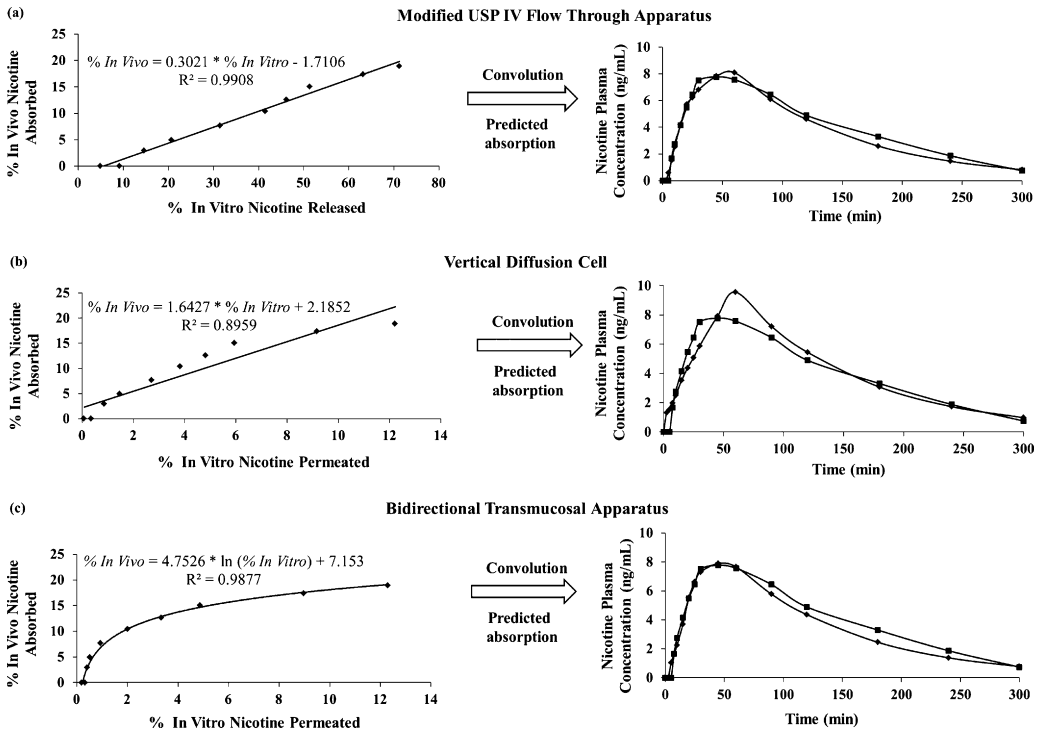

Fig. 8. *In vitro in vivo* relationships (IVIVR) and plasma concentration time profiles of nicotine obtained with three apparatuses (predicted plasma nicotine concentration time profile (♦) and observed plasma nicotine concentration time profile (■)).

Table 4
Observed and predicted pharmacokinetic parameters for snus 1.0 g (nicotine 8 mg).

Apparatus	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng min/mL)	%Prediction errors	
			C_{max}	$AUC_{0-\infty}$
Observed *in vivo* parameters	7.80	1283.50		
Modified USP IV flow-through apparatus	8.09	1208.43	4.03	−5.85
Vertical diffusion cell	9.55	1358.65	22.85	5.85
Bidirectional transmucosal apparatus	7.90	1164.47	1.59	−9.27

Table 5
Simulation of oral cavity conditions by apparatuses.

Apparatus	Media composition and its physical properties	Salivary secretion and swallowing rate	Agitation	Blood flow rate	Bidirectional biorelevant barriers	Permeation
Modified USP IV flow-through apparatus	Yes	Yes	No	No	No	No
Vertical diffusion cell	Yes	No	No	Yes	No	Yes
Bidirectional transmucosal apparatus	Yes	Yes	Yes	Yes	Yes	Yes

As represented in Table 5, the bidirectional transmucosal apparatus allows adjustment of important oral cavity conditions that can affect drug release/permeation. These *in vivo* conditions include salivary secretion and swallowing rate in the donor chamber, blood flow rate in the receptor chambers and agitation in the donor chamber. In addition, it allows study of bidirectional permeation that occurs *in vivo*. The modified USP IV flow-through apparatus and vertical diffusion cell permits adjustment of only few physiological variables as listed in Table 5. The degree of biorelevance achievable with the apparatuses are in the order of modified USP IV flow-through apparatus < vertical diffusion cell < bidirectional transmucosal apparatus.

4. Conclusion

A novel bidirectional transmucosal apparatus was developed and compared to two commercial devices for biorelevant *in vitro* release and permeation testing of oral transmucosal dosage forms. The bidirectional transmucosal system was validated in terms of the orientation of the device, membrane performance and nicotine adsorption on the components. Of the membranes studied, the regenerated cellulose membrane provided consistent permeation and negligible nicotine adsorption. The bidirectional transmucosal apparatus provided linear nicotine permeation profiles with the rate and extent of nicotine permeation similar to the vertical diffusion cell. The modified USP IV and the vertical diffusion cell provided linear relationship between the percent *in vitro* nicotine permeation and *in vivo* nicotine absorption; whereas, the bidirectional transmucosal apparatus demonstrated non-linear relation. Though the modified USP IV flow-through and novel bidirectional transmucosal apparatuses were suitable for predicting the *in vivo* behavior of nicotine from snus based on the pharmacokinetic parameters' prediction error, the novel system allows inclusion of more biorelevant parameters for the oral cavity. In addition,

the work presented here provides a general guide to important steps required for the development and validation of biorelevant systems. The bidirectional transmucosal apparatus is a promising candidate and is appropriate for prediction of the *in vivo* behavior of oral transmucosal dosage forms.

Acknowledgments

The authors gratefully acknowledge The Center of Research and Technology, Altria, Richmond, VA, USA for sharing *in vivo* nicotine pharmacokinetic data. Poonam Delvadia acknowledges the School of Pharmacy, Virginia Commonwealth University for financial support in the form of Graduate Assistantship.

References

Azarmi, S., Roa, W., Löbenberg, R., 2007. Current perspectives in dissolution testing of conventional and novel dosage forms. Int. J. Pharm. 328, 12–21.
Bendas, E.R., 2009. Two different approaches for the prediction of in vivo plasma concentration-time profile from in vitro release data of once daily formulations of diltiazem hydrochloride. Arch. Pharm. Res. 32, 1317–1329.
Brown, C.K., et al., 2011. FIP/AAPS Joint Workshop Report: Dissolution/in vitro release testing of novel/special dosage forms. AAPS Pharm. Sci. Tech. 12, 782–794.
Caka, F.M., et al., 1990. An intercomparison of sampling techniques for nicotine in indoor environments. Environ. Sci. Technol. 24, 1196–1203.
Chidambaram, N., Burgess, D.J., 1999. A novel in vitro release method for submicron sized dispersed systems. AAPS Pharm. Sci. 1 (E11), 1–9.
Crist, G.B., 2009. Trends in small-volume dissolution apparatus for low-dose compounds. Dissolut. Technol. 16, 19–22.
Dressman, J.B., et al., 1998. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm. Res. 15, 11–22.
Emami, J., 2006. In vitro–in vivo correlation: from theory to applications. J. Pharm. Pharm. Sci. 9, 169–189.
Frenning, G., Ek, R., Stromme, M., 2002. A new method for characterizing the release of drugs from tablets in low liquid surroundings. J. Pharm. Sci. 91, 776–784.
Gohel, et al., 2005. Simplified mathematical approach for back calculation in wagner–nelson method. Pharm. Rev. 3 (2) (Accessed on 12/19/2011) http://www.pharmainfo.net/reviews/simplified-mathematical-approach-back-calculation-wagner–nelson-method.
Grubner, O., First, M.W., Huber, G.L., 1980. Gas chromatographic determination of nicotine in gases and liquids with suppression of adsorption effects. Anal. Chem. 52, 1755–1758.
Hanson, R., 2010. A Primer on release-rate testing of semisolids. Dissolut. Technol. 17, 33–35.
Hughes, L., 2003. Tightening up buccal testing. Manuf. Chemist. 74, 28–30.
Iyer, S.S., et al., 2007a. A 'biorelevant' system to investigate in vitro drug released from a naltrexone implant. Int. J. Pharm. 340, 104–118.
Iyer, S.S., Barr, W.H., Karnes, H.T., 2007b. Characterization of a potential medium for 'biorelevant' in vitro release testing of a naltrexone implant, employing a validated stability-indicating HPLC method. J. Pharm. Biomed. Anal. 43, 845–853.
Krebs, H.A., 1950. Chemical composition of blood plasma and serum. Annu. Rev. Biochem. 19, 409–430.
Kvist, C., et al., 1999. Apparatus for studying in vitro drug release from medicated chewing gums. Int. J. Pharm. 189, 57–65.
Kyllonen, H., Pirkonen, P., Nystrom, M., 2005. Membrane filtration enhanced by ultrasound: a review. Desalination. 181, 319–335.
Lestari, M.L.A.D., Nicolazzo, J.A., Finnin, B.C., 2009. A novel flow through diffusion cell for assessing drug transport across the buccal mucosa in vitro. J. Pharm. Sci. 98, 4577–4588.
Luque-Pérez, E., et al., 1999. Analysis of solid samples using supported liquid membranes: a method for the evaluation of the release of nicotine from Swedish snuff. Anal. Chim. Acta. 387, 155–164.
Madhav, N.V., et al., 2009. Orotransmucosal drug delivery systems: a review. J. Control. Release. 140, 2–11.
Malinowski, H., et al., 1997. Draft guidance for industry extended-release solid oral dosage forms. Development, evaluation and application of in vitro–in vivo correlations. Adv. Exp. Med. Biol. 423, 269–288.
Molander, L., Hansson, A., Lunell, E., 2001. Pharmacokinetics of nicotine in healthy elderly people. Clin. Pharmacol. Ther. 69, 57–65.
Morjaria, Y., et al., 2004. In vitro release of nicotine from chewing gum formulations. Dissolution Technolo. 11, 12–15.
Mumtaz, A.M., Ch'ng, H.S., 1995. Design of a dissolution apparatus suitable for in situ release study of triamcinolone acetonide from bioadhesive buccal tablets. Int. J. Pharm. 121, 129–139.
Navazesh, M., et al., 1992. Comparison of whole saliva flow rates and mucin concentrations in healthy Caucasian young and aged adults. J. Dent. Res. 71, 1275–1278.
Patel, V.F., Liu, F., Brown, M.B., 2011. Advances in oral transmucosal drug delivery. J. Control. Release. 153, 106–116.
Pather, S.I., Rathbone, M.J., Senel, S., 2008. Current status and the future of buccal drug delivery systems. Expert Opin. Drug Deliv. 5, 531–542.
Pfister, W., Ghosh, T., 2005. Intraoral delivery systems-an overview, current status, and future trends. In: Pfister, W., Ghosh, T. (Eds.), Drug Delivery to the Oral Cavity-Molecules to Market. CRC Press, Boca Raton, USA, pp. 1–40.
Piade, J.J., D'André s, S., Sanders, E.B., 1999. Sorption phenomena of nicotine and ethenylpyridine vapors on different materials in a test chamber. Environ. Sci. Technol. 33, 2046–2052.
Polli, J.E., 2000. IVIVR versus IVIVC. Dissolut. Technol. 7, 6–9.
Rachid, O., et al., 2011. Dissolution testing of sublingual tablets: a novel in vitro method. AAPS Pharm. Sci. Tech. 12, 544–552.
Rathbone, M.J., Ponchel, G., Ghazali, F., 1996. Systemic oral mucosal drug delivery and delivery systems. In: Rathbone, M.J. (Ed.), Oral Mucosal Drug Delivery. Marcel Dekker Inc., New York, USA, pp. 241–284.
Rehak, N.N., Cecco, S.A., Csako, G., 2000. Biochemical composition and electrolyte balance of unstimulated whole human saliva. Clin. Chem. Lab. Med. 38, 335–343.
Tambwekar, K.R., Kakariya, R.B., Garg, S., 2003. A validated high performance liquid chromatographic method for analysis of nicotine in pure form and from formulations. J. Pharm. Biomed. Anal. 32, 441–450.
Official monograph, 2009a. Ergoloid Mesylate Sublingual Tablets. The United States Pharmacopeia 32 – The National Formulary 27, vol. 2. United States Pharmacopeial Convention Inc., Rockville, USA, pp. 2274–2275.
Official monograph, 2009b. Ergotamine Tartarate Sublingual Tablets. The United States Pharmacopeia 32 – The National Formulary 27, vol. 2. United States Pharmacopeial Convention Inc., Rockville, USA, pp. 2280–2281.
Official monograph, 2009c. Isosorbide Dinitrate Sublingual Tablets. The United States Pharmacopeia 32 – The National Formulary 27, vol. 2. United States Pharmacopeial Convention Inc., Rockville, USA, p. 2716.
Official monograph, 2009d. Nitroglycerine Sublingual Tablets. The United States Pharmacopeia 32 – The National Formulary 27, vol. 2. United States Pharmacopeial Convention Inc., Rockville, USA, pp. 3097–3098.
Van Loy, M.D., et al., 1997. Dynamic behavior of semivolatile organic compounds in indoor air. 1. Nicotine in a stainless steel chamber. Environ. Sci. Technol. 31, 2554–2561.
Wagner, J.G., Nelson, E., 1964. Kinetic analysis of blood levels and urinary excretion in the absorptive phase after single doses of drug. J. Pharm. Sci. 53, 1392–1403.
Wang, Q., Fotaki, N., Mao, Y., 2009. Biorelevant dissolution: Methodology and application in drug development. Dissolut. Technol. 16, 6–12.
Washington, N., Washington, C., Wilson, C., 2001. Drug delivery to the oral cavity or mouth. In: Washington, N., Washington, C., Wilson, C. (Eds.), Physiological Pharmaceutics: Barriers to Drug Absorption. CRC Press, Boca Raton, USA, pp. 37–55.
Zahlsen, K., Nilsen, T., Nilsen, O.G., 1996. Interindividual differences in hair uptake of air nicotine and significance of cigarette counting for estimation of environmental tobacco smoke exposure. Pharmacol. Toxicol. 79, 183–190.