Measurement of Nicotine in Indoor Air Collected by Alkaline-coated Solid Phase Cartridge Followed by GC-MS Analysis

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Nicotine has been used as a selective marker to evaluate environmental tobacco smoke (ETS). In this study, a simple and precise nicotine measurement method using a solid phase cartridge (Sep-Pak® Plus PS-2) followed by GC-MS analysis was evaluated. Prior to use, the solid phase cartridge was coated with potassium hydroxide (KOH), and indoor air was then passed through it. After the air sampling, nicotine was eluted from the cartridge with 10 μg/ml triethylamine/dichloromethane, and the eluent was analyzed by GC-MS. The nicotine recovery from the spiked cartridge was more than 80% for an air sampling time of 12 hr, and the method detection limit (MDL) for the air sampling with an air volume of 72 l was 0.35 μg/m^3. When the cartridge was used for indoor air measurement, the nicotine concentrations in the smoking room ranged from 12.9 μg/m^3 to 86.6 μg/m^3, which were proportional to the number of smoked cigarettes.

Key words —— nicotine, environmental tobacco smoke, indoor air, solid phase cartridge, Sep-Pak® Plus PS-2, smoking room

INTRODUCTION

Environmental tobacco smoke (ETS) is recognized to be an important risk factor for several health problems such as lung cancer,[1,2] coronary heart disease[3,4] and asthma.[5,6] For the investigation of ETS exposure, nicotine has been used as a selective marker of tobacco smoke. Among many reports that proposed measurement methods for indoor air nicotine, XAD-4 resin for active sampling[7–9] and the sodium bisulfate-treated filter for passive sampling[10–12] have been frequently used in recent years. In the active sampling method, it was easy to extract the nicotine from the XAD-4 in the glass tube; which was obtained by ultrasonication of the resin for 30 min;[13] however the nicotine recovery from the spiked sampler was rather low—approximately 70% with the passage of air for 6–8 hr.[9,14] On the other hand, in the passive sampling method, the nicotine in the indoor air is collected on the filters as sulfate salt and the sample preparation procedure is rather complicated since this procedure comprises mainly of three steps: extraction, pH adjustment and liquid/liquid extraction.[15]

In the present study, simple and higher-recovery nicotine measurement method using the solid phase cartridge (Sep-Pak® Plus PS-2) was evaluated. When the solid phase cartridge: containing the same kind of sorbent (styrene-divinylbenzene) with XAD-4, is applied to an active sampling device, nicotine in both vapor-phase and particle-phase is efficiently captured in the cartridge at one time. Furthermore, the sample preparation with use of the cartridge consists of a single elution step, and it requires only a few minutes. To develop the new method, the following two subjects were examined: (1) to improve the peak parameters of nicotine on the gas chromatogram, and to minimize the loss of nicotine during the elution procedure, a suitable solvent for elution was selected. (2) To increase the recovery of nicotine from the air samples, potassium hydroxide (KOH) was used for the cartridge preparation; and the recovery of nicotine from the alkaline-coated cartridge and the non-

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alkaline-coated cartridge were compared. The alkaline coating of the solid phase cartridge was modified for the first time in this study. In addition, the average nicotine concentration in a smoking room in the building during working hours was measured using alkaline-coated cartridges.

MATERIALS AND METHODS

Reagents and Solvents —— Sep-Pak® Plus PS-2 was purchased from Nihon waters K.K. (Tokyo, Japan). The characteristics of the cartridge are as follows; sorbent substrate: styrene-divinylbenzene, sorbent weight: 265 mg/cartridge, surface area: 600 m^2/g, particle size: 50 μm, stable pH range: pH 1–13. Acetone, ethyl acetate, dichloromethane [residual pesticide and polychlorinated biphenyl (PCB) analysis grade], nicotine (analytical grade, 97%), triethylamine and acenaphthene-d_{10} (analytical grade, 99%) were purchased from Wako pure Chemical Industry (Osaka, Japan).

Evaluation of an Eluting Solvent —— Three kinds of solvents—acetone, ethyl acetate and dichloromethane—were tested as the eluting solvent for nicotine from a cartridge. A 1 μg/ml nicotine standard solution was separately prepared with acetone, ethyl acetate and dichloromethane, respectively, and each standard solution was analyzed by GC-MS. From the gas chromatogram, the peak area ratio (peak area of nicotine/peak area of internal standard), the theoretical plates and the tailing factor in each solvent were compared. Then, triethylamine was added to a 0.5 μg/ml nicotine standard solution in the range of 0–50 μg/ml; the nicotine peak area ratios in the solution were analyzed.

Evaluation of Nicotine Recovery from the Solid Phase Cartridge —— The recoveries of nicotine from the spiked cartridges, Sep-Pak® Plus PS-2, without air passed through were evaluated. Prior to use, the solid phase cartridge was washed with 5 ml of dichloromethane and dried with pure nitrogen gas. The cleaned cartridges were spiked with 5 μg of nicotine (10 μl of 500 μg/ml nicotine/dichloromethane solution) and eluted with 5 ml of dichloromethane or 10 μg/ml triethylamine/dichloromethane (TEA-dichloromethane) at a flow rate of 3 ml/min; and the percentage recoveries were then calculated (n = 5).

Evaluation of Nicotine Breakthrough and Recovery After Air was Passed —— The breakthrough and recoveries of nicotine from the spiked cartridges, those were alkaline-coated and the non-alkaline-coated cartridges, after air was passed through were evaluated. For the alkaline modification of the cartridge, 0.7 ml (the bed volume of the cartridge) of 1 M KOH/ethanol was impregnated to the cartridge and then dried with pure nitrogen gas. The cartridge was then washed with 5 ml of dichloromethane and dried again with pure nitrogen gas. After the alkaline coating, cleaning and drying, the cartridge was capped at the orifices and stored in a brown glass flask at room temperature. The breakthrough test and recovery studies were then performed. The alkaline-coated cartridge and non-alkaline-coated cartridges were spiked with 0.5 μg (10 μl of 50 μg/ml nicotine/dichloromethane solution) or 5 μg (10 μl of 500 μg/ml nicotine/dichloromethane solution) of nicotine, and another alkaline-coated cartridge was connected to the back of the spiked cartridge. Nicotine-free indoor air (72 l) was passed through the cartridges (n = 3) at flow rates of 400 ml/min for 3 hr; 200 ml/min, 6 hr; 100 ml/min, 12 hr; and 50 ml/min, 24 hr. The breakthrough was defined as the amount of nicotine collected in the back cartridge when it reached a certain percentage (typically 5%) of the total amount collected by the cartridges. The spiked nicotine was eluted with 5 ml of TEA-dichloromethane from the cartridges at a flow rate of 3 ml/min, and the percentage recoveries were calculated. All the glassware used in this procedure was made of brown glass, and they were rinsed with dichloromethane prior to use.

Indoor Air Nicotine Measurement in the Smoking Room —— In the building of our institute, smoking is not permitted in all area except for smoking rooms, where were completely closed-off and set aside only for smoking. Between July and September 2004, the indoor air of the smoking room was sampled 11 times during working hours at a flow rate of 200 ml/min for approximately 6 hr (72 ± 6 l). Indoor air sampling was carried out using the alkaline-coated cartridge. Along with the nicotine analysis, the number of smoked cigarettes during the sampling period in each measurement was counted. In the smoking room, the floor area, air volume and air ventilation rate (air changes/hr) were 7.5 m^2, 18.0 m^3 and 11.8/hr (all fresh air supply), respectively. Interior materials in the smoking room were polyvinyl chloride (PVC) sheet for the floor, PVC covering for the walls, and gypsum board for the ceiling.

The limit of detection (LOD) was defined as the

absolute amount of nicotine that yields $S/N = 3$, because the alkaline-coated cartridges were devoid of nicotine. The method detection limit (MDL) for the air samples was calculated using the LOD, the volume of the extract (5 ml) and the air sampling volume (72 l).

Instruments —— The nicotine analysis was performed using a HP GC5890/5971A MSD (Agilent Technologies, Palo Alto, CA, U.S.A.). A 12 m × 0.2 mm i.d. HP-Ultra 1 column (100% dimethylpolysiloxane) with a film thickness of 0.33 μm (Agilent Technologies) was used as the GC analytical column. Helium was used as the carrier gas (40 kPa, constant pressure mode). The injector was operated in the splitless mode at a temperature of 250°C (2 μl injection volume). The GC oven temperature was maintained at 70°C for 2 min; it was then increased at a rate of 10°C/min up to 170°C, at 20°C/min to 250°C and maintained for 2 min. A selective ion monitoring mode was used for the nicotine analysis; quantitative ions with m/z of 162 and monitored ions with m/z of 133 and 84 were used. An internal standard, acenaphthene-d_{10} (m/z of 162), was monitored and used for quantification.

RESULTS

Evaluation of an Eluting Solvent

Table 1 shows the peak area ratio (peak area of nicotine/peak area of acenaphthene-d_{10}), theoretical plates and tailing factor of a 1 μg/ml nicotine peak in three kinds of solvents. Though the peak area ratio of nicotine in dichloromethane was the smallest among the three tested solvents, the theoretical plates value and the tailing factor value were best in dichloromethane among them. When a nicotine calibration curve was made with the standard solution in dichloromethane, the obtained calibration curve was not linear (Fig. 1), despite the better peak parameters of nicotine in dichloromethane as compared to the other solvents.

In the National Institute of Occupational Safety and Health (NIOSH) manual of analytical methods for nicotine using XAD-4,[13] 0.01% triethylamine can be added to a desorption solvent (ethyl acetate) to improve the nicotine recovery from the sides of a glass sorbent tube; and the samples are then analyzed by GC-nitrogen-phosphorus detector. Thus, it was thought that the non-linearity of the nicotine calibration curve with regards to dichloromethane was caused by the adsorption of nicotine to the glassware; an adequate concentration of triethylamine then added to dichloromethane for the GC-MS analysis. Figure 2 shows the relationship between the peak area ratio of 0.5 μg/ml nicotine standard solution in dichloromethane and triethylamine concentration. The nicotine peak area ratio was increased with increasing triethylamine concentration up to 10 μg/ml. When the nicotine calibration curve was drawn with regards to TEA-dichloromethane

Table 1. The Peak Parameters of Nicotine in Three Kinds of Solvent (1 μg/ml)

Solvent	Peak area ratio[a)]	Theoretical plates (N)	Tailing factor (T_f)
Acetone	1.23	1070	3.4
Ethyl acetate	0.86	1280	4.2
Dichloromethane	0.75	5560	2.5

a) Peak area ratio; peak area of nicotine/peak area of internal standard (acenaphthene-d_{10}).

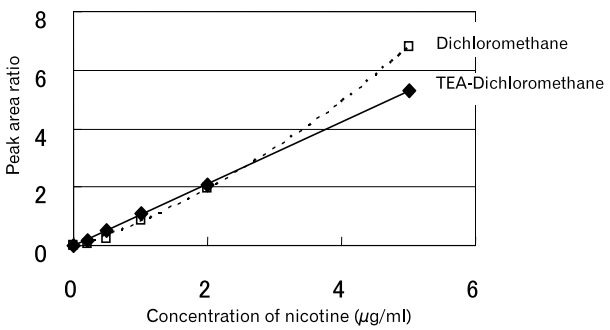

Fig. 1. Calibration Curve of Nicotine Standard Solution
Peak area ratio; peak area of nicotine/peak area of acenaphthene-d_{10}, TEA-Dichloromethane; 10 μg/ml triethylamine/dichloromethane.

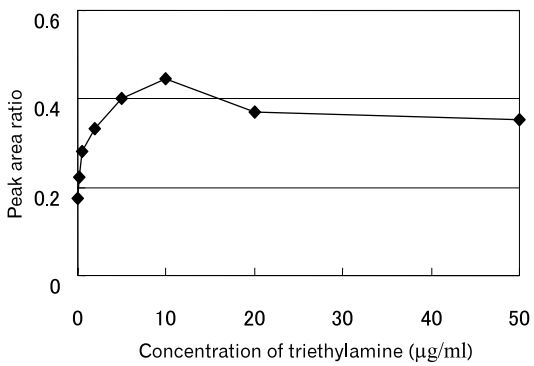

Fig. 2. Relationship between Peak Area Ratio of Nicotine/Acenaphthene-d_{10} and Triethylamine Concentration
Nicotine standard solution in dichloromethane (0.5 μg/ml) was added with triethylamine in the range of 0–50 μg/ml.

(Fig. 1), a linear calibration curve was obtained (r^2 = 0.9997). The peak area ratio, theoretical plates and tailing factor for the 1 μg/ml nicotine solution in TEA-dichloromethane were 1.10, 15, 300 and 1.5, respectively. These parameters improved remarkably as compared to those with regards to dichloromethane without triethylamine (Table 1). When 10 μg/ml triethylamine was added to acetone and ethyl acetate, the tailing factor of the 1 μg/ml nicotine solution was less (approximately 2.5) than those without triethylamine (Table 1); however, the tailing factor were still larger than that of TEA-dichloromethane. The results of this experiment showed that the TEA-dichloromethane was the most suitable for the cartridge eluting solvent.

Usually, deuterated or isotopically labeled nicotine is used as an internal standard in nicotine analysis. Internal standard methods are useful for compensating injection errors and the sensitivity changes in GC-MS. Therefore, it is important to ensure that the same concentration of internal standard in every sample solution. In this study, it was revealed that nicotine is easily adsorbed onto the surface of glass test tubes. When deuterated or isotopically labeled nicotine, which has properties similar to that of nicotine, was used as the internal standard, the concentration of the internal standard in the sample solutions were believed to be different because of the adsorptive property. Acenaphthene-d_{10}, a three-ring polycyclic aromatic hydrocarbon, is a highly stable compound and has the same quantitative ion as nicotine (m/z 162). Therefore, in this study, acenaphthene-d_{10} was adopted as the internal standard for GC-MS analysis instead of deuterated or isotopically labeled nicotine.

Evaluation of Nicotine Recovery from the Solid Phase Cartridge

The recovery of nicotine from the solid phase cartridge (air was not passed through) was then evaluated. The blank cartridge was devoid of nicotine, and the recoveries of nicotine (mean ± SD%) from the spiked cartridges (5 μg of nicotine was added) by using dichloromethane and TEA-dichloromethane (n = 5), were 98.3 ± 3.9% and 82.2 ± 4.9%, respectively.

Evaluation of Nicotine Breakthrough and Recovery After Air was Passed

In the preliminary examination, indoor air was passed through the spiked cartridge (5 μg of nicotine) at a flow rate of 500 ml/min for 6 hr (180 l); nicotine was then eluted from the cartridge with 5 ml of TEA-dichloromethane. In the result of this experiment, there was no breakthrough, but the percentage recovery of nicotine was very low (approximately 10%). It was believed that some modifications were required to prevent the decrease of nicotine during air sampling. Nicotine is a base and is more stable under alkaline conditions; therefore, the alkaline modification of the sampling cartridge was believed to be effective in increasing the recovery of nicotine after passing air. For alkaline modification, KOH dissolved in ethanol was impregnated to the cartridges and dried. Sodium hydroxide (NaOH) is also a strong base, but its solubility in ethanol is much lower than KOH; therefore KOH was selected as the alkaline agent in this study. When the other preliminary recovery test (air flow rate: 200 ml/min for 6 hr) was performed with alkaline-coated cartridges modified with 0.02 M, 0.2 M and 1 M KOH/ethanol, the nicotine recovery was the highest in the cartridge modified with 1 M KOH/ethanol (approximately 85%); therefore 1 M KOH/ethanol was used for the alkaline modification. In the GC-MS analysis, alkaline samples can damage the GC inlet tube or capillary column. However, in this method, KOH impregnated to the solid phase cartridge did not interfere with the GC-MS analysis because KOH is insoluble with dichloromethane.

Table 2 shows the results of the nicotine recoveries from the solid phase cartridge with or without alkaline coating, after air was passed through for various times. The room temperature and the humidity (maen ± S.D.) during the air pass time were 25.7 ± 1.4°C and 54.6 ± 5.5%, respectively. Since nicotine was not detected in the back cartridge, there was no breakthrough of nicotine from the front cartridges. In the other breakthrough test, the air was passed through the spiked cartridge (5 μg of nicotine) at flow rate of 500 ml/min for 24 hr (720 l), there was no breakthrough of nicotine.

The alkaline-coated blank cartridge was also devoid of nicotine as in the case of the non-alkaline-coated cartridge. When KOH was not impregnated to the solid phase cartridge, the nicotine recoveries were low; the highest value was 40.9% with an air passage time of 3 hr in the cartridges added with 5 μg nicotine. The percentage recoveries of nicotine from the alkaline-coated cartridge were more than 80% up to an air passage time of 12 hr in both the cartridges added with 5 μg and 0.5 μg nicotine. The results of the recovery tests showed that

Table 2. Effects of KOH on Recovery of Nicotine from the Solid Phase Cartridge After Air (72 l) was Passed Through

Air flow rate × time	Non-alkaline-coated Cartridge[a] (n = 3)		Alkaline-coated Cartridge[b] (n = 3)	
	Recovery (%)	S.D.	Recovery (%)	S.D.
5 μg nicotine/cartridge				
400 ml/min × 3 hr	40.9	6.8	96.8	3.1
200 ml/min × 6 hr	28.5	9.4	87.5	3.4
100 ml/min × 12 hr	19.4	3.7	81.4	6.4
50 ml/min × 24 hr	NA[c]	NA	70.1	7.7
0.5 μg nicotine/cartridge				
400 ml/min × 3 hr	15.0	5.4	91.3	3.9
200 ml/min × 6 hr	13.5	6.7	84.5	4.5
100 ml/min × 12 hr	8.2	3.2	81.1	2.2
50 ml/min × 24 hr	NA	NA	68.9	8.2

a) Prior to use, the cartridges were washed with 5 ml of dichloromethane and dried with pure nitrogen gas. *b*) Prior to use, the cartridges were impregnated with 0.7 ml of 1 M KOH/ethanol and dried with pure nitrogen gas; they were then washed with 5 ml of dichloromethane and dried again with pure nitrogen gas. *c*) NA: not analyzed.

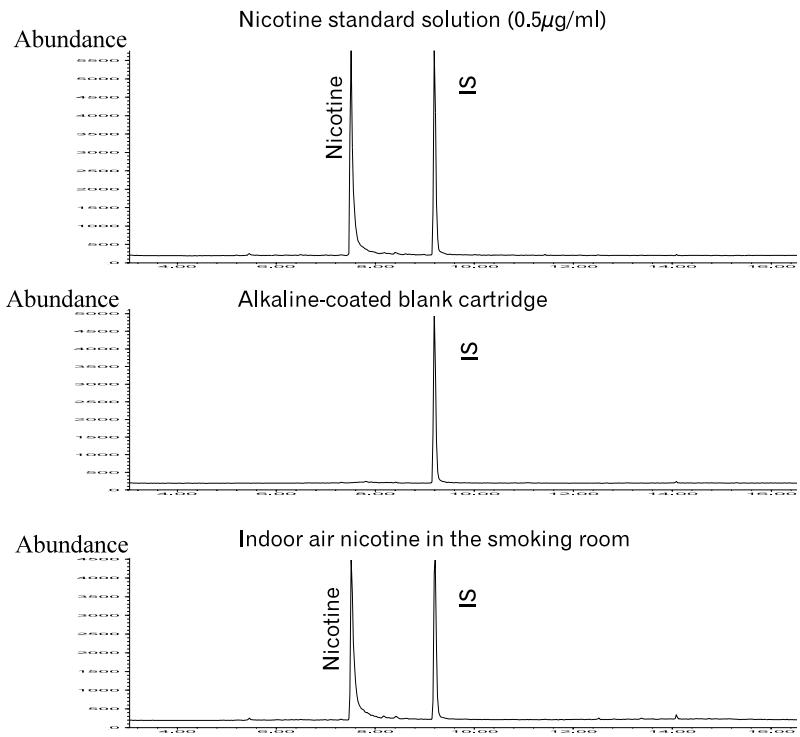

Fig. 3. Gas chromatograms of Nicotine Derived from Different Medium
top; standard solution, middle; eluate of alkaline-coated blank cartridge, bottom; sample solution of indoor air in the smoking room.

the alkaline-coated solid phase cartridge is useful for precise indoor air nicotine measurement within 12 hr of air sampling.

Indoor Air Nicotine Concentrations in the Smoking Room

Figure 3 shows the gas chromatograms of nicotine in the standard solution, alkaline-coated blank cartridge and indoor air sample in the smoking room. The LOD of nicotine by the GC-MS analysis was 0.005 μg/ml, and the MDL for the air samples was 0.35 μg/m^3 with the 72 l air sampling. The indoor air was sampled in early September, and the number of smoked cigarettes during the sampling time (6 hr) was 41. When the duplicate air sampling for 6 hr was carried out in the smoking room, the indoor air nicotine concentrations were very close; they were 73.0 μg/m^3 and 73.5 μg/m^3. Regarding

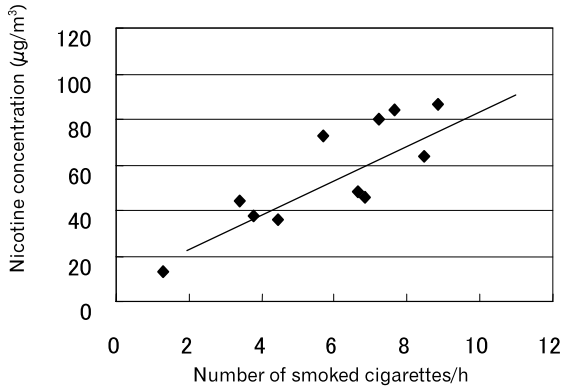

Fig. 4. Relationship between the Number of Smoked Cigarettes and the Nicotine Concentration in the Smoking Room

the storage stability of the air samples, the percentage decrease in nicotine in the cartridge and that in the eluting solvent after storage for 7 days at 4°C was both less than 5%.

Figure 4 shows the relationship between the number of smoked cigarettes and indoor air nicotine concentration in the smoking room. The number of cigarettes and the indoor air nicotine concentrations ranged from 1.3/hr to 8.9/hr and 12.9 μg/m^3 to 86.6 μg/m^3, respectively. The room temperature and the humidity (maen ± S.D.) during the sampling period were 25.3 ± 0.54°C and 62.6 ± 4.5%, respectively. When the correlation analysis was performed with SAS version 6.12 for windows (SAS Institute, Cary, NC, U.S.A.), the indoor air nicotine concentrations had a significant positive correlation with the number of cigarettes (r = 0.841). Along with the nicotine analysis, 50 cigarette butts in the ashtray of the smoking room were measured the length excluding the filter tips, the average smoked length of the cigarettes was 72.1 ± 12.9% of the measured length.

DISCUSSION

The results of this study indicated that two important modifications are required to improve the precision of the indoor air nicotine measurements. The aims of these modifications was (1) to minimize nicotine loss by interaction with a residual silanol group on the glasswase, inlet liner and colum during the extraction and analysis procedure and (2) to minimize the nicotine loss during air sampling. The physical properties of nicotine are as follows: the pH for a 0.005 M solution is 10.96[16] and its dibasic nature is due to the two protonation sites at the pyrrolidine and pyridine nitrogens. The nitrogen protonation occurs in neutral or acidic conditions.[17] It is believed that protonated nicotine is more adsorptive to the residual silanol groups[18] and more reactive with compounds such as hydroxyl radicals and acidic compounds[19] as compared to free-base nicotine. Therefore, in this study, the modifications to maintain an alkaline condition during the measurement procedure and to prevent the protonation of nicotine were evaluated. These modifications comprised the addition of 10 ppm triethylamine to dichloromethane and KOH to the air sampler. In addition, triethylamine was an effective masking agent for the silanol groups on the surface of the glass test tube, the GC inlet tube and the column. It was considered that the silanol masking by triethylamine improved not only the nicotine recovery but also the nicotine peak sharpness; this enabled the GC-MS analysis to be performed with a lower MDL.

When nicotine was eluted from the spiked cartridges (non-alkaline-coated cartridges) with dichloromethane (air was not passed through), the percentage recoveries were higher for the eluent with triethylamine (98.3%) than that without triethylamine (82.2%). After the recovery tests, the eluted cartridges were again eluted with TEA-dichloromethane followed by the GC-MS analysis. The nicotine concentrations in the second round of elutions were below the LOD. The result of the experiment showed that the lower nicotine recovery for the eluent without triethylamine may have been caused by the adsorption of nicotine to the glass tube.

The nicotine recovery from the alkaline-coated solid phase cartridge decreased with increase in the air sampling time. When KOH is exposed to air, it reacts with CO_2 gas, and K_2CO_3 is formed on the surface of KOH. Not only CO_2 but also acidic compounds such as SOx, NOx and organic acids may react with KOH, thereby reducing its alkalinity. In general, a rate of gas-solid reaction (*e.g.*, formation rate of K_2CO_3) depends on a temperature and a concentration of reactants, and it doesn't depend on a gas flow rate when the concentrations are constant. The concentrations of CO_2 and the acidic compounds were believed to be constant in the indoor air used during the recovery tests; therefore, it was considered that a longer sampling time reduced the alkalinity of the cartridge and nicotine recovery less.

The nicotine concentrations detected in

the smoking room ranged from 12.9 μg/m^3 to 86.6 μg/m^3. These levels were considerably higher than the nicotine levels detected in restaurants during the busy serving hours for 3–4 hr, which ranged from 0.04 μg/m^3 to 71.6 μg/m^3.[8] The smoking room that was used in this study had a small floor area of 7.5 m^2, and the highest number of cigarettes smoked per hour during the sampling period was 8.9. Although the air change rate in the room was high (11.8 times/hr), the small area and the high number of cigarettes may have attributed toward increasing the nicotine concentration in the room.

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