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Chemical Characterization of Dissolvable Tobacco Products Promoted To Reduce Harm

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ABSTRACT: In 2009, the R. J. Reynolds Tobacco Co. released a line of dissolvable tobacco products that are marketed as an alternative to smoking in places where smoking is prohibited. These products are currently available in Indianapolis, IN, Columbus, OH, and Portland, OR. This paper describes the chemical characterization of four such products by gas chromatography−mass spectrometry (GC-MS). The dissolvable tobacco products were extracted and prepared by ultrasonic extraction using acetone, trimethylsilyl derivatization, and headspace solid phase microextraction (SPME). The following compounds were identified in the dissolvables using either ultrasonic extractions or trimethylsilyl derivatization: nicotine, ethyl citrate, palmitic acid, stearic acid, sorbitol, glycerol, and xylitol. The following compounds were identified in the dissolvables using headspace SPME: nicotine, ethyl citrate, cinnamaldehyde, coumarin, vanillin, and carvone. With the exception of nicotine, the compounds identified thus far in the dissolvables are either flavoring compounds or binders. The concentration of free nicotine in the dissolvables was determined from the Henderson−Hasselbalch equation and by measuring the pH and nicotine concentration by GC-MS. The results presented here are the first to reveal the complexity of dissolvable tobacco products and may be used to assess potential oral health effects.

KEYWORDS: dissolvable tobacco, solid phase microextraction, Camel Orbs, nicotine, tobacco ingredients

INTRODUCTION

The chemical composition of smokeless tobacco and its effect on health is a well-researched area. For example, several authors have discussed smokeless tobacco products, such as moist snuff, with a particular emphasis on nicotine content[1−7] as well as the presence of tobacco-specific nitrosamines[2,3,5,6,8] and toxic metals.[9] A new development in the smokeless tobacco market occurred in 2009, when the R. J. Reynolds Tobacco Co. released a line of "dissolvable" tobacco products that are currently still in test markets in Indianapolis, IN, Columbus, OH, and Portland, OR. These products are marketed as an alternative to smoking in places where smoking is prohibited.

According to the manufacturer, dissolvable tobacco products are smokeless, spit-free, made from finely milled tobacco, and come in three forms: Camel Orbs, Camel Sticks, and Camel Strips. The dissolvables contain less moisture and salt than moist snuff and, therefore, do not require the user to spit. Orbs are small, oval-shaped pellets that last ∼15 min and come in Mellow and Fresh flavors. Sticks resemble toothpicks, last ∼10−30 min, and come in Mellow flavor. Strips are brown tobacco strips similar to breath freshening strips, last ∼3 min, and come in Fresh flavor. The users of the dissolvable tobacco products are not supposed to swallow the Orb, Stick, or Strip, but allow the tobacco product to dissolve in the mouth. Orbs are used by placing the pellet between the lip and gum, Sticks are to be held like a toothpick or broken into pieces and placed between the lip and gum, and Strips are to be placed on the tongue like a breath strip or placed between the lip and gum.

Concerns have been raised as to whether dissolvable tobacco products are in fact a safe alternative to cigarettes.[10] A particular concern about some dissolvable products is the potential for harming children through unintentional poisoning.[11] For example, the packaging and design of the dissolvables may also appeal to children, and some dissolvables, such as Orbs, may be mistaken for candy. However, the product literature indicates that the level of nicotine in these products is significant (0.6 mg for a Strip, 1 mg for an Orb, and 3.1 mg for a Stick).[11] One independent analysis of the amount of nicotine in an Orb determined that there was 0.83 mg of nicotine per pellet.[11] Nicotine adsorption by the user largely depends on the pH of the dissolvable tobacco product. With increasing pH, more free-base nicotine is present, and therefore more nicotine is absorbed by the user.[7] By measurement of the pH and total nicotine concentration, the percentage of un-ionized (free) nicotine can be calculated from the Henderson−Hasselbalch equation[12]

$$\mathrm{pH} = \mathrm{p}K_a + \log \frac{[\mathrm{B}]}{[\mathrm{BH}^+]}$$

where the pK_a of nicotine is 8.02.

To date, there has not been any published research on mouth diseases that may result from use of the dissolvables, the effect of swallowing a dissolvable, or the effect of using a dissolvable in combination with smoking. Of particular concern are the adverse complications that could result in the oral cavity as a consequence of prolonged use of dissolvable tobacco products. One obstacle to research in this area is an overall lack of information on the chemical composition of the dissolvable tobacco products. In this paper, the components present in the dissolvable tobacco products are analyzed and reported. Chemical characterization of

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the dissolvables was carried out using gas chromatography−mass spectrometry (GC-MS). Three different sample preparation techniques were used: ultrasonic extraction, trimethylsilyl derivatization, and solid phase microextraction (SPME). Quantitative analysis of nicotine was also conducted using GC-MS.

MATERIALS AND METHODS

Dissolvable Tobacco Samples. Dissolvable tobacco products (Mellow and Fresh Camel Orbs, Mellow Camel Sticks, and Fresh Camel Strips) were purchased in January 2010 from gas stations and tobacco shops in Indianapolis, IN. In March 2010, a "new and improved" line of dissolvables was released. For the purposes of this paper, dissolvables purchased prior to March 2010 will be referred to as "old" dissolvables and dissolvables purchased after March 2010 will be referred to as "new" dissolvables. Samples were kept in their packaging at room temperature until needed for analysis.

Ultrasonic Extractions. Orbs (Mellow and Fresh) and Sticks (Mellow) were ground to a powder with a mortar and pestle. The Strips (Fresh) were torn into pieces and broken with a spatula. Approximately 200 mg of each product was weighed out and placed into glass test tubes, and 2 mL of hexane, dichloromethane, acetone, or methanol was added to each test tube. The mixtures were then sonicated in an ultrasonic bath (VWR, Batavia, IL) for 60 min at room temperature, rotating the tube positions every ∼10 min. After sonication, the mixtures were filtered through 0.45 μm PTFE filters (Fisher, Hanover Park, IL) into autosampler vials and analyzed via GC-MS. Standard solutions were made by adding ∼5 mg of the standard into a glass tube and adding 5 mL of methanol. The samples were then sonicated using the same procedure as for the dissolvable tobacco samples.

Derivatization. Five milligrams of each ground tobacco product and 500 μL of Tri-Sil derivatization agent (Thermo, Rockford, IL) were added to 3 mL reaction vials (Thermo, Madison, WI). The vials were then incubated at 80 °C for ∼30 min. After cooling for ∼5 min, the extracts were filtered through 0.45 μm PTFE filters (Fisher). The samples were then transferred to autosampler vials and analyzed via GC-MS. Standard solutions were made by adding ∼0.5 mg of the standard and 500 μL of Tri-Sil reagent. The standard samples were incubated, cooled, and filtered using the same procedure as for the dissolvable tobacco samples.

SPME. The method used for SPME analysis of the dissolvables is a modified version of the SPME methods previously reported for cigarette tobacco.[13,14] In this method, 1 g of each tobacco sample was ground with a mortar and pestle and placed in a 20 mL SPME vial. Standard solutions (1 mg/mL) were analyzed in a similar fashion, where 1 μL of each solution was placed into a 20 mL SPME vial for analysis. All samples and standards were then analyzed via headspace SPME-GC-MS. Two different fiber chemistries were evaluated, polydimethylsiloxane/divinylbenzene (PDMS/DVB) and polyethylene glycol (PEG). The SPME fiber was first conditioned for 30 min at 250 °C (for the PDMS/DVB fiber) or at 240 °C (for the PEG fiber). Prior to fiber absorption, each sample was incubated for 15 min at 100 °C with agitation every 10 s for 10 s. The fiber was inserted into the vial and exposed to the headspace for 5 min, and then the fiber was inserted into the GC column inlet with a desorption time of 1 min. After each injection, the fiber was conditioned for 2 min at 250 °C (for the PDMS/DVB fiber) or at 240 °C (for the PEG fiber).

GC-MS Analysis. A 30 m × 0.25 mm × 0.25 μm capillary column in an Agilent 6890N GC with an Agilent 5975 mass selective detector was used for analysis (Agilent, Wilmington, DE). Helium carrier gas at a flow rate of 1 mL/min and a GC inlet temperature of 250 °C were used. The mass spectrometer was scanned from m/z 50 to 550. Solvent blanks and controls were also prepared and analyzed along with the tobacco samples.

For analysis of the acetone extracts, a split ratio of 20:1 was used. The GC oven temperature program began at 40 °C, was held for 1 min, and was then ramped at 20 °C/min to 320 °C. The total run time was 16 min. For analysis of the trimethylsilyl derivatized samples, a split ratio of 50:1 was used. The GC oven temperature program began at 100 °C, was held for 2 min, was then ramped at 15 °C/min to 325 °C, and was held for 3 min. The total run time was 20 min. For headspace SPME analysis, splitless injection was used with flow rate of 0.8 mL/min. The GC oven temperature program began at 40 °C, was held for 3 min, was then ramped at 6 °C/min to 250 °C, and was held for 3 min. The total run time was 41 min.

pH Analysis. The pH measurement procedure was followed as per the CDC guidelines.[15] The Accumet Basic AB15 digital pH-meter was standardized with 4.00, 7.00, and 10.00 pH buffers. Two grams of each tobacco sample was placed in a 30 mL beaker, and 20.0 mL of deionized water was added via volumetric pipet. The mixture was magnetically stirred, and the pH was measured every ∼5 min for 60 min. Each tobacco sample was analyzed in triplicate. An average pH of each triplicate and an average of each tobacco product were then calculated.

Nicotine Quantification. The nicotine analysis procedure was adapted from the CDC procedure for nicotine analysis of smokeless tobacco[12] and from Stanfill et al.[7] First, a 40 mg/mL internal standard solution was prepared by adding 1.00 g of quinoline to a 25 mL volumetric flask and diluting to the mark with methyl *tert*-butyl ether (MTBE). A 0.4 mg/mL extraction solution was made by diluting 2.5 mL of the internal standard solution to 250 mL with MTBE. A 100 mg/mL nicotine stock solution was then prepared by diluting 2.50 g of nicotine to 25 mL with MTBE in a volumetric flask. Then 0.5 mL of the internal standard was added via autosyringe to five 50 mL volumetric flasks. Standards with nicotine concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL were made by adding 100, 200, 300, 400, and 500 μL, respectively, to the other flasks and diluting to volume with MTBE. Aliquots of each standard were transferred to autosampler vials and analyzed by GC-MS.

Recovery of nicotine was determined by adding 5 mL of the 0.6 mg/mL nicotine standard to three amber tubes containing 2.0 mL of 2 N NaOH and mixing on a vortex for ∼2 min. The tubes then sat to allow the phases to separate, and an aliquot of the upper organic phase was transferred to autosampler vials. The three nicotine recovery samples were then analyzed using the same method as the nicotine standards. The recovery of nicotine was then calculated for each sample using the following equation:[12] recovery = $\text{nicotine}_{\text{calculated}}/\text{nicotine}_{\text{actual}}$.

Standard addition assays were conducted for each tobacco product. One gram of tobacco was added to an amber glass tube. This was repeated for a total of six samples. The first sample was not spiked, but the remaining five samples were spiked with 10, 20, 30, 40, and 50 μL of the 100 mg/mL nicotine stock solution. The samples were then allowed to equilibrate for 10 min. Then, 2 mL of 2 N NaOH was added to each sample. The tubes were swirled to allow the tobacco to be wet with the NaOH. After 15 min, 5.0 mL of extraction solution (0.4 mg/mL quinoline/MTBE) was added to each sample. The tubes were then placed on an orbital shaker table and shaken at ∼200 rpm for 2 h. The tubes were then removed from the shaker table and the phases allowed to separate. An aliquot of the upper organic layer of each sample was filtered through a 0.45 μm PTFE filter and transferred to an autosampler vial and analyzed by GC-MS using the same nicotine method.

Recovery of nicotine for the standards addition assay samples was conducted by adding 30 μL of the nicotine stock to 2.0 mL of 2 N NaOH and 5.0 mL of the 0.4 mg/mL extraction solution in an amber tube. This was repeated for a total of three samples. The tubes were then mixed on a vortex for ∼2 min. After the phases were allowed to separate, an aliquot of the upper organic layer of each sample was transferred to autosampler vials and analyzed via GC-MS using the same nicotine method. The concentration of the recovery samples was then determined from the standard addition assay calibration equation. Recovery of the samples was then calculated from the following equation: recovery =

$nicotine_{calculated}/nicotine_{actual}$. The recovery of nicotine from the nicotine standards and the recovery of nicotine from the standards addition assay samples were compared to determine that the recovery values did not differ by more than 10%. This ensures the aqueous matrix is equivalent to the vegetable matrix of the tobacco product.[12]

Quality control samples at the low and high end of the expected nicotine concentration were prepared. The low control (0.3 mg/mL) was prepared by adding 15 μL of nicotine stock to 2.0 mL of 2 N NaOH and 5.0 mL of extraction solution. The high control (0.7 mg/mL) was prepared by adding 35 μL of nicotine stock to 2.0 mL of 2 N NaOH and 5.0 mL of extraction solution. The tubes were shaken on the shaker table with the standard addition assay samples. An aliquot of the upper organic phase of each sample was filtered through a 0.45 μm PTFE filter and transferred to autosampler vials to be analyzed by GC-MS.

Finally, the tobacco samples were analyzed. One gram of the tobacco sample was added to an amber glass tube. This was done in triplicate. Then, 2 mL of 2 N NaOH was added to each sample. The tubes were swirled to allow the tobacco to be wet with the NaOH. After 15 min, 5.0 mL of extraction solution (0.4 mg/mL quinoline/MTBE) was added to each sample. The tubes were then placed on an orbital shaker table and shaken at ~200 rpm for 2 h. The tubes were then removed from the shaker table and the samples sat to allow the phases to separate. An aliquot of the upper organic layer of each sample was filtered through a 0.45 μm PTFE filter, transferred to an autosampler vial, and analyzed by GC-MS using the same nicotine method used for the nicotine standards, recovery samples, standards addition assay samples, and quality control samples.

GC-MS Analysis of Nicotine. The GC-MS method used is an established method for the rapid and selective quantification of nicotine in tobacco.[7] A 25 m × 0.32 mm × 0.52 μm Agilent Ultra 2 capillary column in a Thermo Trace GC Ultra with a DSQ II mass selective detector was used for analysis (Thermo, Madison, WI). Helium carrier gas at a flow rate of 1.7 mL/min and a GC inlet temperature of 230 °C were used. An injection volume of 1.0 μL and a split ratio of 50:1 were used. The GC oven temperature program began at 175 °C, was held for 1 min, was then ramped at 5 °C/min to 180 °C, and was then ramped at 35 °C/min to 240 °C. The total run time was 3.7 min.

The mass spectrometer transfer line was held at 250 °C with a 1.0 min solvent delay. Selected ion monitoring (SIM) was (mass; dwell; ion type) as follows: quinoline (102.00 amu; 10 ms; quantification), nicotine (133.00 amu; 10 ms; quantification), nicotine (162.00 amu; 35 ms; confirmation), quinoline (129.00 amu; 10 ms; confirmation), and nicotine (161.00 amu; 35 ms; additional). Two solvent blanks were analyzed before each sample.

RESULTS AND DISCUSSION

Chemical Characterization. Table 1 summarizes the major compounds identified in the dissolvables using each extraction method. Extracting the dissolvable tobacco products using the solvent polarity series of hexane, dichloromethane, acetone, and methanol was carried out to provide a comprehensive view of the extractables present in the products. A comparison of the results from the four different solvents determined that the less polar solvents (hexane and dichloromethane) extracted few, if any, sample components. In contrast, acetone allowed for more compounds to be seen in the chromatogram without extracting compounds such as carbohydrates, which exhibit poor chromatographic behavior in the form of peak fronting. These highly polar compounds were present in the methanol extracts, however.

Figure 1 shows a comparison of the chromatograms of the four dissolvables in acetone. Nicotine, ethyl citrate, palmitic acid, and stearic acid were confirmed by mass spectral library search and by comparison to the retention times and mass spectra of authentic standards. As would be expected, all of the dissolvable products contain nicotine (peak 3), and those products denoted as "Fresh" flavor contain menthol (peak 2). Perhaps due to its tougher matrix, the extraction efficiency for the Mellow Stick was lower than that of the other products, as evidenced by the lower signal-to-noise in its chromatogram. It should also be noted that ethyl citrate (peak 4), palmitic acid (peak 6), and stearic acid (peak 7) were found only in the Orb dissolvables.

Table 1. Major Compounds Identified by GC-MS Using Each Extraction Method for Mellow Orb (MO), Fresh Orb (FO), Mellow Stick (MS), and Fresh Strip (FS)

	acetone extract				derivatization				SPME			
compound	MO	FO	MS	FS	MO	FO	MS	FS	MO	FO	MS	FS
menthol		X		X						X		X
nicotine	X	X	X	X					X	X	X	X
ethyl citrate	X	X							X	X		
palmitic acid	X	X										
stearic acid	X	X										
glycerol							X	X				
xylitol							X					
sorbitol					X	X	X	X				
carvone										X		X
cinnamaldehyde									X			
vanillin									X	X	X	
coumarin									X			

Peak 5 was found in all of the dissolvable products, and it was identified as phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) by a mass spectral library search. However, the retention time of a standard solution of phytol was significantly greater than that of peak 5 in the dissolvable tobacco chromatograms. Therefore, it is possible that this compound is in the same terpenoid structural class as phytol but is of lower molecular weight. Finally, the peak at ~4.8 min (peak 1) in the Fresh Strip sample was identified as glycerin.

Although liquid chromatography has been used to profile carbohydrates such as glucose, fructose, and sucrose in various types of tobacco,[16] derivatization useful in this work as any polar compound including sugars, alcohols, and amines can be made amenable to GC-MS. Figure 2 shows a comparison of the chromatograms of the four dissolvables analyzed by derivatization. The identities of all derivatives were confirmed by mass spectral library search and the retention times of derivatized standards of each compound. In contrast to conventional tobacco, significant amounts of sorbitol (peak 3), a sugar alcohol widely used an artificial sweetener, were identified in all products. The Mellow Stick was the only product found to contain the sugar alcohol xylitol (peak 2). Peak 4 was not identified by a mass spectral library search, but appears to be a di- or trisaccharide. Palmitic acid and stearic acid were also found as minor constituents in both the Mellow Orb and the Fresh Orb, consistent with the analysis of the acetone extracts. Threitol and malic acid, having retention times of 7.6 and 7.4 min, respectively, were found as minor compounds in all of the dissolvables.

SPME analysis was conducted using PDMS/DVB and PEG fibers. The PEG fiber was found to be better suited for the volatile components in the dissolvables because it is polar, whereas the

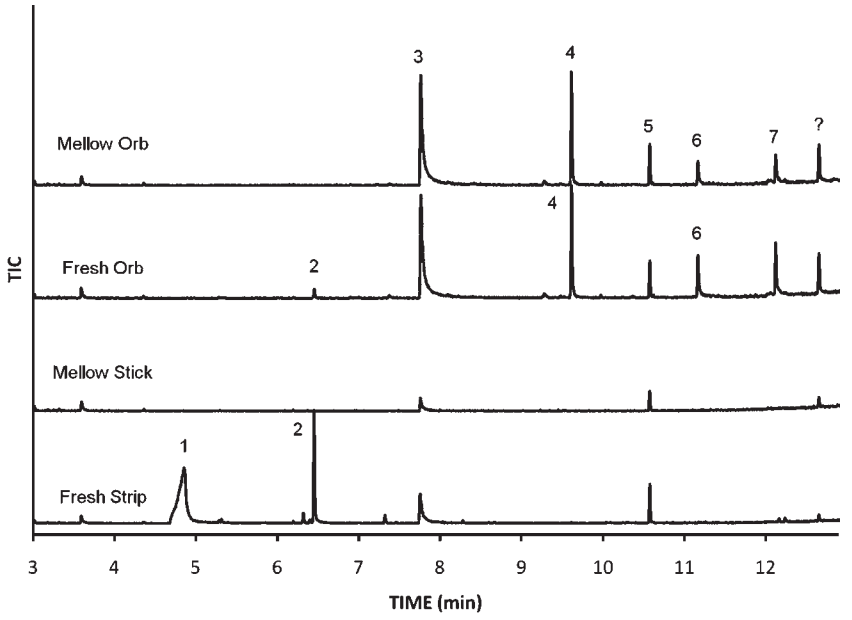

Figure 1. Acetone extracts of dissolvables. Peaks: (1) glycerin; (2) menthol; (3) nicotine; (4) ethyl citrate; (5) possible terpenoid (see text); (6) palmitic acid; (7) stearic acid; (?) unknown.

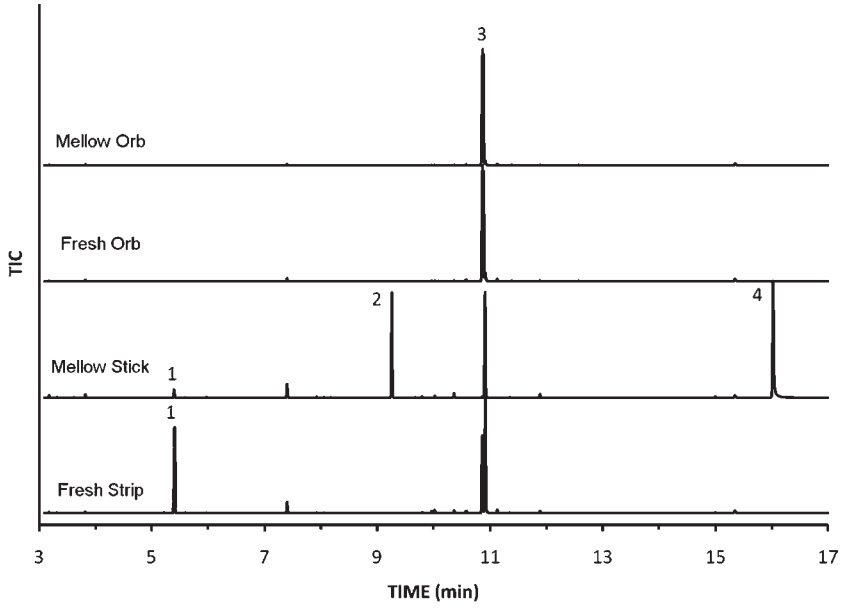

Figure 2. Trimethylsilyl derivatization of dissolvables. Peaks: (1) glycerol; (2) xylitol; (3) sorbitol; (4) di- or trisaccharide.

PDMS/DVB fiber is nonpolar and better suited for adsorption of neutral components and hydrocarbons. Figure 3 shows the chromatograms of the dissolvables analyzed with the PEG fiber. Each of the chromatograms is clearly dominated by nicotine (peak 4), as it has appreciable vapor pressure in free base form. Also, and as was seen previously, ethyl citrate was identified in the Orbs, and menthol was identified in products with Fresh flavor. Other compounds that were identified include carvone (peak 2), cinnamaldehyde (peak 3), vanillin (peak 5), and coumarin (peak 6). It should also be noted that vanillin was found only in the Orbs and the Mellow Stick and that carvone was found only in the Fresh flavor dissolvables (Orbs and Strips). Peak 8 was identified as phytol from a mass spectral library search, but its retention time does not match that of a standard solution of phytol. Hence, as was stated above, it is suggested that this compound is in the same terpenoid structural class as phytol, but is of lower molecular weight.

The complete chemical characterization was conducted for both the old and new dissolvable tobacco products. Upon comparison of the major compounds detected via acetone extraction, derivatization, and SPME, there was no qualitative difference between the old and new dissolvables.

From the results of the acetone extractions, derivatization, and SPME, the following major compounds have been accurately identified in the dissolvables: nicotine, ethyl citrate, palmitic acid, stearic acid, sorbitol, glycerol, xylitiol, cinnamaldehyde,

coumarin, vanillin, and carvone. Aside from nicotine, the compounds identified in the dissolvables are known to be used as sweeteners (e.g., sorbitol), flavoring compounds (e.g., menthol), binders (palmitic and stearic acid), or humectants (e.g., glycerin).

pH and Nicotine Quantification. pH measurements and nicotine quantification of the dissolvables were also conducted. From total nicotine concentration, free-base nicotine (nicotine readily absorbed by the user) was calculated from pH and the Henderson−Hasselbalch equation. Results of pH for the old and new products can be seen in Table 2. These results demonstrate that the percentage of free nicotine in the old dissolvables ranges from 23.5 to 50.2% and in the new dissolvables from 23.2 to 29.4%.

Nicotine quantification results for the new dissolvables can be found in Table 3. Calibration curves for nicotine standards and the standard addition assay were performed with each dissolvable product analyzed. All of the calibration curves had R^2 values of >0.99, indicating good correlation of the data. Recovery of nicotine ranged from 92 to 96% with recovery of nicotine from the standard addition assay always within ±5%. This indicates that there were minimal matrix effects in the analysis.

Product literature states total nicotine concentration for Mellow and Fresh Orbs is 1 mg/Orb, for Mellow Stick is 3.1 mg/Stick, and Fresh Strips is 0.6 mg/Strip. The results from the nicotine quantification found total nicotine concentrations lower than the product literature. The nicotine analysis found 0.82 mg/Orb for Mellow Orb, 0.77 mg/Orb for Fresh Orb, 0.91 mg/Stick for Mellow Stick, and 0.21 mg/Strip for Fresh Strip. These total nicotine concentrations correspond to free nicotine concentrations, respectively, of 0.19, 0.17, 0.25, and 0.0058 mg per dissolvable.

Table 2. pH Measurements and Percent Un-ionized (Free) Nicotine in the Old and New Dissolvable Tobacco Products

	old dissolvables		new dissolvables	
dissolvable	pH	% free nicotine	pH	% free nicotine
Mellow Orb	7.82 ± 0.04	38.5	7.50 ± 0.03	23.2
Fresh Orb	7.61 ± 0.17	28.0	7.50 ± 0.03	23.2
Mellow Stick	7.51 ± 0.07	23.5	7.64 ± 0.07	29.4
Fresh Strip	8.02 ± 0.06	50.2	7.53 ± 0.20	24.6

Toxicology of Dissolvable Tobacco Components. Dissolvable tobacco products have the potential to cause mouth diseases and complications in the users of these products. It is therefore important to understand some of the potential toxicological effects of some of the ingredients of these products, particularly nicotine.

The toxicity of nicotine has been extensively researched, and the complications associated with nicotine are widely accepted. Nicotine is a tertiary amine and can be converted into *N*-nitrosamines within the body. Tobacco-specific *N*-nitrosamines (TSNAs) are carcinogenic, particularly 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*′-nitrosonornicotine (NNN). Once TSNAs are metabolically activated, they can form DNA adducts, which can eventually lead to cancer.[17] Nicotine also has adverse effects on the oral cavity, such as inhibited gingival fibroblast growth and collagen production.[18] Nicotine can also inhibit mineralization of human dental pulp cells and inhibit apoptosis in oral cancer cells.[19−21]

Sorbitol and xylitol are sugar alcohols that are commonly used as sucrose substitutes in foods such as sugar-free chewing gums. Frequently, both sorbitol and xylitol are added together as a sweetner because xylitol is expensive but allegedly has better health effects than sorbitol alone.[22] Frequent exposure to sorbitol can lead to an increase in tooth demineralization and can be potentially carcinogenic when regularly used by people with low salivary secretions.[23] Xylitol has not shown any harmful effects to the oral cavity, but, contrary to popular belief, it also has not been shown to be beneficial. Consumption of xylitol for long periods of time may result in selection of xylitol-resistant *Streptococcus mutans* (micro-organisms in dental plaque), leading to increased oral bacteria.[24]

Additional ingredients found in dissolvable tobacco products are ethyl citrate, cinnamaldehyde, and coumarin. Ethyl citrate is

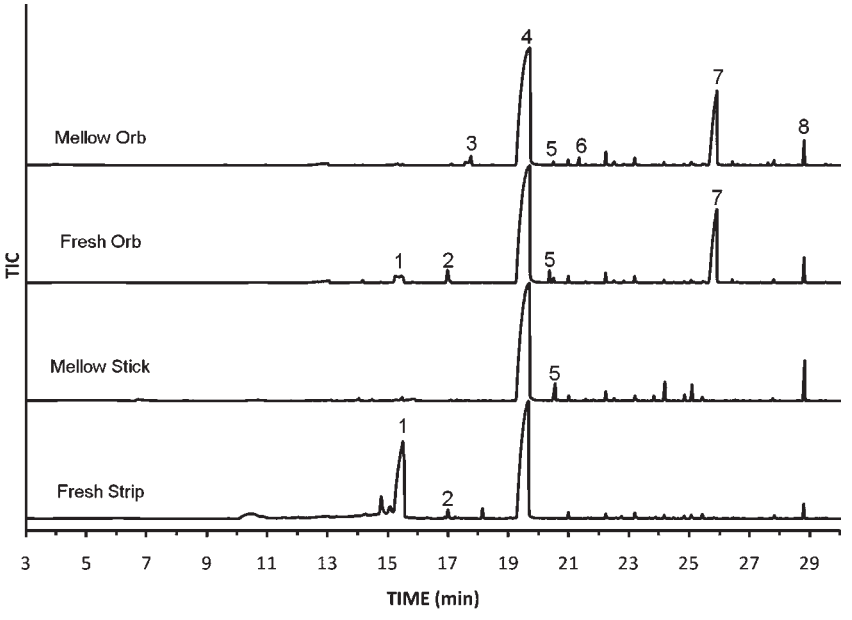

Figure 3. SPME analysis with PEG fiber of dissolvables. Peaks: (1) menthol; (2) carvone; (3) cinnamaldehyde; (4) nicotine; (5) vanillin; (6) coumarin; (7) ethyl citrate; (8) possible terpenoid (see text).

Table 3. Nicotine Quantification of the New Dissolvable Tobacco Products

		total nicotine		free nicotine	
dissolvable	av mass/dissolvable (g)	mg/g	mg/dissolvable	μg/g	μg/dissolvable
Mellow Orb	0.225	3.65 ± 0.08	0.821 ± 0.02	847 ± 20	190 ± 4
Fresh Orb	0.225	3.42 ± 0.1	0.77 ± 0.02	750 ± 20	170 ± 5
Mellow Stick	0.513	1.78 ± 0.12	0.91 ± 0.06	490 ± 30	250 ± 20
Fresh Strip	0.1	2.12 ± 0.02	0.212 ± 0.002	58 ± 0.5	5.8 ± 0.05

acutely toxic when delivered orally.[25] Cinnamaldehyde is also an oral irritant with desensitization recovery time of >10 min.[26] Coumarin is also a harmful ingredient and causes liver damage in rodents. Coumarin is found in tonka beans and has been banned as a flavor additive to food.[27]

Overall, it is important to monitor the concentration of nicotine in the dissolvables to understand the potential toxicity of nicotine in humans. The route of administration of nicotine, such as orally, dermally, or intravenously, is also very important. Exposure of nicotine is largely dependent on how the user consumes the nicotine-containing product. Rapid injection of nicotine leads to the highest blood and brain concentrations at the lowest doses of nicotine. However, oral administration requires higher doses of nicotine to produce the same toxic effects. It has been estimated that an oral dose of 5 mg/kg (2.3 mg/Lbs.) of nicotine is lethal to an average 70 kg (~155 lb) person.[28]

To conclude, the chemical characterization, pH measurements, and nicotine quantification analyses of the dissolvable tobacco products have revealed important information about the complexity of dissolvable tobacco products. In particular, the ingredients in dissolvable tobacco are flavor compounds (e.g., menthol, ethyl citrate, cinnamaldehyde, coumarin, vanillin, and carvone), sweeteners (e.g., sorbitol and xylitol), binders (e.g., palmitic acid and stearic acid), humectants (e.g., glycerin), and nicotine. The percentage of free nicotine in these products ranges from 23 to 29% in the new dissolvables, and total nicotine concentrations range from 0.21 to 0.91 mg/dissolvable.

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