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Portland State University

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ANALYZING FREE-BASE NICOTINE CONTENT IN THE
PARTICULATE MATTER OF MAINSTREAM TOBACCO SMOKE
USING A HEADSPACE SOLID-PHASE MICROEXTRACTION
GC/MS METHOD

BY

CHRISTOPHER S. MOTTI

A research project report submitted in partial fulfillment
of the requirement for the degree of

MASTER OF SCIENCE
IN
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Project Advisor:
James F. Pankow

Portland State University
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The faculty and staff of Maseeh College of Engineering & Computer Science, Civil & Environmental Engineering Department

My fellow engineering students

My parents

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ABSTRACT

The acid/base chemistry of cigarette smoke allows nicotine, the primary drug in mainstream tobacco smoke, to exist in protonated and free-base forms. Evidence suggests that most of the nicotine present in mainstream tobacco smoke is in the particle phase. α_{fb} is the fraction of particle-phase nicotine in the free-base form. The magnitude and rate of nicotine deposition in the respiratory tract is dependent on α_{fb} because only the free-base form of nicotine can volatilize from the particle phase of an inhaled aerosol and, once gaseous, rapidly deposit. This study develops a headspace solid-phase microextraction technique coupled with gas chromatography/mass spectrometry analysis that effectively quantifies α_{fb} . The developed method is used to study the α_{fb} of several commercial cigarette brands. This research has important implications on the drug delivery rate and addiction potential of inhaled mainstream tobacco smoke particulate matter.

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1.0 INTRODUCTION

1.1 ACID/BASE CHEMISTRY OF NICOTINE

Due to its acid/base chemistry, nicotine can exist in three forms in the particulate matter of (PM) mainstream tobacco smoke (MTS), a diprotonated form, a monoprotated form, and an unprotonated or free-base (FB) form. The protonated forms are non-volatile and therefore only occur in the PM of the smoke. Evidence suggests that most of the nicotine in MTS is found in the PM phase (1). Smoke particles have been observed to deposit in the respiratory tract (RT) with about 50% efficiency, while gas phase nicotine deposits rapidly. Since protonated nicotine is not volatile, only the free-base form may volatilize from the PM phase to the gaseous phase of an inhaled aerosol. Thus, the fraction of nicotine that exists in the free-base form is important when understanding the magnitude and rate of nicotine absorbed by a smoker.

Figure 1. The three forms of nicotine (1).

As the effective pH of MTS PM increases from above about 6.0, an increasing amount of nicotine occurs in the free-base form (2). This volatile form is rapidly absorbed and believed to be perceived by the smoker as a nicotine “impact” or “kick”. The greater the impact, the more addictive the cigarette. The free-base form is denoted as Nic, the monoprotated form as NicH^+ , and the deprotonated as NicH_2^{2+} (Figure 1). Due to its relatively low concentrations for typical effective pH ranges of MTS, the diprotonated form can be neglected. Therefore, the fraction of volatile, free-base nicotine (α_{fb}) in the PM phase of tobacco smoke is approximated by:

$$\alpha_{\text{fb}} = \frac{[\text{Nic}]}{[\text{Nic}] + [\text{NicH}^+]}$$

(1)

1.2 THEORY OF SPME

Headspace solid-phase microextraction (HS-SPME) is a useful sample extraction technique that has unique capabilities for the chromatographic analysis of chemicals in difficult matrices (3), making it well suited for measuring α_{fb} . The technique relies on the extraction of analytes from a headspace (HS) of an sample. The fiber is held over a sealed sample for a set period of time at a constant temperature. The equilibrated sample will predictably partition between the liquid phase and the gas phase. A sorptive coating on the SPME fiber absorbs analytes from the HS. The exposure time selected is affected by the Henry's Constant (K_h) of the analyte. Substances with high K_h values require a shorter exposure time because there will be an abundance of analyte in the gaseous HS. Conversely, substances with low K_h values can require longer exposure times because the process of diffusion from the condensed phase to the gas phase will be slow (4). After fiber exposure, solutes are transferred from the SPME coating to an inlet system that desorbs the solutes into a flowing gas phase for determination by gas chromatography/mass spectrometry (GC/MS) (3).

Figure 2. Schematic of HS-SPME extraction from a sealed vial.

1.3 HS-SPME APLIED TO TOBACCO SMOKE

The evaluation of the FB nicotine content of MTS PM collected on a Cambridge filter pad (CFP) can proceed by the HS-SPME process. After MTS PM is collected on the CFP, the filter is sealed retained in the filter holder, and a septum is added at the top. After allowing sufficient time for particles to settle and analytes to equilibrate between the PM phase (on the filter) and the gas phase (in the HS), the SPME fiber is exposed to the HS for a set period of time. Any nicotine present in the gas phase is in the FB form (5). Analysis by GC/MS can then separate, identify, and quantify the compounds that were sorbed on the fiber, including nicotine. Finally, the base ammonia is injected as a gas into the HS. This process strips the protonated nicotine trapped within the PM on the CFP of its charge, chemically transforming it to FB nicotine, allowing some to volatilize into gas phase. After allowing for the requisite equilibration time, the fiber is again exposed for the same set period of time to the HS above the filter pad now containing more gaseous nicotine. GC/MS analysis yields a new, larger nicotine response. The original nicotine response over the post-ammonia addition response represents the α_{fb} of the tobacco smoke PM.

2.0 METHODS

2.1 PARTICLE COLLECTION/EXTRACTION PROTOCOL

In order to determine the FB nicotine content of various brands of commercial cigarette PM, MTS PM was collected using a mechanical smoking device and a CFP. The CFP was held in a filter holder, with sealable openings on either side. The front of the filter holder allowed for an attachment to be installed to hold the cigarettes, while the back connected to the pump. The pump was calibrated to take a 2 second, 35 mL puff every minute. A dual cigarette holder insured that a total of 70 mL of smoke were drawn through the filter every puff. Before smoking, the sealed holder with CFP was weighed to determine its mass. Cigarettes were then lit after attaching the filter holder to the smoking device, inserting cigarettes into the cigarette holder, and turning on the pump. The smoking process was concluded after a 23 mm butt length remained for non-filtered cigarettes or the length of the filter overwrap plus 3 mm remained for filtered cigarettes. At this point, the pump was turned off and the filter holder was removed from the smoking device, resealed, and reweighed. The mass before smoking was subtracted from the mass after smoking producing the total particulate matter (TPM) mass of the MTS. After smoking, the front end of the filter holder allowed use of a septum to let the SPME fiber enter the HS and for a syringe to introduce ammonia.

Figure 3. The smoking apparatus.

Supelco (Bellefonte, PA) was the source of the SPME fiber, purchased from Sigma-Aldrich (Milwaukee, WI). The 1 cm fiber was coated with a polydimethylsiloxane/divinylbenzene (PDMS/DVB) (65 μm thick coating, and 24 gauge needle). Nicotine is a semi-volatile, polar compound with a molecular weight (MW) of 162.23 g mol^{-1} . This particular fiber is compatible for volatile analytes with MW from 50 to 300 g mol^{-1} , making it a good choice for determining MTS volatiles, and specifically FB nicotine.

Accurate results for FB nicotine extraction with HS-SPME require equilibrium for the FB nicotine on the filter pad, the (HS), and the SPME fiber. Based on previous studies (5,6), a minimum aerosol pre-equilibration time of 1-hour was proposed for this study, determined to be adequate time for the polar, semi-volatile compound to reach partitioning equilibrium between the CFP and HS. After this time, the FB nicotine present in the HS has been found to remain constant for up to 20-hours (6), meaning that the window for performing HS-SPME may be 1 to 20 hours after collecting MTS PM on a CFP.

2.2 METHOD DEVELOPMENT

Before a finalized HS-SPME method could be followed, certain developmental experiments were conducted to ensure that FB nicotine measurements would be accurate. Multiple brands of cigarettes were selected in order to ensure that differing brands would not yield inconsistent results for method conditions. The first experiment focused on confirming that the SPME fiber produced similar responses for a constant exposure time. To test this, the fiber was exposed to the HS of the equilibrated sample for 20 minutes, after which GC/MS data was obtained. This process was repeated six times. Next, the system received 6.0 mL of ammonia and sat for the minimum 1-hour equilibration time. The process was repeated six more times.

The next experiment involved assessing whether a SPME fiber carried a proportional response for varied nicotine content. Differing amounts of nicotine were dissolved in pyridine (0.1, 0.5, 1.0, 2.0, 4.0, and 6.0% nicotine by mole fraction) and HS-SPME/GC/MS tests were conducted for each sample. A linear response for peak area versus nicotine percentage would confirm that the SPME fiber's response to FB nicotine both before and after the ammonia addition would be proportional to the amount of FB nicotine present in the HS for each case.

Another method development experiment involved collecting data that varied SPME fiber exposure time and examining the GC/MS nicotine response. This would help determine the appropriate length of exposure for the final experiments. Responses should increase linearly at first and then may begin to approach a constant after a long enough exposure time as the sorptive fiber reaches a state of equilibrium with the surrounding HS.

Figure 4. The SPME fiber holder and filter holder during SPME fiber headspace exposure.

After the pre-ammonia addition FB nicotine response was determined, gaseous ammonia was introduced to the sealed filter holder. It was imperative that the amount of ammonia added to the system be sufficient to completely deprotonate all of the nicotine within the PM trapped on the CFP, transforming it to FB nicotine. Some of this newly formed FB nicotine could then volatilize and reach a new equilibrium between the PM and the HS. To determine the proper amount to add, 3 mL volumes of ammonia were incrementally added to the HS. After each

addition, the 1-hour minimum equilibration time interval passed and the system was reanalyzed using HS-SPME/GC/MS. Chromatogram results were compared for nicotine for each ammonia addition. At a certain point, the peak area for nicotine stopped increasing. The total volume of ammonia added at that point was selected as the amount to add for the main experiment, the volume needed to completely deprotonate the nicotine in the system. (Note that not all of the ammonia added actually participated in deprotonation: some remained as NH_3 .)

In order to assess whether filter holder material had an effect on the amount of ammonia needed to deprotonate the nicotine present on the CFP, metal filter holders were used to perform the same experiment as the previous paragraph. One had a conical HS volume and the other a cylindrical shape. This was done to prove that the ammonia was not permeating into the walls of the plastic filter holder, meaning more would be needed to deprotonate the nicotine on the CFP. To further test required ammonia addition, a CFP with MTS PM was placed inside a volatile organics analysis (VOA) vial. The same amount of ammonia was needed to stabilize post-ammonia addition GC/MS nicotine response for both the metal and glass holders would confirm that the plastic filter holder could be used to measure FB nicotine content.

In the final method development experiment, a CFP coated with MTS was cut into four equal-sized pieces. Each piece was inserted into a separate VOA vial, allowed at least a 1-hour equilibration time, and twice analyzed by HS-SPME/GC/MS. After analysis, 12 mL of ammonia was injected into each vial and each sample re-equilibrated and reanalyzed. Minimal variation in FB nicotine content would further validate the method.

2.3 FINAL METHOD

The final method for our experiment consisted of smoking dual cigarettes in the way previously discussed and allowing a minimum of 1-hour to elapse for the closed system to reach partitioning equilibrium. Next, the SPME fiber was exposed to the HS in the filter holder for 20 minutes, immediately after which it was analyzed by GC/MS. This exposure/GC/MS process was immediately repeated to verify consistency in the data. After the second run, 12 mL of ammonia was injected into the HS via a syringe inserted into the septum at the top of the filter holder and the system was allowed to reach a new partitioning equilibrium. The SPME fiber was again exposed to the HS above the MTS PM containing filter for 20 minutes and subsequently

analyzed by GC/MS. Exposure and GC/MS were repeated to verify consistency. This process was repeated for a total of three times for each brand of cigarette. FB nicotine content (α_{fb}) was calculated as the average of the nicotine peak areas before the ammonia addition over the average of the peak areas post-ammonia addition. Percent error was calculated for both pre-ammonia and post-ammonia cases.

2.4 GC/MS PARAMETERS

The GC/MS analysis was carried out on an Agilent-6890N (G 1540N) GC coupled to a Leco Pegasus IV mass spectrometer. After HS exposure, the PDMS/DVB fiber was thermally desorbed in the GC injection port. The GC injector was run in split mode at 250 °C, with a 30:1 split ratio. Complete desorption of analytes was accomplished after 4 minutes at 250 °C. The GC Agilent DB-5 column had a length of 30 m, an internal diameter of 250 μm , and a film thickness of 0.25 μm . The carrier gas flow rate was 1.2 mL min⁻¹ for the duration of the run, with helium as the carrier gas. The GC oven temperature was 80 °C for 2 minutes, increased to 200 °C at a rate of 10 °C min⁻¹, and finally increased to 260 °C at a rate of 20 °C min⁻¹. The GC method total run time was 1020 seconds. The MS operated from a starting mass of 34 amu to an ending mass of 400 amu with a 1450 acquisition voltage and -70 volts electron energy. The ion source was operated at 225 °C.

3.0 RESULTS AND DISCUSSION

3.1 DEVELOPMENT PHASE

A 65 μm PDMS/DVB SPME fiber sampled the HS of a filter holder with CFP containing the MTS PM from two cigarettes. Before HS sampling, the closed system was allowed to reach partitioning equilibrium between gas phase and PM phase nicotine. Method development efforts yielded the following results.

Marlboro Special Blend 100s (red, flip-top pack) were smoked for the first experiment.

Table 1. HS-SPME/GC/MS replicate results for Marlboro Special Blend 100s.

Before NH₃ addition, 20 minutes fiber exposure	
Sample #	Nicotine peak area
1	lost
2	5.07E+06
3	5.50E+06
4	5.54E+06
5	5.47E+06
6	5.94E+06
After 6mL NH₃ addition, 20 minutes fiber exposure	
Sample #	Nicotine peak area
1	6.53E+07
2	6.52E+07
3	6.08E+07
4	5.93E+07
5	6.01E+07
6	6.09E+07
Average response (before NH₃ addition):	
5.51E+06	
Average response (after NH₃ addition):	
6.19E+07	
Free-base nicotine (%):	
8.9	
Standard deviation (before NH₃ addition):	
3.07E+05	
Standard deviation (after NH₃ addition):	
2.63E+06	
CV (before NH₃ addition):	
5.6	
CV (after NH₃ addition):	
4.2	

Results from the multiple tests show excellent agreement with 5.6 and 4.2% coefficient of variation (CV) for before and after ammonia addition, respectively. As expected the nicotine response increased after the addition of the ammonia and α_{fb} equaled 0.089 (8.9% FB nicotine).

The GC/MS machine was recalibrated after analyzing sample #1 before the addition of ammonia leading to an unusable peak area. All other results display desired agreement between data points.

Figure 5. HS-SPME/GC/MS FB nicotine peak area variations with increasing nicotine content.

Figure 5 plots the results of FB nicotine peak area versus percent by mole fraction nicotine in pyridine solution. The linear response for peak area confirms that the HS-SPME/GC/MS analysis correctly measured the proportional amount of volatile nicotine in the HS above solutions containing various amounts of total nicotine. The R^2 value of 0.9867 confirmed that these data points fit a linear trendline. For validation, the same experiment was repeated at a later date. Results are displayed in Figure 11 (appendix).

After a certain length of exposure time, peak areas will stop increasing over increasing HS extraction times because the gases present will reach partitioning equilibrium between the SPME fiber and HS. However, it can be impractical to wait the requisite time for this equilibrium to be achieved. Thus, the next experiment involved measuring the nicotine response for differing

exposure times for both before and after ammonia addition in order to determine an appropriate exposure time. Marlboro Special Blend 100s were smoked for these results.

Figure 6. Pre-ammonia addition nicotine peak areas for a range of exposure times (Marlboro Special Blend 100s).

Figure 7. Post-ammonia addition peak areas for a range of exposure times (Marlboro Special Blend 100s).

Both pre-ammonia and post-ammonia regimes displayed linearity for up to 30 minute exposure times. A slight drop in peak area after a 50 minute exposure time was observed for a post-ammonia sample. However, any exposure under the 30 minute mark should be within the linear period. Thus, a 20 minute exposure was selected as optimal for our final α_{fb} tests. These linear results confirmed that equal exposure times legitimized the comparison of FB nicotine and total nicotine peaks. A similar experiment was conducted at a later date with Silver Eagle Gold Cigarettes (100s, flip-top box). Exposure times ranged from 5 to 80 minutes. Results are shown in Figures 12 and 13 (appendix).

Figure 8. GC/MS nicotine peak area variations over multiple gas phase ammonia additions (Marlboro Special Blend 100s).

After some volume of gas phase ammonia injected into the filter holder, the system had enough base to completely deprotonate all PM phase nicotine. According to Figure 8, that point occurred from 12 mL to 15 mL, when the nicotine peak area stopped increasing. These results, obtained from Marlboro Special Blend 100s, confirmed that 12 mL of ammonia would be adequate to deprotonate all PM phase nicotine and therefore accurately assess MTS PM FB

nicotine content. Figures 14 and 15 (appendix) display the results for a similar experiment run with American Spirit Natural (blue, flip-top box, full-bodied taste, made with organic tobacco) and Silver Eagle Gold Cigarettes (100s, flip-top box), respectively. Results show that between 12 mL to 18 mL there was little increase in nicotine peak for American Spirit and a slight decrease for Silver Eagle Gold.

The following figures display results from the ammonia addition experiments with metal and glass as the filter holders. Silver Eagle Gold 100s were used to obtain this data.

Figure 9. GC/MS nicotine peak area variations over multiple gas phase ammonia additions using a metal filter holder with conical headspace (Silver Eagle Gold 100s).

Figure 10. GC/MS nicotine peak area variations over multiple gas phase ammonia additions using a glass VOA vial to contain the filter (Silver Eagle Gold 100s).

Figure 16 (appendix) displays the results for the metal filter holder with cylindrical HS. The data for this particular filter holder shows that less ammonia was required to deprotonate the nicotine on the CFP, possibly due to the different HS shape. Figures 9 and 10 show that 12 mL of ammonia was still required to deprotonate the nicotine trapped on the CFP for both the VOA vial and the metal filter holder with conical HS, meaning that the plastic filter holders did not require more ammonia compared to metal or glass.

Table 2 presents the data from the experiment in which a CFP with MTS PM was cut into 4 pieces and each piece placed inside a separate VOA vial.

Table 2. Free-base nicotine percentage for four vials containing PM from the same filter (Silver Eagle Gold 100s).

	Vial 1	Vial 2	Vial 3	Vial 4
FB nicotine %:	5.5	3.6	7.0	6.6
% error (before NH ₃ addition):	11.1	3.7	19.0	19.9
% error (after NH ₃ addition):	6.9	9.9	8.9	9.9

Silver Eagle Gold 100s were smoked during this experiment. Vials 1, 3 and 4 show similar FB nicotine content. Vial 2 is an outlier, possibly because not enough PM was collected by that

portion of the CFP. The CG/MS nicotine response for Vial 2 was lower than for the other 3 vials. This result further confirms that these methods yield consistent results.

3.2 FINAL EXPERIMENTAL PHASE

Finally, the main experiment determined the α_{fb} of the smoke PM for multiple brands of commercial cigarette and one type of cigarette-like “cigars”. The brands investigated for this report include: Marlboro Special Blend 100s, Silver Eagle Gold 100s, American Spirit Natural, Marlboro Red Label 100s (soft pack), and Cheyenne Full Flavor 100s “Cigars”. Table 3 presents the α_{fb} for each of these tobacco products.

Table 3. Mainstream tobacco smoke particulate matter free-base nicotine content for various brands/types of cigarettes and “cigars”.

Cigarette/cigar brand/type	α_{fb}
Marlboro Special Blend 100s	0.051
Silver Eagle Gold 100s	0.069
American Spirit Natural	0.185
Marlboro Red Label 100s	0.065
Cheyenne Full Flavor 100s cigars	0.057

The α_{fb} for the Marlboro Special Blend 100s, Silver Eagle Gold 100s, and American Spirit Natural were inferred from the method development phase of this research. α_{fb} values for the other two products were determined using the final method protocol. The details of these tests are displayed in the following tables.

Table 4. Free-base nicotine percentage, percent errors, and α_{fb} values for Marlboro Red Label 100s (soft-pack).

	Sample 1	Sample 2	Sample 3	Average
FB nicotine %:	5.4	6.3	7.9	6.5
% error (before NH3 addition):	2.1	6.6	2.1	% error
% error (after NH3 addition):	3.3	1.8	1.7	5.1
α_{fb} :	0.054	0.063	0.079	0.065

Table 5. Free-base nicotine percentage, percent errors, and α_{fb} values for Cheyenne Full Flavor 100s “Cigars”.

	Sample 1	Sample 2	Sample 3	Average
FB nicotine %:	5.2	6.5	5.3	5.7
% error (before NH3 addition):	3.9	8.8	1.1	% error
% error (after NH3 addition):	5.3	3.4	2.5	7.9
α_{fb} :	0.052	0.065	0.053	0.057

According to these results, American Sprit Natural cigarettes contain the largest amount of FB nicotine. This result indicates that the MTS from these cigarettes has the highest pH_{eff} because a more basic smoke will have less hydrogen ions available to protonate nicotine. Smoke PM from the other cigarettes/cigar investigated does not show an extreme range in α_{fb} , suggesting that these products have similar smoke pH_{eff} values. In any case, the results indicate that there is a measurable and significant amount of FB nicotine in MTS PM. Furthermore, commercial cigarettes/cigars can show a considerable range in α_{fb} values. As previously discussed, a higher α_{fb} translates into a greater magnitude and rate of nicotine uptake by smokers because PM phase FB nicotine can volatilize and then efficiently deposit in the RT. Calculated α_{fb} values support the concept that the volatilization of FB nicotine from inhaled particles can occur within the RT. Therefore, the amount of FB nicotine in MTS PM carries important implications for how cigarette smoke nicotine is deposited in the RT.

4.0 CONCLUSION

Deposition rate in the RT is influenced by the composition of the inhaled aerosol. Soluble gases will deposit with high, nearly complete, efficiency, while only 50% of particles typically deposit (the other 50% are exhaled.) Due to its acid/base chemistry, nicotine present in MTS can be in either protonated forms or FB form. Furthermore, only the FB nicotine can volatilize from smoke PM into the gas phase of an inhaled stream of MTS. Nicotine delivery magnitude and rate due to changes in the fraction of FB nicotine are associated with addiction potential. These phenomena indicate the importance of the question: how much gaseous nicotine is present in a stream of inhaled MTS?

In order to answer this question, this study focused on determining α_{fb} , the fraction of FB nicotine in MTS. Several brands of cigarettes were tested using HS-SPME/GC/MS analysis. This method requires exposing a PDMS/DVB SPME fiber to the HS of a MTS PM CFP sample, allowing some of the FB nicotine in the HS to sorb onto the fiber. After GC/MS analysis, a base (ammonia) is injected into the filter holder containing the smoke PM, stripping the protons from the protonated nicotine and transforming it to FB nicotine. However, before an exact method could be finalized, a method development stage refined the procedures needed to accurately assess α_{fb} . Fiber testing, exposure time, and ammonia addition were all details that needed calibration. Various filter holders were tested to ensure that their composition did not affect ammonia addition. Results of this testing process yielded that the fiber produced a repeatable, linear response to varying exposure times; a 20 minute fiber exposure time was sufficient to produce a desired nicotine response, 12 mL of ammonia was adequate to strip the H^+ ions from the protonated nicotine and transform it to FB nicotine, and the plastic filter holder did not absorb appreciable amounts of ammonia and was fit to conduct the testing.

This study examined the PM FB nicotine of four brands of commercial cigarettes and one cigar brand. Results presented α_{fb} values that ranged from 0.051 for Marlboro Special Blend 100s to 0.185 for American Spirit Natural cigarettes. These FB nicotine totals indicate that the volatilization of significant amounts of FB nicotine can occur from the PM of MTS. Thus, assessed α_{fb} values from each brand/type of commercial tobacco product have important implications on the addiction potential and delivery magnitude and rate of the nicotine present in the tobacco smoke.

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6.0 APPENDIX

6.1 TABLES

The following tables contain the raw data for the figures or tables listed in each table caption.

Table 6. HS-SPME/GC/MS FB nicotine peak area variations with increasing nicotine content. Data corresponds to Figure 5.

Date:	1/18/2015
Nicotine in pyridine solution (20 min fiber exposure)	
Sample (% by mole fraction)	Nicotine response
0.1% Nic in PY	170336
0.1% Nic in PY	205703
0.5% Nic in PY	985809
0.5% Nic in PY	1105946
1.0% Nic in PY	2541416
1.0% Nic in PY	2315977
2.0% Nic in PY	7870046
4.0% Nic in PY	11863309
6.0% Nic in PY	21118027
6.0% Nic in PY	21455564

Table 7. HS-SPME/GC/MS FB nicotine peak area variations with increasing nicotine content. Data corresponds to Figure 11.

Date:	2/19/2015
Nicotine in pyridine solution (20 min fiber exposure)	
Sample (% by mole fraction)	Nicotine response
0.1% Nic in PY	570128
0.5% Nic in PY	1576115
1.0% Nic in PY	2863703
2.0% Nic in PY	6681879
4.0% Nic in PY	13935252
6.0% Nic in PY	26480621

Table 8. Nicotine response for various SPME fiber exposure times. Data corresponds to Figures 6 and 7.

Date:	4/23/2015	
Marlboro Special Blend 100s (Red Pack, Flip-top Box, Rich Mellow Flavor)		
Sample	Exposure time (min)	Nicotine response
Before NH ₃	5	1429503
Before NH ₃	10	3040507
Before NH ₃	20	6337767
Before NH ₃	20	6418110
Before NH ₃	30	9714887
Before NH ₃	50	16018857
After 6 mL NH ₃	5	15614541
After 6mL NH ₃	10	32149525
After 6 mL NH ₃	20	63515364
After 6mL NH ₃	20	62570828
After 6mL NH ₃	30	89975155
After 6mL NH ₃	50	132471277

Table 9. Nicotine response for various SPME fiber exposure times. Data corresponds to Figures 12 and 13.

Date:	5/21/2015	
Silver Eagle Gold 100s		
	TPM (mg):	29.1
Sample	Exposure time (min)	Nicotine response
Before NH ₃	5	523259
Before NH ₃	10	1026767
Before NH ₃	20	2369229
Before NH ₃	20	2383240
Before NH ₃	30	3997392
Before NH ₃	50	6874814
Before NH ₃	80	10784202
After 12 mL NH ₃	5	4295423
After 12 mL NH ₃	10	8361114
After 12 mL NH ₃	20	17533885
After 12 mL NH ₃	20	16999222
After 12 mL NH ₃	30	25587019
After 12 mL NH ₃	50	41077394
After 12 mL NH ₃	80	61409770

Table 10. GC/MS nicotine peak area variations over multiple gas phase ammonia additions. Data corresponds to Figure 8.

Date:	4/28/2015	
Marlboro Special Blend 100s		
	TPM (mg):	39.7
Sample	Exposure time (min)	Nicotine response
Before NH ₃	20	3370339
Before NH ₃	20	3214994
Before NH ₃	20	3045942
After 1 mL NH ₃	20	29363202
After 1 mL NH ₃	20	26489884
After 3 mL NH ₃	20	47762130
After 3 mL NH ₃	20	44777014
After 6 mL NH ₃	20	54138809
After 6 mL NH ₃	20	51159987
After 12 mL NH ₃	20	63690924
After 12 mL NH ₃	20	61752140
After 15 mL NH ₃	20	64733070
After 15 mL NH ₃	20	61935874

Table 11. GC/MS nicotine peak area variations over multiple gas phase ammonia additions. Data corresponds to Figure 14 and Table 3.

Date:	5/12/2015	
American Spirit Natural		
	TPM (mg):	42.9
Sample	Exposure time (min)	Nicotine response
Before NH ₃	20	21069592
Before NH ₃	20	27187681
Before NH ₃	20	MS power failure
After 6 mL NH ₃	20	115035516
After 6 mL NH ₃	20	116955155
After 9 mL NH ₃	20	128664795
After 9 mL NH ₃	20	125249071
After 12 mL NH ₃	20	131876284
After 12 mL NH ₃	20	128791413
After 15 mL NH ₃	20	133969305
After 15 mL NH ₃	20	132719467
After 18 mL NH ₃	20	137180259
After 18 mL NH ₃	20	135881151

Table 12. GC/MS nicotine peak area variations over multiple gas phase ammonia additions. Data corresponds to Figure 15.

Date:	5/14/2015	
Silver Eagle Gold 100s		
	TPM (mg):	27.9
Sample	Exposure time (min)	Nicotine response
Before NH ₃	20	3457871
Before NH ₃	20	3228456
Before NH ₃	20	3021259
After 6 mL NH ₃	20	40984771
After 6 mL NH ₃	20	39178112
After 9 mL NH ₃	20	44841187
After 9 mL NH ₃	20	41256803
After 12 mL NH ₃	20	45162143
After 12 mL NH ₃	20	42140565
After 15 mL NH ₃	20	44676202
After 15 mL NH ₃	20	40738908
After 18 mL NH ₃	20	43984651
After 18 mL NH ₃	20	43263592

Table 13. GC/MS nicotine peak area variations over multiple gas phase ammonia additions using a metal filter holder with conical headspace. Data corresponds to Figure 9.

Date:	5/28/2015	
Silver Eagle Gold 100s		
Sample	Exposure time (min)	Nicotine response
Before NH ₃	20	4746974
Before NH ₃	20	4920165
Before NH ₃	20	5387388
After 6 mL NH ₃	20	54157254
After 6 mL NH ₃	20	51580431
After 9 mL NH ₃	20	55833343
After 9 mL NH ₃	20	53535514
After 12 mL NH ₃	20	58364085
After 12 mL NH ₃	20	57202031
After 15 mL NH ₃	20	58302540
After 15 mL NH ₃	20	55192397

Table 14. GC/MS nicotine peak area variations over multiple gas phase ammonia additions using a glass VOA vial as the filter holder. Data corresponds to Figure 10.

Date:	6/2/2015	
Silver Eagle Gold 100s		
	TPM (mg):	18.0
Sample	Exposure time (min)	Nicotine response
Before NH ₃	20	3070047
Before NH ₃	20	3341565
Before NH ₃	20	3944610
After 6 mL NH ₃	20	38178270
After 6 mL NH ₃	20	38657661
After 9 mL NH ₃	20	47600050
After 9 mL NH ₃	20	47777551
After 12 mL NH ₃	20	55857029
After 12 mL NH ₃	20	55510099
After 15 mL NH ₃	20	51804071
After 15 mL NH ₃	20	54248616

Table 15. GC/MS nicotine peak area variations over multiple gas phase ammonia additions using a metal filter holder with cylindrical headspace. Data corresponds to Figure 16.

Date:	5/29/2015	
Silver Eagle Gold 100s		
	TPM (mg):	32.6
Sample	Exposure time (min)	Nicotine response
Before NH ₃	20	3184724
Before NH ₃	20	3372906
Before NH ₃	20	3544608
After 6 mL NH ₃	20	70866234
After 6 mL NH ₃	20	66243481
After 9 mL NH ₃	20	71558681
After 9 mL NH ₃	20	68343152
After 12 mL NH ₃	20	70124849
After 12 mL NH ₃	20	65387401

Table 16. Nicotine response for four vials containing PM from the same filter. This data corresponds to Table 2.

Date:	6/3/2015	
Silver Eagle Gold 100s		
	TPM (mg):	29.7
Sample	Exposure time (min)	Nicotine response
Vial 1, Before NH ₃	20	1753947
Vial 1, Before NH ₃	20	1498783
Vial 2, Before NH ₃	20	1247279
Vial 2, Before NH ₃	20	1183702
Vial 3, Before NH ₃	20	2564553
Vial 3, Before NH ₃	20	1957553
Vial 4, Before NH ₃	20	2430578
Vial 4, Before NH ₃	20	1831342
Vial 1, After 4 mL NH ₃	20	31049689
Vial 1, After 4 mL NH ₃	20	28152042
Vial 2, After 4 mL NH ₃	20	36631344
Vial 2, After 4 mL NH ₃	20	31834434
Vial 3, After 4 mL NH ₃	20	34087407
Vial 3, After 4 mL NH ₃	20	30060038
Vial 4, After 4 mL NH ₃	20	34762282
Vial 4, After 4 mL NH ₃	20	30234611

Table 17. Nicotine response for three samples of MTS PM from Marlboro Red Label 100s. This data corresponds to Table 4.

Date:	6/4/2015	
Marlboro Red Label 100s		
TPM _{#1} : 44.4 mg	TPM _{#2} : 35.6 mg	TPM _{#3} : 39.5 mg
Sample	Exposure time (min)	Nicotine response
#1, Before NH ₃	20	2889625
#1, Before NH ₃	20	2977588
#2, Before NH ₃	20	3893802
#2, Before NH ₃	20	3548529
#3, Before NH ₃	20	5735646
#3, Before NH ₃	20	5570997
#1, After 12 mL NH ₃	20	55539409
#1, After 12 mL NH ₃	20	53034835
#2, After 12 mL NH ₃	20	59943696
#2, After 12 mL NH ₃	20	58440816
#3, After 12 mL NH ₃	20	72271360
#3, After 12 mL NH ₃	20	70593847

Table 18. Nicotine response for three samples of MTS PM from Cheyenne Full Flavor 100s Cigars. This data corresponds to Table 5.

Date:	6/5/2015	
Cheyenne Full Flavor 100s Cigars		
Sample	Exposure time (min)	Nicotine response
#1, Before NH ₃	20	1013498
#1, Before NH ₃	20	1070887
#2, Before NH ₃	20	1287636
#2, Before NH ₃	20	1458936
#3, Before NH ₃	20	1292106
#3, Before NH ₃	20	1271360
#1, After 12 mL NH ₃	20	20639697
#1, After 12 mL NH ₃	20	19156104
#2, After 12 mL NH ₃	20	20537518
#2, After 12 mL NH ₃	20	21554980
#3, After 12 mL NH ₃	20	23706113
#3, After 12 mL NH ₃	20	24546500

6.2 FIGURES

Figure 11. HS-SPME/GC/MS FB nicotine peak area variations with increasing nicotine content (second run).

Figure 12. Pre-ammonia addition nicotine peak areas for a range of exposure times Silver Eagle Gold Cigarettes (100s, flip-top box).

Figure 13. Post-ammonia addition nicotine peak areas for a range of exposure times Silver Eagle Gold Cigarettes (100s, flip-top box).

Figure 14. GC/MS nicotine peak area variations over multiple gas phase ammonia additions (American Spirit Natural).

Figure 15. GC/MS nicotine peak area variations over multiple gas phase ammonia additions (Silver Eagle Gold 100s).

Figure 16. GC/MS nicotine peak area variations over multiple gas phase ammonia additions using a metal filter holder with cylindrical headspace (Silver Eagle Gold 100s).