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Changes in Unprotonated Nicotine Concentration in Cigarette Mainstream Smoke with Three Machine-Smoking Conditions

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ABSTRACT : This study was conducted to determine the amount of unprotonated nicotine in cigarette mainstream smoke and to investigate its relationship to level of filter ventilation and machine smoking conditions. Unprotonated nicotine from TPM trapped on a Cambridge filter pad(CFP) was adsorbed by Carboxen/PDMS SPME fiber, thermally desorbed and determined by GC/MS. 2R4F reference cigarette, twelve commercial brands from the Korean market and five test cigarette samples, which had the same tobacco blend with different levels of filter ventilation, were analyzed for unprotonated nicotine. In commercial brands, the amount of unprotonated nicotine changed slightly depending on the pH values of smoke, and decreased as the tar level increased. Filter ventilation in these commercial cigarettes was 28~80 % and the higher filter ventilation increased relative unprotonated nicotine levels, but not significantly. However, in five test cigarettes with different filter ventilation(0~70 %), unprotonated nicotine levels increased almost linearly with the level of filter ventilation. Concentrations of unprotonated nicotine in mainstream smoke generally increased in order HC < ISO ≤ MDPH machine smoking conditions. The ratio of unprotonated nicotine to total nicotine among cigarettes(α_{fb}) increased in order HC < MDPH < ISO conditions. Concentrations of unprotonated nicotine varied with three machine smoking conditions.

Key words **: unprotonated nicotine, filter ventilation, smoking condition, SPME, mainstream smoke**

Nicotine can be found in both the smoke particulate matter(PM) and the gas phase of the smoke(Pankow, 2001). Most of the nicotine in the cigarette mainstream smoke is in the PM phase, with 1 % or less initially in the gas phase(Armitrage and Turner, 1970; Lauterbach, 2000). In smoke PM, nicotine can exist in protonated(mono, di) and unprotonated forms and affects important acid/base chemistry in tobacco smoke(Pankow, 2001)(Fig. 1).

In highly acidic aqueous conditions(pH < 3), the nicotine exists almost as a diprotonated form. However, low pH conditions are typically not encountered in tobacco or smoke, and the diprotonated nicotine has usually been assumed to be of negligible importance(Morie, 1972). The monoprotonated nicotine exist primarily tobacco filter materials and has strong ionic forces

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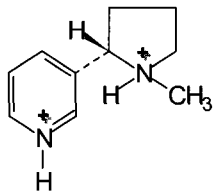
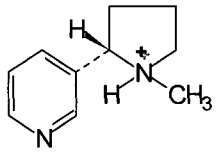
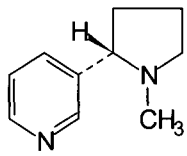

Diprotonated nicotine Monoprotonated nicotine Unprotonated nicotine

Fig. 1. Three forms of nicotine.

minimizing evaporative loss(Brunnemann and Hoffmann, 1974; Sensabugh and Cundiff, 1967). The unprotonated nicotine can volatilize to the gas phase, but protonated nicotine can not. Also, unprotonated nicotine can move into and through tissue lipid layers, but protonated nicotine in largely excluded from such layers (Pankow, 2001).

Changes of acid base smoke chemistry can cause shifts in equilibrium between protonated and unprotonated nicotine(Armitrage and Turner, 1970; Schievelbein *et al.,* 1973). That is, the acid dissociation of protonated nicotine to return the unprotonated nicotine plus a proton occurs according to [Protonated nicotine = Unprotonated nicotine + H^+](Teague, 1973). The changes of equilibrium would be expected to alter the concentrations of protonated and unprotonated nicotine.

In previous studies, researchers have measured the smoke pH to estimate the percentage of nicotine present in the unprotonated form. The relative ratio of unprotonated and protonated nicotine could be determined from pH, total nicotine content and acid base equilibrium data (Morie, 1972). However, the pH measurement provided relative values of the smoke's acid base properties, and those for comparing different styles or types of cigarettes.

Recently the unprotonated nicotine has been studied by direct sampling approach. Pankow *et al.*(2003) has attempted to examine unprotonated nicotine in mainstream smoke. The unprotonated nicotine in mainstream smoke was collected using glass dual cigarette holder and Teflon bag, adsorbed by Tenax TA cartridge, and then analyzed by thermal desorption and GC/MS. Watson *et al.*(2004) studied to determine directly unprotonated nicotine by solid phase micro-extraction(SPME) after smoking under FTC condition. The SPME technique is ideally suited for headspace analysis of unprotonated nicotine because of the SPME fiber's ability to preconcentrate while sampling volatile components.

Therefore this study was performed to quantify the unprotonated nicotine in the mainstream smoke of 2R4F reference cigarette and seventeen cigarette samples using SPME-GC/MS and to investigate its relationship to smoke pH, level of filter ventilation and machine smoking conditions.

MATERIALS AND METHODS

Reagents and materials

Nicotine(98 %) was purchased from Sigma Co. (USA) and an anhydrous NH_3(gas) was purchased from Air Product Company(Korea). Toluene d_8, the primary labeled internal standard, was purchased from CDN Isotopes Inc.(Canada). To prepare stock solutions, chemical reagents at the microliter level were transferred using positive displacement pipettes. 2R4F reference cigarette, 12 commercial brands from the Korean market and 5 test cigarettes, which had the same tobacco

blend with different levels of filter ventilation, were used for this experiment. 2R4F reference cigarette was obtained from the Kentucky Tobacco Research & Development Center, University of Kentucky, Lexington, KY, USA. The unopened cigarette packs were individually sealed in plastic bags and stored at 0℃ until used.

Standard preparation

Analyte standards were prepared by dilution in methanol after the neat compounds had been weighed to the nearest 0.1 mg, and final dilution was made by water. Anhydrous NH_3(gas) was used to convert all of the nicotine to the unprotonated nicotine for calibration. Triplicate samples were analyzed over the maximum range expected for unprotonated nicotine. Concentrations of calibration standards ranged from 0.01 to 1.0 mg of nicotine. To convert nicotine to unprotonated nicotine, blank CFP in a 20 mL vial was placed, spiked with nicotine and the internal standard, and sealed the vial with the septum lined top. The lower three fourths of each vials was submerged in liquid N_2 and the air removed by puncturing the septum with a needle attached by tubing to a mechanical low vacuum pump. The air was replaced with 20 mL of NH_3(gas) from a Tedlar bag filled with NH_3(gas) using a gas tight syringe and the vial was resealed with a new unpunctured septum top.

Cigarette machine-smoking condition

Cigarette samples were smoked on the three machine-smoking conditions(Table 1), such as International Organization of Standardization (ISO), the Massachusetts Department of Public Health(MDPH) and Health Canada(HC) conditions by using ASM 500(Cerulean, UK) and RM20/CS(Heinr Borgwaldt, Germany), and TPM were collected in 44 mm and in 92 mm CFPs, respectively. Table 1 indicated puffing parameters for the three machine-smoking conditions.

Table 1. Puffing parameters for three machine-smoking conditions

Condition	Puff Interval (sec)	Puff Duration (sec)	Puff Volume (mL)	Ventilation blocking (%)
ISO[1)]	60	2	35	0
MDPH[2)]	30	2	45	50
HC[3)]	30	2	55	100

[1)] International Organization of Standardization.
[2)] Massachusetts Department of Public Health.
[3)] Health Canada.

Sample preparation

For unprotonated nicotine analysis, each 44 mm CFP, which had collected the mainstream smoke of a maximum of five cigarettes, was placed in a 20 mL SPME vial and spiked with 50 μL of internal spiking solution(1 μg/mL). For nicotine analysis, ISO 10315(2000) method was used.

SPME-GC/MS analysis

SPME-GC/MS analysis was carried out on a Varian 1200 GC/MS(USA) equipped with a Leap CTC CombiPAL autosampler(USA), which automated the SPME headspace analysis. A 75 μm Carboxen/PDMS fiber(Supelco, USA) was used for the headspace sampling. After 2 min exposure in the headspace above the CFP sample, the fiber was introduced into the inlet of the GC/MS.

The chromatograph was equipped with a DB-624 column(30 m×0.32 mm i.d., 1.8 μm film thickness, J&W Sci., USA). The following oven temperature program was used: hold at 50 ℃ for 2 min, ramp to 210 ℃ at 30 ℃/min, and hold at 210 ℃ for 5 min. Helium was used as the carrier gas at a flow rate of 2.0 mL/min. The injection inlet was used a narrow bore(75 μm) inlet liner, and operated in splitless mode.

Source, transfer line and injector temperatures kept at 200 ℃, 230 ℃ and 285 ℃, respectively.

pH measurement

The smoke pH was measured by whole smoke in an aqueous solution with no CFP using pH meter(Thermo Orion, Model 720, USA), after smoking one cigarette. Smoke was collected by using glass impingers filled with 20 mL deionized water. The measurement of smoke pH was taken using two impingers per cigarette sample, and used only tapered glass gas sampling tubes(no frits). The smoke pH measured 5 min after sample collection(Mellinger, 1997).

RESUTLS AND DISCUSSION

We focused on nicotine types associated with the particulate portion of smoke aerosol trapped on a CFP. Nicotine measured using the SPME headspace method was attributed to the gaseous portion of unprotonated form because the protonated form has substantially lower volatility. Using this technique, unprotonated nicotine in headspace above the CFP was measured for 2R4F reference cigarette, twelve commercial brands from the Korean market and five test cigarettes.

Effect of pH and filter ventilation at ISO machine-smoking condition

Cigarette samples were sorted by tar levels and measured nicotine concentrations and pH values (Table 2), and investigated influence of filter ventilation level on the amount of unprotonated nicotine(Fig. 2).

As the tar level increased, the concentration of total nicotine increased, pH value and the

Table 2. Tar, nicotine, pH and filter ventilation for cigarette samples on three machine-smoking conditions

Cigarette	Ventilation (%)	ISO			MDPH			HC		
		Tar (mg/cig.)	Nicotine (mg/cig.)	pH	Tar (mg/cig.)	Nicotine (mg/cig.)	pH	Tar (mg/cig.)	Nicotine (mg/cig.)	pH
2R4F	28.3	8.90	0.72	5.05	23.16	1.76	5.06	35.37	1.91	5.02
A	34.1	7.51	0.60	5.07	22.75	1.45	5.10	36.80	1.78	5.07
B	34.4	6.43	0.57	5.10	18.59	1.39	5.13	31.06	1.54	5.02
C	41.2	6.13	0.56	5.19	17.70	1.43	5.17	34.85	1.76	5.14
D	49.0	6.24	0.54	5.18	18.35	1.38	5.06	29.78	1.70	5.06
E	53.7	5.66	0.53	5.11	17.20	1.61	5.06	38.31	2.00	5.07
F	59.3	4.88	0.49	5.18	17.81	1.49	5.11	32.67	1.97	5.04
G	60.1	2.86	0.25	5.13	11.27	0.88	5.16	26.51	1.25	5.02
H	63.1	3.17	0.30	5.14	12.68	1.09	4.99	29.88	1.67	5.02
I	73.8	0.82	0.09	5.18	6.14	0.52	5.03	19.79	0.88	5.01
J	76.9	1.04	0.09	5.24	7.67	0.67	5.09	28.27	1.27	5.07
K	80.1	0.77	0.07	5.24	6.93	0.57	5.02	24.30	1.27	4.97
L	81.8	1.25	0.11	5.26	7.91	0.72	5.02	29.49	1.29	4.98
Test cig. 1	0	11.66	0.88	5.03	25.07	1.82	5.06	32.77	1.92	4.98
Test cig. 2	10	10.38	0.82	5.08	23.32	1.75	5.04	31.22	1.89	-
Test cig. 3	30	8.51	0.71	5.12	21.69	1.70	5.02	31.62	1.90	-
Test cig. 4	50	5.77	0.52	5.12	18.39	1.49	5.06	30.66	1.84	-
Test cig. 5	70	3.52	0.31	5.19	14.24	1.26	4.98	30.34	1.82	-

unprotonated nicotine level decreased. In the selected commercial cigarettes, the amount of unprotonated nicotine was not significantly changed, but it was affected by the pH of cigarette smoke. It demonstrated Teague(1973)'s report that protonated nicotine returns to the unprotonated nicotine plus a proton by the acid dissociation.

Filter ventilation in these commercial cigarettes was 28~80 % and the higher filter ventilation decreased both total nicotine and tar, but increased relative unprotonated nicotine levels. Especially, the fraction of the PM nicotine that is in the unprotonated form(α_{fb}) increased with the filter ventilation levels(Fig. 2), and its linear relationship(R^2) was 0.654.

To further study, five test cigarette samples, which had different levels of filter ventilation, were analyzed. Relationship(R^2) between α_{fb} and the filter ventilation rate for five test cigarettes was 0.835. Also the amount of unprotonated nicotine almost linearly increased as the filter ventilation rate increased.

Those results were previously observed in the denuder work by Lewis *et al.*(1995). We confirmed that filter ventilation was an important factor on the unprotonated nicotine deliveries. The effect of-filter ventilation on changes of unprotonated nicotine levels could be partially related to "off gassing" (Hurt and Robertson, 1967; Teague, 1973). As the filter ventilation level increases, the smoke aerosol is increasingly diluted and resulting in increased surface area and surface residence times for evaporation. This could tend to maximize the particulate surface area, enhancing the evaporation of volatile species from the CFP(Watson *et al.*, 2004). Cigarette with lower tar may have a significant fraction of their total nicotine present as the unprotonated form.

Changes in unprotonated nicotine levels with the machine-smoking condition

To further investigate the effect of smoking parameters, the mainstream smoke of cigarette samples at three machine-smoking conditions were analyzed(Table 2, Fig. 3).

Concentrations of unprotonated nicotine in

α_{fb} = [Unprotonated nicotine] / [Total nicotine]

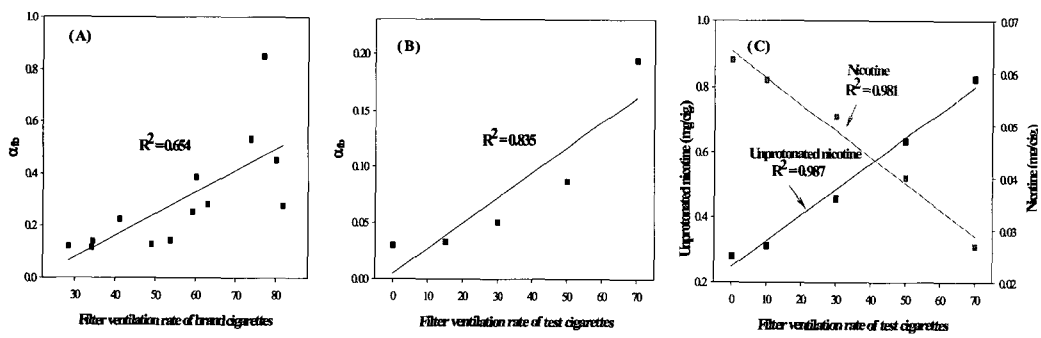

Fig. 2. Relationship between the unprotonated nicotine and the filter ventilation rate for cigarette samples. A : Relationship between α_{fb} and the filter ventilation rate for 13 cigarette brands, B : Relationship between α_{fb} and the filter ventilation rate for 5 test cigarettes, C : Relationship between unprotonated nicotine and total nicotine amount by the filter ventilation rate for 5 test cigarettes.

cigarette mainstream smoke generally increased from MDPH to ISO to HC conditions. The ratio of unprotonated nicotine to total nicotine among cigarettes(α_{fb}), however, generally decreased from ISO to MDPH to HC conditions. Its tendency was similar to smoke pH which was tend to decrease in order ISO > MDPH > HC conditions(Table 2). It demonstrates that some relationship between smoke pH and unprotonated nicotine exist.

As the tar decreased, α_{fb} increased. Especially I~L cigarette samples(approximately 1 mg/cig. of tar) were showed the dramatic increasing of unprotonated nicotine level at ISO condition. But concentrations of unprotonated nicotine had a little change at MDPH and HC conditions.

In five test samples with different levels of filter ventilation, unprotonated nicotine levels were no significant differences at MDPH and HC conditions. The linear relationship(R^2) between levels of filter ventilation and unprotonated nicotine amount was 0.987 at ISO condition, 0.752 at MDPH condition and 0.185 at HC condition. The linear relationship(R^2) between levels of filter ventilation and α_{fb} was 0.835 at ISO condition, 0.968 at MDPH condition and 0.185 at HC condition.

Pankow(2001) published that up to 40 % or more of the nicotine present in mainstream smoke could be in the unprotonated form for some brands. Our results indicate that the percentage of unprotonated nicotine in mainstream smoke ranged from 12 % to 39 % for cigarettes of tar 3~8 mg/cig, and from 27 % to 85 % for cigarettes of tar approximately 1 mg/cig, supporting Pankow (2001)'s report that a significant fraction of the nicotine could be present in the unprotonated form.

Percentage of unprotonated nicotine in mainstream smoke of commercial cigarettes ranged from 4 % to 10 % except two brands(G : 14 %, J : 20 %) at MDPH condition, and those ranged from 1.6 % to 4.5 % at HC condition. Percentage of unprotonated nicotine for five test samples ranged from 3 % to 20 % at ISO condition, from 2 % to 5 % at MDPH condition and 1.5 % at HC condition.

Compared to absolute unprotonated nicotine level at three machine-smoking conditions, half of cigarette brands at MDPH condition were a little lower or similar levels compared to ISO condition. Differences of ISO and MDPH conditions are blocking %, puff volume and puff interval.

It is demonstrated that concentration of unprotonated nicotine increased as filter ventilation rate increased. Even though cigarettes were 50 % blocked, unprotonated nicotine levels increased or was the same. It would be affected by puff volume(45 mL) and puff interval(30 s) at MDPH condition.

CONCLUSIONS

In this study, we have directly determined the concentration of unprotonated nicotine in trapped mainstream smoke particulates using SPME method. In 2R4F reference cigarette, twelve commercial brands and five test cigarettes, the concentration of unprotonated nicotine varied slightly depending on smoke pH value. The higher filter ventilation increased unprotonated nicotine levels, but not significantly. In five test cigarettes, unprotonated nicotine levels increased almost linearly with increasing level of filter ventilation. The concentration and percentage of unprotonated nicotine changed by the machine-smoking conditions(ISO, MDPH and HC). Those of unprotonated nicotine were strongly influenced by the filter ventilation. However, many factors will contribute to the smoke delivery achieved by an individual smoker. We need to further study on the effects of chemicals naturally present in tobacco, additives, physical properties and actual smoking condition.

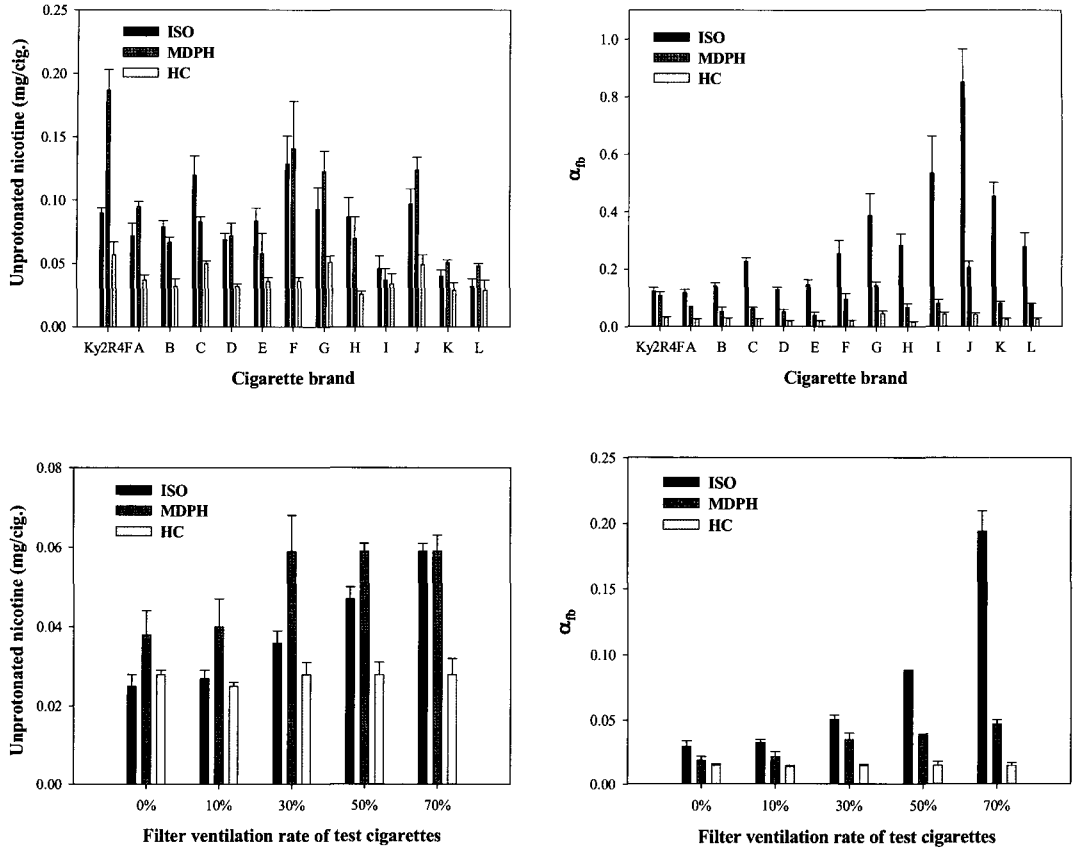

Fig. 3. Comparison of unprotonated nicotine amount and α_{fb} measured in the mainstream smoke of cigarette samples by three machine smoking conditions.

REFERENCES

Armitrage, A.K. and Turner D.M. (1970) Absorption of nicotine in cigarette and cigar smoke through the oral mucosa. *Nature* 226: 1231-1232.

Brunnemann, K.D. and Hoffmann, D. (1974) The pH of tobacco smoke. *Food Cosmet. Toxicol.* 12: 115-124.

Hurt, R.D. and Robertson, C.R. (1967) Prying open the door to the tobacco industry's secrets about nicotine: The Minnesota Tobacco Trial. *J. Am. Med. Assoc.* 280: 1173-1181.

ISO (2000) 10315: Cigarette Determination of nicotine in smoke condensate gas chromatographic method.

Lauterbach, J.H. (2000) A critical assessment of recent work on the application of gas/particle portioning theories to cigarette smoke. *Beitr. Tabakforsch. Int.* 19: 65-83.

Lewis, D.A., Collbeck, I. and Mariner, D.C. (1995) Diffusion of mainstream tobacco smoke and its effect upon the evaporation and diffusion of nicotine. *J. Aerosol Sci.* 26: 841-846.

Mellinger, K.H. (1997) Analysis of pH and redox potential in mainstream smoke collected in an

aqueous solution. PM Document Code AC-3A3P-ØØ.

Morie, G.P. (1972) Fraction of protonated and unprotonated nicotine in tobacco smoke at various pH values. *Tob. Sci.* 16: 167.

Pankow, J.F. (2001) A consideration of the role of gas/particle partitioning in the deposition of nicotine and other tobacco smoke compounds in the respiratory tract. *Chem. Res. Toxicol.* 14: 1465-1481.

Pankow, J.F., Tabakoli, A.D., Luo, W. and Isabelle, L.M. (2003) Percent free base nicotine in the tobacco smoke particulate matter of selected commercial and reference cigarettes. *Chem. Res. Toxicol.* 16: 1014-1018.

Schievelbein, H., Eberhardt, R., Loschenkohl, K., Rahlfs, V. and Bedall, J.K. (1973) Absorption of nicotine through the oral mucosa : I. Measurement on nicotine concentration in the blood after application of nicotine and total particulate matter. *Agents Actions* 3: 254 258.

Sensabaugh, A.J. and Cundiff, R.H. (1967) A technique for determining the pH of whole tobacco smoke. *Tob. Sci.* 11: 25-30.

Teague, C.E. (1973) Implication and activities arising from correlations of smoke pH with nicotine impact, other smoke qualities and cigarettes sales. R. J. Reynolds internal report; Minnesota Trial Exhibit, Bates no. 500136994-7023.

Watson, C.H., Trommel, J.S. and Asley, D.L. (2004) Solid phase microextraction based approach to determine free base nicotine in trapped mainstream cigarette smoke total particulate matter. *J. Agric. Food Chem.* 52: 7240-7245.