The Relative Buffering Capacities of Saliva and Moist Snuff: Implications for Nicotine Absorption

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Abstract

Commercial moist snuff products are used by placing a portion of tobacco inside the mouth between the inner cheek or lip and gum. Nicotine is absorbed into the blood stream via transfer across various oral membranes including the buccal mucosa (cheek lining). The resulting salivary pH when a given moist snuff product is placed in the mouth is an important factor for nicotine absorption because it will affect the proportion of free base nicotine that is readily available for absorption. The resulting salivary pH for a given moist snuff product will be determined in part by the relative acid-base buffering capacities of the saliva and moist snuff, as well as the pHs of the saliva and moist snuff prior to coming in contact with one another. In the current study, the acid-base buffering capacities (µeq/g) of a series of commercial moist snuff products were determined and compared to the acid-base buffering capacity for unstimulated, whole human saliva. The buffering capacities of the moist snuff products were determined to be 10–20 times higher than the buffering capacity of human saliva. The resulting salivary pH ranges after contact between an artifical saliva and the various moist snuff products were also determined; the results were used to predict the proportion of free base nicotine that can be expected to occur in the mouth during the first few minutes of product use. These studies provide a basis for examining and understanding the effects that moist snuff product pHs and buffering capacities may be expected to have on nicotine absorption.

Introduction

Commercial moist snuff products have traditionally been used by placing a portion or "pinch" of tobacco inside the mouth between the inner cheek or lip and gum. Nicotine is absorbed into the blood stream via transfer across various oral membranes including the buccal mucosa (cheek lining). The rate and extent of nicotine absorption from moist snuff products into the blood stream has been studied (1–4). Plasma nicotine concentrations rapidly increased within the first few minutes of product use (3,4) and reached levels typically obtained from smoking cigarettes within 30 min of product use (2–4). Measurement of plasma nicotine concentrations after a single day of moist snuff consumption also yielded levels similar to cigarette use (1).

One of the primary mechanisms for drug transfer across the oral mucosa is passive diffusion (5–7). One factor that is considered critical for passive diffusion of many drug compounds is the proportion of drug present in the un-ionized or uncharged form (5–8). Un-ionized drugs undergo passive diffusion much more readily than their corresponding ionized forms because of the greater solubility of uncharged molecules in lipophilic cellular membranes. The proportion of drug present in the un-ionized form is determined by the dissociation constant of the drug and the pH of the medium in which the drug is found. This results in a pH dependence for the absorption of many drugs across the oral mucosa and forms the basis for affecting drug delivery via manipulation of the oral pH (9–14).

For nicotine, the un-ionized form is the free base form, and the relevant medium for transfer across the buccal mucosa is saliva. In earlier studies, the pH dependence of nicotine absorption across the buccal mucosa was examined using the "buccal absorption test" in which buffered solutions of nicotine were placed in the mouth (8,15). Nicotine absorption, measured as the disappearance of nicotine from the mouth, increased with increasing pH over the pH ranges tested (pH 5–9). More recently, in vitro studies of nicotine permeation across porcine buccal mucosa demonstrated that the rate of nicotine permeation was pH dependent, increasing exponentially with increasing pH of the donor solution (16). Several commercial pharmaceutical products are formulated with buffers and/or pH adjusters to affect the delivery of nicotine across the buccal mucosa (17–23).

Both commercial moist snuff products and human saliva have finite acid-base buffering capacities. The resulting salivary pH when a given moist snuff product is placed in the mouth will be determined in part by the relative acid-base buffering capacities of the saliva and moist snuff, as well as the pHs of the saliva and moist snuff prior to coming in contact with one another. There have been a number of reports in the literature in which the physical or chemical properties of commercial moist snuff products have been determined and/or compared including nicotine content, product pH, and/or moisture content

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(24–27). Some studies have examined the correlation between smokeless tobacco/moist snuff product pH and nicotine absorption (25,26,28,29). However, we are aware of no studies in which the relative buffering capacities of saliva and commercial moist snuff have actually been measured, or in which the resulting pHs for mixtures of commercial moist snuff and saliva have been determined.

Parts 1 and 2 of this study were designed to measure and compare the pHs and acid-base buffering capacities of unstimulated, whole human saliva and six different brands of commercial moist snuff. The nicotine and moisture contents of the commercial products were also determined. Part 3 of this study was designed to measure the resulting salivary pH after contact between saliva and the six brands of commercial moist snuff under conditions related to actual use. An artificial saliva (30) was used in this part in order to determine the effect of saliva volume, and to compare the resulting salivary pHs for the six different brands. These studies provide a basis for examining and understanding the effects that moist snuff product pHs and buffering capacities may have on nicotine absorption.

Materials and Methods

Overall study design

There were three major parts to the study: (1) determination of acid-base buffering capacity and pH for unstimulated, whole saliva obtained under both restricted and unrestricted consumption conditions; (2) determination of nicotine content, moisture content, product pH, and acid-base buffering capacity for six different brands of commercial moist snuff products; and (3) determination of observed solution pH for mixtures of artificial saliva and commercial moist snuff products under simulated use conditions.

Subjects

Twenty-two adult volunteer subjects ages 24–49 (17 males, 5 females) participated in the first half of the saliva study (restricted consumption). Twenty adult volunteer subjects ages 24–49 (14 female, 6 male) participated in the second half of the saliva study (unrestricted consumption). Both halves included three regular cigarette smokers, one regular chew user, and one occasional cigar smoker. Eleven of the adult volunteer subjects (nine male, two female) were common to both halves. All subjects were employees of the Food and Drug Administration in Cincinnati, OH.

Procedures—part 1

Saliva study first half (restricted consumption). Each subject was assigned to a specific collection time frame (morning or afternoon) and instructed to refrain from eating, drinking, chewing gum, or using any tobacco products for 1 h prior to their scheduled collection time. Each subject provided a saliva sample on two consecutive days at approximately the same time of day (within 50 min). For each subject, one of the saliva samples was titrated to determine acidic buffering capacity and the other saliva sample was titrated to determine basic buffering capacity. The study was designed so that equal numbers of both morning and afternoon saliva samples were used for the determination of acidic buffering capacity and for the determination of basic buffering capacity. The study was conducted over 4 days, with 10 subjects participating the first 2 days, and 12 subjects participating the latter 2 days.

Saliva was collected under unstimulated conditions. Subjects were instructed to spit three times into a specimen cup, wait 5 min, and then spit three more times into the cup. The weight of saliva collected was recorded. After saliva collection, 70 mL of water was added to the specimen cup, and the saliva solution was titrated with standardized acid or base. The pH of the saliva solution was also measured prior to titration for the latter two days of the study.

Saliva study second half (unrestricted consumption). Subjects were called at random to participate in the study. There were no restrictions on eating, drinking, chewing gum, or using tobacco products. However, subjects were asked to record anything they had been eaten, drunk, or consumed 1 h prior to collection. The study was conducted over two days (10 different subjects per day) with samples collected equally divided between morning and afternoon and between determination of acidic buffering capacity and basic buffering capacity. Each subject provided a single saliva sample, and measurement of saliva weight, pH measurement, and acid-base titration were conducted as described in the first half.

Procedures—part 2

Commercial moist snuff: product pH, nicotine content, moisture content, and acid-base buffering capacity. Ten tins of six different commercial moist snuff products were purchased from a retail store in Dallas, TX, in November 1997. In total, six tins per product were used in the study; the other four tins were kept as reserve samples. The study was conducted over six days using one tin per product per day. Immediately after opening the product tin, moist snuff samples were weighed out for determination of moisture content, product pH, nicotine content, acid-base buffering capacity, and for use in the third part of the study (observed solution pH after mixing with artificial saliva). The commercial moist snuff brands were Copenhagen, Skoal Long Cut Classic, Original Fine Cut Wintergreen, Skoal Long Cut Cherry, Skoal Bandits Wintergreen, and Skoal Bandits Straight.

Determination of product pH. Product pH was determined by adding 10 mL water to 1.5 g moist snuff in a glass scintillation vial, sonicating for 2 min, and then measuring the solution pH. For pouch-type moist snuff products (Bandits brands), a second product pH was determined by adding 10 mL water to 1 pouch (which contains about 0.5 g moist snuff), sonicating for 2 min, and then measuring the solution pH. Experiments were conducted using water at ambient temperature (24–27°C), or water preequilibrated to 37°C (three tins per product tested under each set of temperature conditions).

Determination of nicotine content. Nicotine content was determined using reversed-phase ion-pair liquid chromatography (31). Nicotine determinations were made on moist product from freshly opened tins; all values for nicotine content are reported in units of milligrams per gram on a "wet product weight" basis.

Determination of moisture content. Percent nonvolatiles were determined by drying a weighed portion of moist snuff (approximately 1 g) in a laboratory oven to a constant weight (4 h at 105°C), cooling the samples in a desiccator, and reweighing. The percent by weight volatile material (100% – %nonvolatiles) was used as an estimate of percent by weight moisture.

Determination of acidic or basic buffering capacity. For determination of acidic buffering capacity, samples were titrated with standardized sodium hydroxide, and the volume titrant corresponding to a final pH of 8.02 was determined from the titration data. For determination of basic buffering capacity, samples were titrated with standardized hydrochloric acid, and the volume titrant corresponding to a final pH of 6.02 was determined from the titration data.

Both acidic and basic buffering capacity were separately determined for moist snuff products under fairly exhaustive extraction conditions: 0.25–0.35 g moist snuff, 15 mL water, sonication for 30 min. Basic buffering capacity only was determined for moist snuff products under conditions related to actual use: 1 pouch for pouch-type products (Bandits brands), 1.5 g moist snuff (representing a "pinch") for all other products, 10 mL water, sonication for 2 min, and water preequilibrated to 37°C.

Procedures—part 3

Artificial saliva. Artificial saliva was prepared according to the procedure described by Chou and Hee (30). In this approach, the final pH of the saliva is adjusted to pH 7.0 at room temperature. In the present study, the saliva pH was remeasured after equilibration to 37°C and just prior to use in the moist snuff experiments. Observed pHs were 6.97 and 6.89 for two separate batches. A third batch had an observed pH of 6.89, but the pH was subsequently adjusted to pH 5.54 with 0.1M HCl for purposes of the experiment.

Observed pH for mixtures of artificial saliva and commercial moist snuff. For pouch-type moist snuff products (Bandits brands), a specified volume of artificial saliva was added to one product pouch in a glass scintillation vial. For all other moist snuff products, a specified volume of artificial saliva was added to 1.5 g moist snuff (representing a "pinch") in a glass scintillation vial. After addition of the artificial saliva, the vial was sonicated for 2 min, and then the pH of the solution was measured. Saliva volumes of 15 mL, 10 mL, 7.5 mL, 5 mL, and 3 mL were tested. Experiments were conducted using saliva preequilibrated to 37°C and with initial measured saliva pHs of 6.97, 6.89, and 5.54.

Chemicals. Standardized sodium hydroxide and hydrochloric acid solutions (0.05N each) were used for titration. Sodium hydroxide titrant was standardized against potassium acid phthalate (Aldrich ACS Acidimetric Standard). Hydrochloric acid titrant was standardized against previously standardized sodium hydroxide titrant.

Apparatus

pH measurement. All pH measurements were made using a Corning model 245 pH meter with a Corning Rugged Bulb combination electrode (cat. no. 476296). Measurements at 37°C were made using the automatic temperature compensation (ATC) probe. The electrode was calibrated a minimum of once daily.

Measurement of acidic or basic buffering capacity. All titrations were conducted with a Mettler DL 25 Titrator with a Mettler DG 111-SC combination electrode. The electrode was calibrated daily. Acidic buffering capacity was measured by titrating with standardized 0.05N NaOH and obtaining discreet measurements of pH versus titration volume. The acidic buffering capacities were calculated to a pH endpoint of 8.02. Basic buffering capacity was measured by titrating with standardized 0.05N HCl and obtaining discreet measurements of pH versus titration volume. The basic buffering capacity was calculated to a pH endpoint of 6.02.

Statistical tests. All statistical tests (ANOVA, *t*-tests, linear regression) were conducted using SYSTAT software (version 6.0).

Results

Part 1. Unstimulated, whole saliva amount, pH, and buffering capacity

Table I summarizes the measurements made on unstimulated, whole saliva collected throughout the six days of the study. Saliva was collected under both restricted (Days 1 through 4, 22 subjects) and unrestricted (Days 5 and 6, 20 sub-

Table I. Real Saliva Measurements Summary

Day of study*	Amount collected (g)		Saliva pH		Buffering capacity (µeq/g)				
					Acidic (to pH 8.02)		Basic (to pH 6.02)		Total
	average	(range)	average	(range)	average	(range)	average	(range)	(pH 6.02–8.02)
1	1.1	(0.1–2.0)	—	—	7.1	(0.0–23)	6.6	(0.0–14)	14
2	1.3	(0.2–2.7)	—	—	4.5	(0.0–8.4)	6.4	(0.0–11)	11
3	1.5	(0.5–3.2)	6.82	(6.08–7.25)	8.9	(2.4–18)	6.2	(2.6–2.9)	15
4	1.8	(0.6–5.1)	6.80	(6.46–7.18)	5.2	(1.7–7.5)	4.8	(0.0–9.2)	10
5	1.5†	(0.6–3.5)	6.74	(5.97–7.46)	3.5	(0.7–7.0)	9.0	(0.0–20)	13
6	1.3†	(0.3–2.6)	6.37	(5.39–7.03)	3.8	(1.8–6.8)	4.2	(0.0–6.3)	8.0

* Days 1–4 represent restricted consumption conditions. Days 5 and 6 represent unrestricted consumption conditions. Data from days 1 and 2 are based on 10 subjects. Data from days 3 and 4 are based on 12 subjects. Data from days 5 and 6 are based on 10 subjects each. For buffering capacity measurements, half of the subjects were used to measure acidic buffering capacity and half of the subjects were used to measure basic buffering capacity.

† Amount collected data from these days was based on only 9 subjects.

jects) consumption conditions. Of the 20 subjects in the unrestricted consumption portion of the study, 17 subjects consumed food within 1 h prior to providing the saliva sample, and 2 subjects smoked cigarettes. Food intake varied and included chicken, sandwiches, yogurt, rice, muffin, crackers, fruit, and chocolate. Seven of the 17 subjects who consumed food also consumed either a carbonated beverage (4 subjects) or coffee (3 subjects).

Overall, the amount of saliva collected per subject ranged from 0.1 to 5.1 g. The amount of saliva collected under restricted consumption conditions (overall average 1.4 g, overall range 0.1–5.1 g) was similar to the amount collected under unrestricted consumption conditions (overall average 1.4 g, overall range 0.3–3.5 g). For the restricted consumption portion of the study (Days 1 through 4), subjects gave saliva samples on two consecutive days. The amount of saliva collected for a given subject was similar from one day to the next, suggesting that the amount of saliva was dependent on the subject.

Table I also provides the averages and ranges for saliva pH collected under both restricted and unrestricted consumption conditions. Under unrestricted consumption conditions (Days 5 and 6), the pH ranges extended below pH 6.0, and the average pHs were slightly lower compared to restricted consumption conditions (Days 1 through 4). The average saliva pH from the seven subjects who consumed either a carbonated beverage or coffee prior to providing saliva samples was pH 6.15 (range 5.39–6.79). The lower pHs observed for these subjects could be due to the consumption of acidic beverages prior to providing saliva samples; however the data is limited and does not include a saliva pH measurement prior to consumption of the beverage. The overall average pH (Days 1 through 6) for unstimulated, whole saliva was pH 6.69, with an overall pH range of 5.39–7.46 (32 subjects). This range is consistent with reported pH ranges for unstimulated saliva (32–34).

For each day of the study, half of the saliva samples were used to measure acidic buffering capacity (pH endpoint 8.02), and half of the saliva samples were used to measure basic buffering capacity (pH endpoint 6.02). These pH endpoints were chosen for study based on the pH properties of commercial moist snuff products and the acid-base properties of nicotine (see Discussion section for a more detailed explanation).

All acidic buffering capacities for unstimulated, whole saliva, whether under restricted or unrestricted consumption conditions, fell within the range 0.0–23 µeq/g (see Table I). The overall average under restricted consumption conditions was 6.6 µeq/g (22 subjects), and under unrestricted consumption conditions was 3.7 µeq/g (10 subjects). Thus, although the subjects consumed food and beverages which may have been acidic, no increase in acidic buffering capacity was observed under unrestricted consumption conditions. All basic buffering capacities for unstimulated, whole saliva, whether under restricted or unrestricted consumption conditions, fell within the range 0.0–20 µeq/g (see Table I). The overall average under restricted consumption conditions (6.0 µeq/g, 22 subjects) was similar to the overall average under unrestricted consumption conditions (5.7 µeq/g, 10 subjects).

The total acid-base buffering capacity for unstimulated, whole saliva over the range pH 6.02 to 8.02 was calculated by summing the acidic and basic buffering capacities (see Table I, last data column). No trends associated with morning versus afternoon collection were observed. The overall average total buffering capacity obtained under restricted consumption conditions (12 µeq/g) was slightly higher than that obtained under unrestricted consumption conditions (9.2 µeq/g). The overall range was 8.0–15 µeq/g.

It has been reported that approximately 60% of the buffer capacity for unstimulated saliva is due to its bicarbonate content (35). Values for the measurement of bicarbonate in unstimulated saliva have been reported in the range 2–13 meq/L or mmol/L (33,35). Correcting for the specific gravity of saliva (1.00–1.02) (32,33) and applying the proper unit conversions, the overall range for total buffering capacity (pH 6.02–8.02) of unstimulated saliva from the current study was 8.1–15 meq/L.

Table II. Commercial Moist Snuff Product Properties

Product	Product pH* 37°C	Product pH* ambient	Nicotine content† (mg/g)	Moisture content† (percent by weight)	Buffering capacity (µeq/g)‡ Acidic (to pH 8.02) average	Acidic (to pH 8.02) (range)	Basic (to pH 6.02) average	Basic (to pH 6.02) (range)	Total (pH 6.02–8.02)
Skoal Long Cut Classic	8.04	8.10	13.7	56%	0.0	(all 0.0)	170	(120–220)	170
Copenhagen	7.99	7.98	13.9	54%	11	(0.0–36)	170	(110–210)	180
Skoal Original Fine Cut Wintergreen	7.18	7.28	13.6	56%	38	(26–51)	70	(48–86)	110
Skoal Long Cut Cherry	7.26	7.31	12.6	55%	35	(27–47)	81	(63–93)	120
Skoal Bandits Wintergreen (1.5 g)	6.45	6.37	9.7	51%	54	(51–58)	56	(49–60)	110
Skoal Bandits Wintergreen (pouch)	6.46	6.40							
Skoal Bandits Straight (1.5 g)	5.43	5.38	11.6	51%	130	(130–140)	0.0	(all 0.0)	130
Skoal Bandits Straight (pouch)	5.54	5.47							

* Values are average of measurements from 3 product tins at each temperature.
† Values are average of measurements from 6 product tins. Nicotine content reported on a "wet weight" basis.
‡ Buffering capacities measured under "exhaustive" extraction conditions at ambient temperature. See text for discussion. Values are average of measurements from 6 product tins.

Part 2. Commercial moist snuff: product pH, nicotine content, moisture content, and acid-base buffering capacity

Product pH, nicotine content, moisture content, and acid-base buffering capacity for six commercial brands of moist snuff are given in Table II. For comparison, pH measurements were obtained under both ambient conditions and at 37°C; only slight differences in measured pH values were obtained. Two products (Skoal Long Cut Classic and Copenhagen) were observed to have a higher pH (pH near 8.0), two products (Skoal Original Fine Cut Wintergreen and Skoal Long Cut Cherry) had intermediate pHs (pH 7.2–7.3), and two products (Bandits Wintergreen and Bandits Straight) had lower pHs (acidic, pH 6.5 or 5.5). The nicotine contents of the six products ranged from 9.7 to 13.9 mg/g, and the moisture content ranged from 51 to 56%. The two Bandits brands had both the lowest nicotine and lowest moisture contents.

The acidic, basic, and total buffering capacities for the six commercial moist snuff products are given in an analogous manner to the saliva results. For these measurements, the moist snuff product was extracted under fairly exhaustive conditions (0.25–0.35 g moist snuff, 15 mL water, sonication for 30 min). The six products could be divided into two groups with respect to their average total acid-base buffering capacities: (1) those products with a higher total acid-base buffering capacity in the range 170–180 µeq/g, which included Copenhagen and Skoal Long Cut Classic, and (2) those products with a lower total acid-base buffering capacity in the range 110–130 µeq/g, which included Skoal Original Fine Cut Wintergreen, Skoal Long Cut Cherry, Skoal Bandits Wintergreen, and Skoal Bandits Straight. The highest acidic buffering capacity (130 µeq/g) was obtained for Skoal Bandits Straight, the product with the lowest pH. The highest basic buffering capacities (both 170 µeq/g) were obtained for Skoal Long Cut Classic and Copenhagen, the products with the highest pHs. Because the buffering capacities of the moist snuff products were 10–20 times higher than the buffering capacity range for saliva, it is feasible that these moist snuff products can affect the pH of saliva depending on the relative amounts of moist snuff and saliva, the pHs of the moist snuff product and saliva prior to contacting one another, and other factors.

Additional measurements of the basic buffering capacities for the six commercial moist snuff products were made under conditions simulating the first few minutes of product use (1 pouch for Bandits pouch-type products, 1.5 g "pinch" moist snuff for all other products, 10 mL water, sonication for 2 min, water preequilibrated to 37°C). Results are given in Table III. Under these conditions, the basic buffering capacities were 0.0 or 5.1 µeq/pouch for the two Bandits products, and ranged from 48 to 102 µeq/"pinch" for the other four products. The basic buffering capacities obtained under these latter conditions are also expressed in microequivalents per gram (Table III) in order to allow a comparison with the basic buffering capacities reported in Table II. The buffering capacity values in the latter experiment are lower which, given the shorter extraction time, probably indicates incomplete extraction of the components with acid-base buffering capacity. However, the product trends are the same in both experiments with the highest basicity observed for Copenhagen and Skoal Long Cut Classic; intermediate basicity for Skoal Original Long Cut Wintergreen and Skoal Long Cut Cherry; and the least basicity for the two Bandits products.

Table III. Commercial Moist Snuff Basic Buffering Capacity Under Simulated Use Conditions*

Product	Basic buffering capacity 37°C (to pH 6.02) µeq/g Average	(Range)	µeq/pinch	µeq/pouch
Skoal Long Cut Classic	68	(50–81)	102	—
Copenhagen	57	(39–67)	86	—
Skoal Original Fine Cut Wintergreen	32	(35–36)	48	—
Skoal Long Cut Cherry	34	(31–40)	51	—
Skoal Bandits Wintergreen	10	(9.7–12)	—	5
Skoal Bandits Straight	0.0	(all 0.0)	—	0

* Buffering capacities measured under conditions simulating first few minutes of product use. See text for discussion. Values are average of measurements from three product tins.

Table IV. Artificial Saliva pH and Acid-Base Buffering Capacity*

Batch	Saliva pH (37°C)	Buffering capacity (µeq/g) Acidic (to pH 8.02)	Basic (to pH 6.02)	Total (pH 6.02–8.02)
1	6.97	4.1	1.2	5.3
2	6.89	4.0	6.8	11
3	5.54	3.1	6.2	9.3

* Buffering capacity measured under ambient conditions (all batches), and prior to pH adjustment for Batch 3 only.

Part 3. Determination of observed solution pH for mixtures of artificial saliva and commercial moist snuff

The pH and acid-base properties for the three batches of artificial saliva used in this part of the study are summarized in Table IV. The artificial saliva pHs and total acid-base buffering capacities (pH 6.02–8.02) are representative of the ranges reported for unstimulated, whole saliva (32–35).

Figure 1 shows the observed pHs that resulted for mixtures of artificial saliva with the six commercial tobacco products (initial saliva pH 6.97). pH was measured on the liquid portion after 2 min of contact between the saliva and the moist snuff (samples sonicated during that period). Saliva volumes ranging from 3 to 15 mL were tested, and the saliva was preequilibrated to 37°C. One "pinch" (1.5 g) or pouch (containing about 0.5 g) of moist snuff was used. The product pH for the specific tin of product used in the experiment is given on the *y*-axis (i.e., saliva volume = 0 mL). The results show that the pH of the saliva is altered after contact with the moist snuff and is similar to the product pH at the lower saliva volumes. This experiment was repeated with a second batch of artificial saliva and second set of tins for each product (initial saliva pH 6.89). Similar results were obtained.

In order to test the influence of a more acidic saliva pH, the

experiment was conducted with a third batch of saliva with an initial saliva pH of 5.54 (Figure 2). The buffering capacities for the four moist snuff products with basic pHs (Copenhagen, Skoal Long Cut Classic, Skoal Long Cut Wintergreen, and Skoal Long Cut Cherry) were sufficient to overcome the acidity of the saliva, and to maintain a resulting saliva pH at or near the product pH over the entire range of saliva volumes tested. The resulting saliva pH for Skoal Bandits Wintergreen was intermediate between the product pH (6.48) and the initial saliva pH (5.54) over the entire range. For Skoal Bandits Straight, the product pH (5.53) was similar to the initial saliva pH (5.54), and all of the resulting saliva pHs were measured at pH 5.3. This pH is slightly lower than either the product pH or the initial saliva pH, which could indicate that a chemical reaction occurred.

In order to further examine the influence of the initial saliva pH, the data curves obtained in the experiments with saliva pHs near 7.0 are overlaid with the data curves obtained in the saliva pH 5.54 experiment (Figures 3A and 3B). The comparison is made for product tins with matching manufacturing code dates in order to minimize or eliminate lot to lot variations in product pH; hence, the comparison was possible for five of the six products only. Copenhagen and Skoal Long Cut Classic, the two products with the higher pHs and buffering capacities had a marked effect on saliva pH. For these products, the resulting saliva pH after contact with the moist snuff ranged from pH 7.1 to 8.0 correlating with the individual tin product pHs which ranged from pH 7.7 to 8.3. This correlation was observed regardless of whether the starting pH of the saliva was neutral (pH 6.9–7.0) or acidic (pH 5.5) as shown in Figures 3A (Copenhagen) and 3B (Skoal Long Cut Classic).

The resulting saliva pH was also influenced by saliva volume; as the volume of saliva was increased, the resulting saliva pHs gradually became less basic. These results make sense because of the relative amounts of moist snuff and saliva tested in the experiment: 1.5 g moist snuff, representing a "pinch", and from 3 mL to 15 mL saliva. For Copenhagen and Skoal Long Cut Classic, the basic buffering capacity measured under the conditions of the mixed moist snuff/saliva was 86 or 102 µeq, respectively (see Table III). For the three batches of saliva used in the study, the total µeq of acid-base buffering capacity should range from 15 to 33 µeq for 3 mL saliva, and from 75 to 165 µeq for 15 mL saliva (based on results from Table IV, calculated

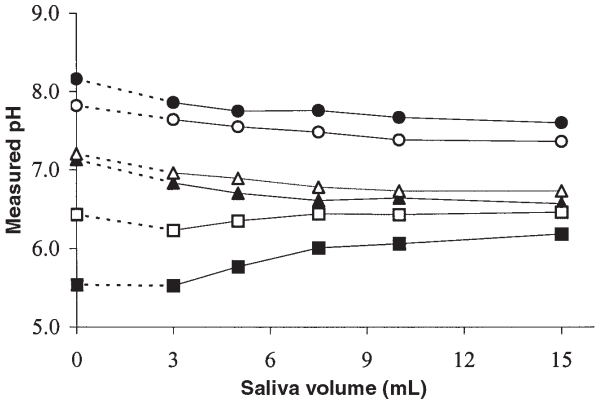

Figure 1. Resulting saliva pHs for commercial moist snuff products as a function of saliva volume with an initial saliva pH of 6.97. The product pH for the specific tin used in the experiment is given on the y-axis. Products: Copenhagen (●); Skoal Long Cut Classic (○); Skoal Long Cut Cherry (△); Skoal Original Fine Cut Wintergreen (▲); Skoal Bandits Wintergreen (□); Skoal Bandits Straight (■).

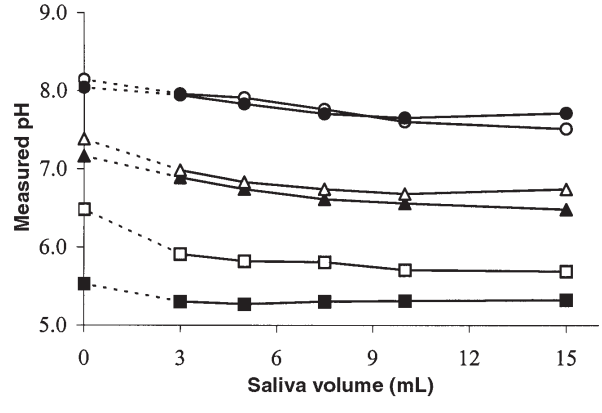

Figure 2. Resulting saliva pHs for commercial moist snuff products as a function of saliva volume with an initial saliva pH of 5.54. The product pH for the specific tin used in the experiment is given on the y-axis. Products: Copenhagen (●); Skoal Long Cut Classic (○); Skoal Long Cut Cherry (△); Skoal Original Fine Cut Wintergreen (▲); Skoal Bandits Wintergreen (□); Skoal Bandits Straight (■).

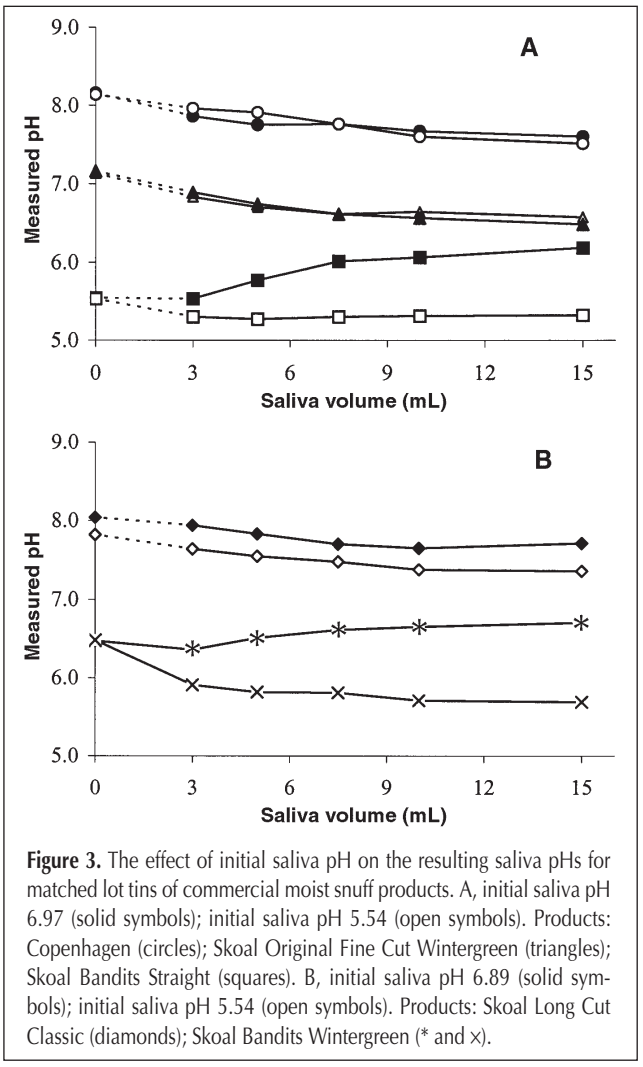

Figure 3. The effect of initial saliva pH on the resulting saliva pHs for matched lot tins of commercial moist snuff products. A, initial saliva pH 6.97 (solid symbols); initial saliva pH 5.54 (open symbols). Products: Copenhagen (circles); Skoal Original Fine Cut Wintergreen (triangles); Skoal Bandits Straight (squares). B, initial saliva pH 6.89 (solid symbols); initial saliva pH 5.54 (open symbols). Products: Skoal Long Cut Classic (diamonds); Skoal Bandits Wintergreen (* and ×).

using a saliva density of 1.0 g/mL). Thus, the total microequivalents of acid-base buffering capacity from saliva becomes more significant with respect to the moist snuff buffering capacity as the saliva volume increases. The current study tested saliva volumes up to 15 mL. The actual volume of saliva that contacts the moist snuff product when it is first placed in the mouth may be less. In Part 1 of the current study, the maximum volume of unstimulated, whole saliva collected from one subject was 5 mL, and the overall average was less than 1.5 mL (see Table I).

The remaining four products (Skoal Original Fine Cut Wintergreen, Skoal Long Cut Cherry, Skoal Bandits Wintergreen, and Skoal Bandits Straight) had similar buffering capacities to one another, but differed according to their product pHs. The two products Skoal Original Fine Cut Wintergreen and Skoal Long Cut Cherry had slightly basic product pHs with individual tins ranging from pH 7.1 to 7.4. For these products, the resulting saliva pH after contact with the moist snuff ranged from pH 6.5 to 7.0, again regardless of whether the saliva was initially "neutral" or "acidic" (see Figure 3A, results shown for Skoal Original Fine Cut Wintergreen only). The effect of saliva volume was similar to that observed for Copenhagen and Skoal Long Cut Classic.

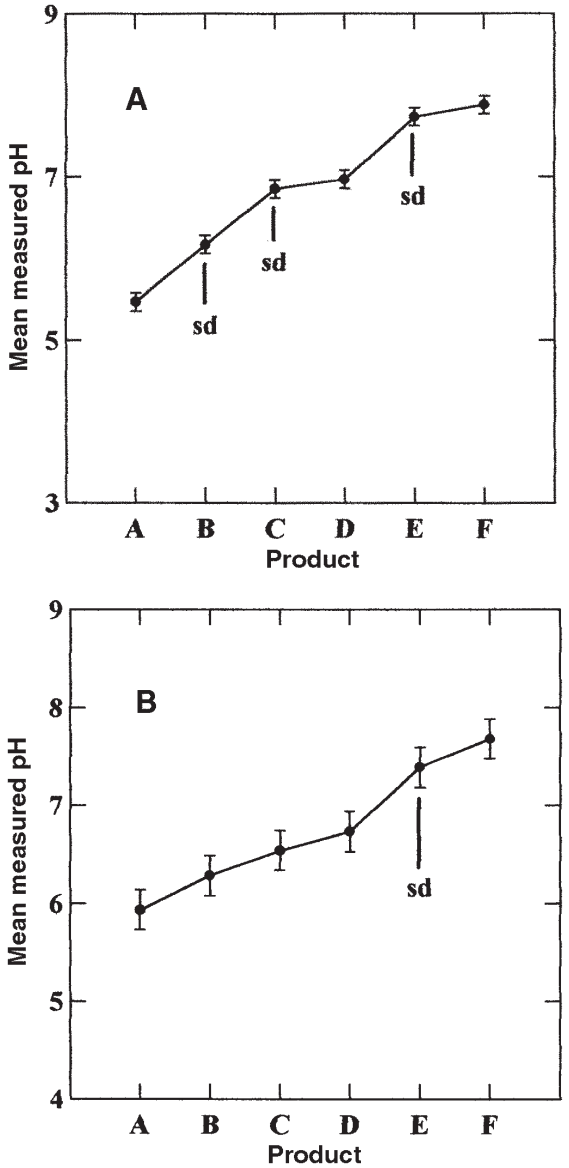

Figure 4. ANOVA least-square means and residuals for the six commercial moist snuff products. A, 3-mL saliva volume experiment; significant differences (p = 0.05, 95% confidence) between products with adjacent means indicated in figure. B, 15-mL saliva volume experiment; significant differences observed between the two high-pH products and the two low-pH products. Product identification: A, Skoal Bandits Straight; B, Skoal Bandits Wintergreen; C, Skoal Original Fine Cut Wintergreen; D, Skoal Long Cut Cherry; E, Copenhagen; and F, Skoal Long Cut Classic.

Finally, Skoal Bandits Wintergreen and Skoal Bandits Straight had acidic product pHs with individual tins ranging from pH 6.4 to 6.5 and pH 5.5 to 5.6, respectively. For these products, the resulting saliva pH after contact with the moist snuff varied according to the initial pH of the saliva relative to the product pH and saliva volume. This is especially evident from the data curves in Figures 3A (Skoal Bandits Straight) and 3B (Skoal Bandits Wintergreen). It is important to note that the Bandits products were tested using one pouch, which contains approximately 0.5 g of moist snuff or about one third of the amount used for the other four products. The lower weight of moist snuff for the pouch products is expected to decrease the total microequivalent buffering capacity available in this experiment by a factor of 3 relative to the "pinch" products.

In order to determine if the resulting salivary pHs observed for the six products in the three batches of artificial saliva represented statistically significant differences, the data was subjected to statistical analysis. p = 0.05 (95% confidence) was taken as the minimum confidence level for defining significant differences. A separate analysis of variance (ANOVA) was conducted for each of the saliva volumes tested using resulting salivary pH as the dependent variable and product brand as the independent variable. Statistically significant differences were observed among the six products at all of the saliva volumes tested (F-ratios ranged from 69.58 for 3 mL experiment to 10.59 for 15 mL experiment; df_{num} = 5; df_{den} = 12). ANOVA least square means and residuals for the six products are plotted in Figures 4A and 4B for the lowest (3 mL) and highest (15 mL) saliva volumes tested, respectively.

Pairwise comparisons (two sample t-tests) between product brands with adjacent means for the 3-mL saliva volume experiment showed statistically significant differences (see Figure 4A) between Copenhagen and Long Cut Cherry (t = 4.3, df = 4); between Original Fine Cut and Bandits Wintergreen (t = 5.1, df = 4); and between Bandits Wintergreen and Bandits (t = 4.5, df = 4). As saliva volume increased, the differences between product brands with adjacent means decreased, and for the 15-mL saliva volume experiment, no statistically significant differences (p = 0.05, 95% confidence) were observed between "adjacent product brands". However, statistically significant differences were observed in pairwise comparisons between either of the two high pH products (Copenhagen and Long Cut Classic) and either of the two low pH products (Bandits Wintergreen and Bandits Straight) at all saliva volumes tested (see Figure 4B for 15 mL experiment).

A larger variation in individual tin pH was observed for both of the high pH products (Copenhagen and Long Cut Classic) relative to the other four products. For example, the individual tin

product pH for Copenhagen in the first experiment (Figure 1) was pH 8.16. The individual tin product pH for Copenhagen in the second experiment (data not shown) was pH 7.66. The resulting saliva pHs were observed to track the individual tin product pH. As a result, the correlation between resulting salivary pH and individual tin pH was examined.

Resulting saliva pH versus individual tin pH is plotted in Figures 5A and 5B for the 3-mL and 15-mL saliva volume experiments, respectively. Inspection of the plots shows an obvious correlation across the six products between resulting saliva pH and individual tin pH for the 3-mL saliva volume (linear regression gave a correlation coefficient [*R*] of 0.986). However, the correlation between resulting saliva pH and individual tin pH appears to be product dependent for the 15-mL saliva volume. Little to no correlation was observed for the two Bandits products, whereas a noticable correlation was still observed for the other four products. This interpretation was confirmed by testing the correlation both with and without the two Bandits products: (1) $R = 0.873$ (15 mL data, all 6 products) and (2) $R = 0.975$ (15 mL data, excluding the two Bandits products). Recall that the two Bandits products had the least basic buffering capacity (see Table III) and were tested with a single tobacco pouch, which contains only about 0.5 g of tobacco.

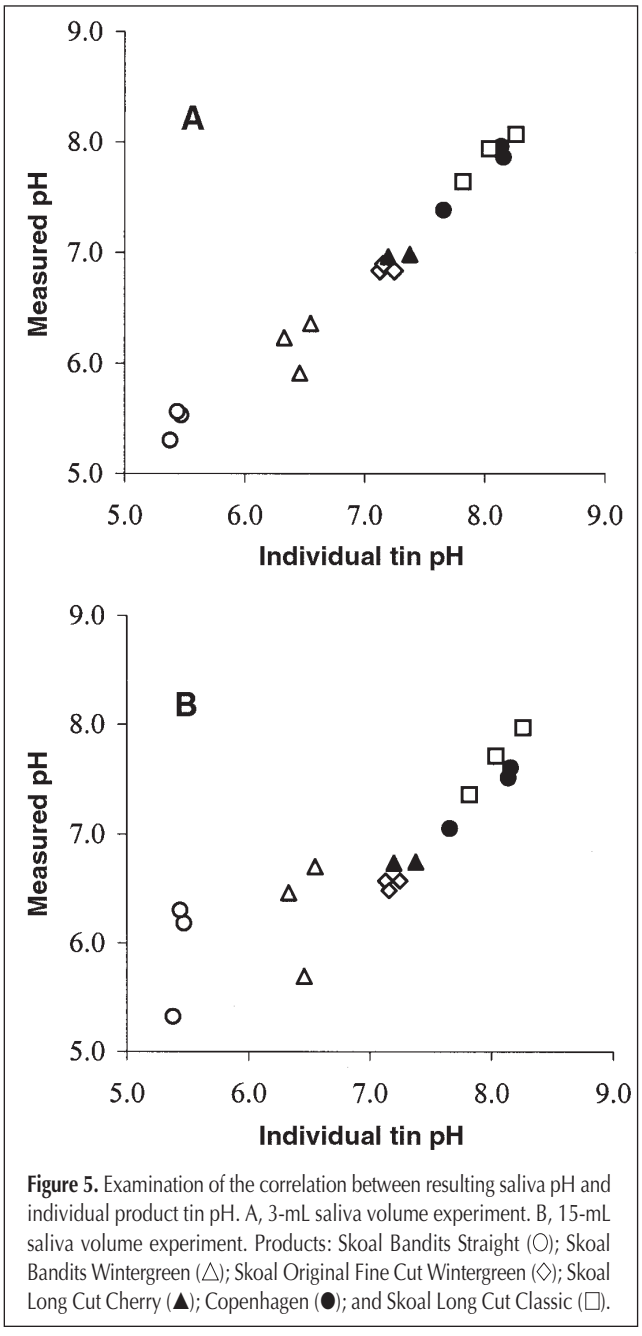

Figure 5. Examination of the correlation between resulting saliva pH and individual product tin pH. A, 3-mL saliva volume experiment. B, 15-mL saliva volume experiment. Products: Skoal Bandits Straight (○); Skoal Bandits Wintergreen (△); Skoal Original Fine Cut Wintergreen (◇); Skoal Long Cut Cherry (▲); Copenhagen (●); and Skoal Long Cut Classic (□).

Discussion

Nicotine is a diprotic base with pK_as of 3.12 (pyridine ring) and 8.02 (pyrrolidine ring) (36). The higher pK_a of 8.02 is associated with conversion between the monoprotonated and free base forms of nicotine. In model experiments with porcine buccal mucosa, the free base form was demonstrated to permeate the buccal membrane much more readily than the monoprotonated form (16). In solutions or media at pH 6.02 or below, the proportion of free base nicotine will be virtually zero. As the pH increases, the proportion of free base nicotine will increase according to the Henderson-Hasselbach equation. The steepest increase in the nicotine free base proportion occurs above pH 7.0 and reaches a proportion of 50% when the pH equals the pK_a of 8.02.

Based on measurements taken in the current and other studies (24–26), moist snuff products with average product pHs in the range 5.4–8.2 are commercially available. On the upper pH end, individual product tins have been observed with product pHs near 9.0 (24,25). As demonstrated in the current study, resulting saliva pHs were observed in the pH range 5.3–8.1 when varying volumes of saliva were mixed with commercial moist snuff products. Thus, the pH range 6.02–8.02 is meaningful with respect to the absorption of nicotine from commercial moist snuff products.

In studying the absorption of nicotine from commercial moist snuff products, it is important to consider the nature and role of human saliva, including its pH, buffering capacity, rate of pro-

Table V. Summary of Literature Measurements for Unstimulated and Stimulated Saliva

	Unstimulated saliva		Stimulated saliva		
Parameter	average	range or std. dev.	average	range or std. dev.	(Reference)
pH	6.7	5.6–7.6	7.4	7.2–7.6	(32)
	6.4	5.8–7.1	—	—	(33)
	—	—	7.07	6.37–7.57	(37)
	7.34	6.62–8.54	—	—	(34)
Buffering capacity (meq/L or mmol/L) as bicarbonate	—	2–13	—	up to 60	(33)
	2.9	±2.4	21	±2.4	(35)
Rate of production (mL/min)	0.57	0.1–1.8	1.9	0.4–4.8	(32)
	—	0.35–0.38	—	0.5–7.0	(33)
	0.29	±0.15	1.8	±0.8	(35)
	—	—	—	0.3–2.6	(37)

duction, and how these parameters change as a function of stimulation. Table V provides a summary of pH, buffering capacity, and production rates given in the literature (32–35,37) for unstimulated and stimulated saliva. Stimulated saliva is generally reported (32,38,39) to have a higher average pH (above 7.0) relative to unstimulated saliva (in the range 6.0–7.0), although a couple of more recent reports have not shown this trend (34,37). The buffering capacity is also higher for stimulated saliva, reported in the range 20–60 meq/L versus a range of 2–13 meq/L for unstimulated saliva (33,35); buffering capacity reported as bicarbonate content. Finally, production rates for stimulated saliva are reported (32–35,37) in the range 0.3–7.0 mL/min, compared to 0.06–1.8 mL/min. for unstimulated saliva (32,33,35). The average rate of production for stimulated saliva is reported as 1.8–1.9 mL/min (32,35).

When moist snuff products are placed in the mouth, the relative contributions of the product and saliva to the resulting salivary pH are likely to change as a function of time as the production of saliva is stimulated. Given the production rates for stimulated saliva, it will take several minutes to accumulate significant volumes of saliva. During these first few minutes of product use, the impact of the product pH and buffering capacity properties is expected to be the greatest. These first few minutes of product use are important because they represent the initial "hit" of the product. It has been demonstrated that nicotine is rapidly absorbed into the bloodstream from U.S. moist snuff products (3), producing peak blood nicotine concentrations within 5 min of placing the product in the mouth.

The current mixed saliva/moist snuff experiments were conducted under conditions that are most related to the first few minutes of product use. An artificial saliva was used which most closely models unstimulated saliva. These studies have demonstrated the in vitro effects of moist snuff products on saliva pH and predict that different moist snuff products will produce different salivary pHs within the first few minutes of contact within the mouth. The resulting salivary pH will affect the proportion of the total nicotine that is present in the free base form.

Experiments with commercial moist snuff products in direct contact with artificial saliva have shown that nicotine is rapidly released from the tobacco, with 90% or more of the total nicotine in the tobacco portion released within the first minute of contact (29). Experiments in our laboratory (unpublished) have shown that a substantial portion of the total nicotine is released from moist snuff products immediately upon contacting aqueous media such as water and artificial saliva (first measurements taken after 5 s of contact). Given the rapid release of nicotine from the tobacco into saliva within the first few minutes of contact, it is predicted that when moist snuff is placed in the mouth, a nicotine concentration gradient is rapidly established in which the initial percent free base proportion will vary according to the specific product pH and product buffering capacity characteristics. Nicotine will be absorbed more rapidly from products which produce higher nicotine free base proportions.

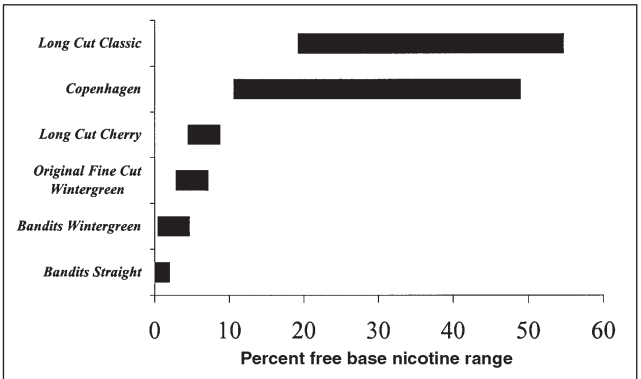

Figure 6. Percent free base nicotine ranges calculated for commercial moist snuff products based on the resulting saliva pH ranges measured after 2 min of contact between the moist snuff and saliva.

Given the ranges of resulting saliva pHs which were observed for the various products, these products can now be compared for the proportion of free base nicotine which can be expected to occur in the mouth during the first few minutes of product use. These results are depicted in Figure 6, in which a range of percent free base nicotine is calculated for each product based on the range of resulting saliva pHs which was observed for that product. These ranges encompass all of the variables tested including the variations in the initial saliva pHs and the full range of saliva volumes. The nicotine free base proportion is greatest for Skoal Long Cut Classic and Copenhagen ranging from 11 to 55%. The nicotine free base proportion for all four of the other products is less than 10%; the nicotine free base proportion for Skoal Bandits Straight is the least at 0–2%. The different ranges of percent free base nicotine predicted for the various commercial moist snuff products are expected to affect the relative absorption of nicotine from these products.

Because the percent free base nicotine depends on the solution pH relative to the pK_a of nicotine, a given pH range can result in either a narrow or wide percent free base nicotine range. For example, the resulting saliva pH range for both of the Bandits products was 1 full pH unit (pH 5.7–6.7 for Bandits Wintergreen and pH 5.3–6.3 for Bandits Straight), but the corresponding ranges in percent free base nicotine are narrow as shown in Figure 6. By contrast, the resulting saliva pH ranges for Skoal Long Cut Straight and Copenhagen are both less than 1 pH unit (pH 7.4–8.1 for Skoal Long Cut Straight and pH 7.1–8.0 for Copenhagen), but the corresponding ranges in percent free base nicotine are wide (see Figure 6).

Other product characteristics or product use factors are expected to affect the rate and extent of nicotine absorption by affecting the rate and extent of nicotine release from the tobacco. Experiments with a pouch type product showed that the effect of the pouch was to slow the release of nicotine during the first few minutes of contact between the tobacco-containing pouch and saliva (29). The nicotine content of the product and the actual size of the "pinch" placed in the mouth will determine the total nicotine amount available for release. The cut of tobacco (long vs. fine) should affect the exposed surface area of the tobacco, resulting in faster absorption for higher exposed surface area. The extent to which the user works the "pinch" should also affect the release rate, and some of the nicotine that has been released from the tobacco will be lost if/when the user expectorates or swallows. There is also some evidence that the buccal mucosa barrier itself is altered during use of smokeless

tobacco products (40–42), affecting its permeability to nicotine (40).

Physiological factors will also play a role in nicotine absorption. The specific pH and buffering capacity characteristics of a particular user's saliva, as well as the amount of saliva contacting the tobacco, should affect the resulting salivary pH. As discussed, the impact of these characteristics on the resulting salivary pH is likely to increase with time because of saliva stimulation as the moist snuff product remains in the mouth. Other physiological factors that should also affect nicotine absorption include the surface area of the buccal tissues and the rate of blood flow to the tissues. As a whole, these physiological factors can be expected to have an impact on the rate and extent of nicotine absorption for a given user from a given moist snuff product. However, these physiological factors would be expected to have a lesser impact for a given user who is switching among various commercial moist snuff products or brands. For this latter scenario, the physiological factors may be relatively constant, whereas the moist snuff product characteristics or factors vary from brand to brand. These product factors are expected to have a direct impact on the rate and extent of nicotine absorption.

Conclusions

The resulting salivary pH when a given moist snuff product is placed in the mouth will be determined in part by the relative acid-base buffering capacities of the saliva and moist snuff, as well as the pH of the saliva and moist snuff prior to coming in contact with one another. The salivary pH which results throughout product use will affect the proportion of free base nicotine which is readily available for absorption across the buccal mucosa. In the current study, the acid-base buffering capacities (µeq/g) of a series of commercial moist snuff products were determined to be 10–20 times higher than the acid-base buffering capacity of unstimulated, whole human saliva. The resulting salivary pH ranges after contact with moist snuff varied among the different commercial moist snuff products tested and were used to predict the proportion of free base nicotine that can be expected to occur in the mouth during the first few minutes of product use. The nicotine free base proportion was greatest for Skoal Long Cut Classic and Copenhagen, ranging from 11 to 55% regardless of whether the initial pH of the saliva was neutral or acidic. The nicotine free base proportion for the other four products tested in the study (Skoal Original Fine Cut Wintergreen, Skoal Long Cut Cherry, Skoal Bandits Wintergreen, and Skoal Bandits Straight) was less than 10%; Skoal Bandits Straight had the least free base proportion at 0–2%. Given the rapid release of nicotine from moist snuff products, it is expected that nicotine will be absorbed more rapidly from products which produce higher nicotine free base proportions.

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