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RESEARCH PAPER

Effect of household air pollutants on the composition of exhaled breath characterized by solid-phase microextraction and needle-trap devices

Shakiba Zeinali[1] · **Janusz Pawliszyn**[1]

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Abstract
Exposure to household air pollutants is becoming a serious environmental health risk. Various methods can be applied to assess humans' exposure status to indoor pollutants, with breath monitoring being among the best options. Breath sampling is fast and non-invasive, and contains compounds that can be used as markers for evaluating exposure length and estimating internal concentrations of pollutants. However, the distribution of compounds between gas and droplets in breath samples represents one of the key challenges associated with this analytical method. In this work, a needle-trap device (NTD) was prepared by packing the needle with a porous filter, divinyl benzene, and Carboxen to enable the exhaustive capture of both droplet-bound and gaseous components. Furthermore, fiber-based solid-phase microextraction (SPME) was also applied to extract compounds from only the gas phase to distinguish this portion of analytes from the total concentration in the sample. Dynamic, real-time breath sampling was enabled via a new sampling tube equipped with 2 one-way valves, which was specially designed for this work. Both methods provided satisfactory reproducibility, repeatability, and sensitivity, with detection limits as low as 0.05 ng mL^{-1}. To investigate the real-world applicability of the proposed devices, breath samples were obtained from volunteers who had been exposed to candle and incense smoke and aerosol sprays, or had smoked cannabis. The results revealed the high concentration of organic air pollutants in inhaled air (maximum of 215 ng mL^{-1}) and exhaled breath (maximum of 14.4 ng mL^{-1}) and a correlation between the components in inhaled air and exhaled breath. Significantly, the findings further revealed that the developed NTD has enhanced breath-sample determinations, especially for polar compounds, which tend to remain trapped in breath droplets.

Keywords Exposome · Air pollutants · Needle-trap devices · Solid-phase microextraction · Breath analysis

Introduction

The importance of breath composition and its relation to human health has been known for a long time; however, advanced technologies enabling the analysis of breath composition have only emerged over the past few decades. While more than 1000 volatile organic compounds (VOC) have since been detected in breath samples, only a few of these VOCs are common to human samples [1]. The non-invasive nature of breath sampling makes it an excellent candidate for monitoring health status, particularly with respect to clinical diagnosis (endogenous compounds) and exposure analysis (exogenous compounds).

Previously, most breath-sample studies have focused on identifying biomarkers that can be used to determine disease stages [2–11], with little attention being given to the use of breath biomarkers as a tool for the rapid determination of levels of potentially noxious compounds in humans due to exposure, specifically via inhalation [12, 13]. According to the National Academy of Sciences, exposure is defined as "an event that occurs when there is contact at a boundary between a human and the environment with a contaminant of specific concentration for an interval of time" [14]. Since it is a simpler matrix, expired breath is preferred for measuring

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✉ Shakiba Zeinali
szeinali@uwaterloo.ca

[1] Department of Chemistry, University of Waterloo, Ontario N2L 3G1 Waterloo, Canada

exposure to VOCs [15]. Additionally, breath analysis can be used to monitor the decay and degradation of volatile toxic substances in the body in real time [13, 16].

The use of biomarkers in exposome studies was developed to estimate the relationship between occupational/environmental exposure and its effect on people, with the goal of preventing diseases by reducing exposure through early identification [17]. Since there is an equilibrium between alveolar air and pulmonary capillary blood, breath exposome studies enable the estimation of the internal concentration and distribution of chemicals in the body [18].

Most exposure studies consider industrial environments with high levels of exposure; however, it has been shown that long-term exposure to low concentrations of some VOCs can be carcinogenic or result in allergic reactions [19, 20].

Another issue with breath analysis is the low concentration of VOCs/biomarkers in breath samples and their distribution between the gas and droplet phases. Previously, extraction methods focusing on gas-phase composition have been reported using solid-phase microextraction (SPME) [9, 21–24] and solid sorbents [25–27] for preconcentration of breath biomarkers. Nearly all studies in the area of breath analysis have been limited to the investigation of either aerosol/condensate phase [28, 29] or gas phase [30], which highlights the need for an integrated and comprehensive method for studying biomarkers in breath samples.

It is possible to trap exhaled breath aerosol and extract exhaled breath vapor using a single needle-trap device (NTD) [31–36]. While the design of commercial NTDs allows them to act as a filter for trapping particles, their filtration efficiency is rather low due to the large size of the packing material. This deficiency can be remedied by adding a proper filter to the NTD. Furthermore, SPME can be applied to distinguish the aerosol portion of a breath sample from the vapor portion, as it is capable of extracting only from the gas phase.

To address the aforementioned issues, we packed an NTD with an electrospun heated polyacrylonitrile (H-PAN) filter and commercial divinyl benzene (DVB) and Carboxen (CAR) sorbent particles to enable the trapping of aerosol particles and the extraction of gaseous components, respectively. Additionally, a DVB/CAR SPME fiber was applied to study the gaseous components in breath samples. The developed methods were used to study the relationship between the composition of inhaled air and exhaled breath following exposure to cannabis cigarette/candle/incense smoke and aerosol sprays. To facilitate this study, a breath sampling tube was designed to enable the real-time dynamic monitoring of respiration.

Materials and methods

The detailed explanation on the materials and instruments is provided in supporting information, methods and material section. The protocol for breath sampling was based on the ethical clearance approved by University of Waterloo #42853.

Preparation of H-PAN filter

An extensive study on the preparation procedure and characteristics of the filter has been reported previously [31]. A schematic of the filter-preparation process is shown in Fig. 1.

Extraction devices and procedure

Gas mixtures were prepared via direct injection of the pure liquid analytes into a glass bulb. For this process, A 1-L glass bulb was washed, dried, vacuumed, injected with 1 µL of each analyte, and then heated. Nitrogen gas was added to compensate for the pressure difference between the air in the bulb and the external atmosphere. The concentrations of each analyte in the bulb were calculated according to the equations in [37] and can be found in Table S1. For extraction, appropriate amounts of the standard gas mixture were transferred from the 1-L glass bulb to a 125-mL glass bulb

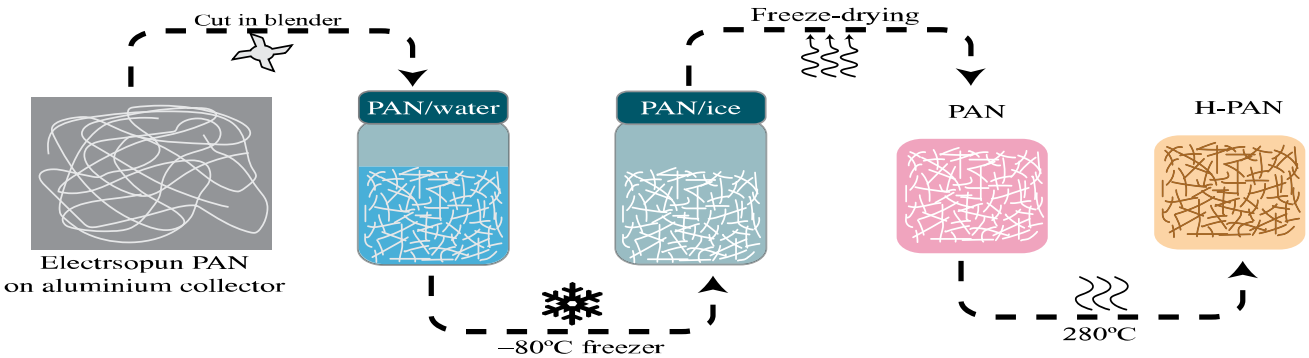

Fig. 1 Schematic of filter-preparation steps

using gas-tight syringes in order to obtain the desired concentrations (Fig. S1a).

A DVB/CAR SPME fiber (50/30 µm, SUPLECO) and a home-made H-PAN/DVB/CAR NTD were applied for the extraction of gaseous compounds during the optimization and calibration steps. The NTD was packed with 5 mm of DVB and 5 mm of CAR, which were sandwiched between two filter plugs (2 mm). The SPME fiber was left inside the mixture for a pre-defined time period, while NTD extractions were performed by using a pump to draw the sample through the needle (flow rate = 20 mL min^{-1}). A schematic of the extraction devices is presented in Fig. 2.

Volunteers were lab members and staff from University of Waterloo. For the extraction of gaseous compounds from breath samples, a volunteer (with ethical approval from University of Waterloo #42853) was asked to exhale into the sampling tube. As shown in Fig. 3, the initial exhaled breath sample fills discard bag #1 (pink path #1), which ensures that any stagnant mouth air is removed and that the sample consists entirely of alveolar air. After filling the first discard bag, the breath pressure opens the one-way valve and enters the sampling tube, before being pushed into discard bag #2 (green path #2). The sampling process continues until discard bag #2 is full. The incorporation of the second discard bag is significant, as it enables the reproducible sampling of alveolar air. Additionally, based on the size of the discard bags (400 mL) and the tube volume (125 mL), it is possible to be sure that the air in the tube has been fully replaced by breath when the second discard bag is full. In addition, the tubes can be cleaned by passing clean nitrogen gas through them for 30 min after each sampling run, thus making it possible to reuse the same tube for multiple runs. A full diagram of the sampling device can be found in Fig. 3. The breath samples were extracted by inserting the developed extraction devices into the sampling portal located on the tube (green septum). Additionally, to control for the inhaled air, the air surrounding the volunteer during the experiment was studied by performing the extraction procedure under optimum conditions with the DVB/CAR SPME fiber.

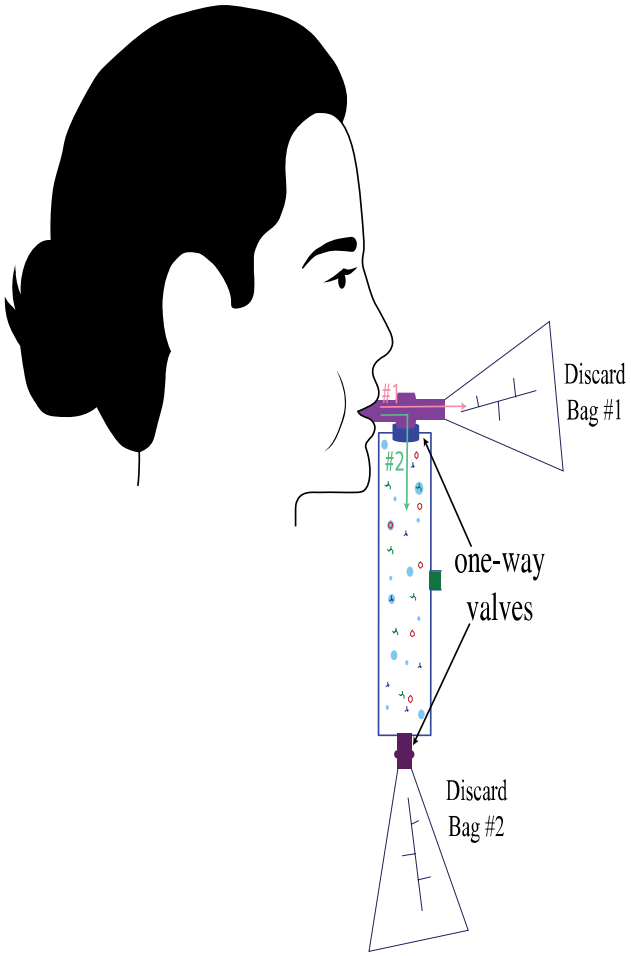

Fig. 3 Sampling process for breath analysis

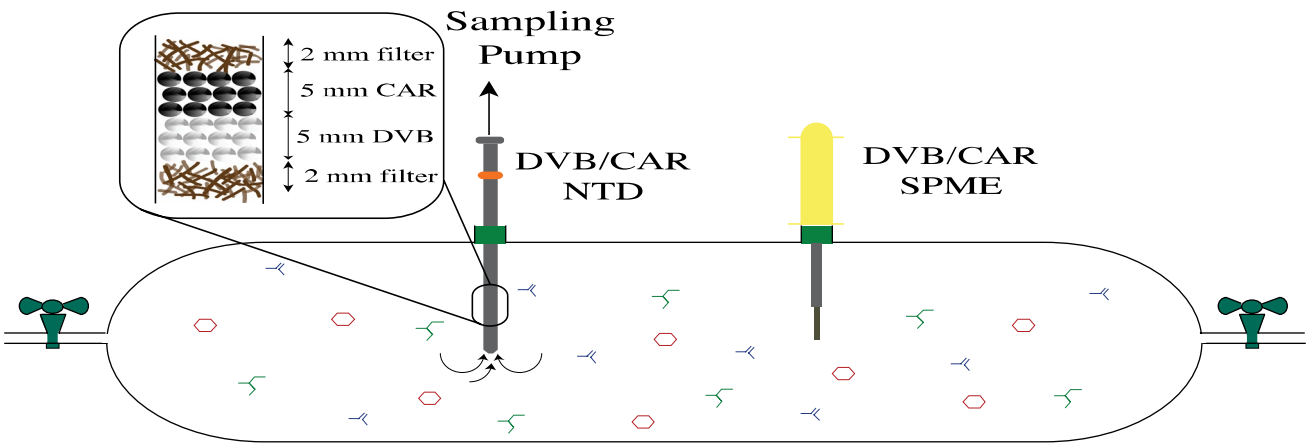

Fig. 2 Schematic of extraction procedure using SPME and NTD during optimization and calibration

Filtration efficiency

The filtration efficiency of the developed NTD was assessed using a scanning mobility particle sizer (SMPS). Specifically, the H-PAN/DVB/CAR NTD was inserted into the SMPS, with the filtration efficiency being defined as the difference in the instrument's particle count before and after insertion. To make up for the high flow rate required by the SMPS, 6 parallel needles were inserted into it during these tests.

Extraction time: gas/droplet stability and equilibrium time

Two important factors were considered in determining the optimum sampling time: the stability of the gas/droplets in the sampling device and the equilibrium time required for SPME. Ideally, the extraction time for SPME should be long enough to achieve equilibrium, as this will ensure maximum sensitivity; however, in gas mixtures, analytes can be lost due to attachment to the chamber wall, diffusion, or escape through valves/connections. This phenomenon is more significant for low sampling volumes, as they are generally accompanied by high ratios between the container surface area and gas volume. Therefore, it was important to carefully consider equilibrium time and sample stability when determining the optimum sampling time. The stability of VOCs in glass bulbs has been studied previously, with findings showing that a gas mixture can remain stable inside a glass bulb for at least a few hours [38–40].

1. To check the stability of the gas mixture, a home-made sampling tube was spiked with gas mixture and a 1-min extraction by SPME fiber was applied (Fig. S1c). The extraction was performed over a 30-min time period after injection while the tube was left capped in room temperature (Fig. S1b) and the relative signals of the two volatile components were followed and reported as an indicator of the stability. The signals were adjusted to compensate for the depletion after each SPME extraction.
2. Acetone was chosen as the target compound for studying the stability of aerosol droplets in breath due to its polarity and presence in droplet phase. Multiple breath samples were obtained (Fig. 3), and extractions were performed at different time points after sampling using NTD (1 min, 20 mL min^{-1}).
3. The equilibrium time for the SPME method was determined by exposing an SPME fiber to the gas mixture for different amounts of time (Fig. 2). The equilibrium time was considered to have been achieved when the extraction signal remained constant despite further increases to the extraction time.

Finally, the optimal sampling time was selected by considering the stability of the gaseous mixture and droplets, as well as the equilibrium time profile.

Breakthrough volume (BTV)

Breakthrough volume is defined as the sampling volume at which the NTD reaches its full capacity or equilibrium. It is important to study BTV when using NTDs, as the linear relationship between the extracted amount and sample concentration is lost after the BTV has been reached. If two needles are connected in series, the BTV is assumed to have been reached when compounds start escaping from the 1st needle and are detected in the 2nd needle. Therefore, the DVB/CAR NTD under study was connected to a secondary commercial needle to determine the BTV. The signal of the compounds in the secondary needle was monitored while increasing the sample volume up to 250 mL (sample concentration ~ 500 ng mL^{-1}); if no compounds were detected at a given sample volume, the BTV was not considered to have been reached, as the primary NTD was still functioning as an exhaustive sampler.

Method validation

To validate and calibrate the developed DVB/CAR NTD and DVB/CAR SPME methods, gas mixtures with varying concentrations were prepared by spiking the glass bulb with different volumes of stock mixture and humid air. The humid air was prepared in a separate 1-L glass bulb, after injection and heating of 40 µL of Milli-Q water. To check the repeatability of the developed method, inter-day and intra-day relative standard deviations (RSD) were investigated. The linear dynamic range (LDR) was chosen based on previous reports detailing the possible concentrations of pollutants in breath after exposure and calculated with the external calibration method. The limits of detection (LODs) and limits of quantification (LOQs) were investigated using signal-to-noise ratios of 3 and 10, respectively. All optimization was performed in single ion monitoring (SIM) mode for optimum sensitivity, and the selected m/z values are provided in Table S1.

Real sample analysis

Breath samples were obtained from volunteers following exposure to smoke from wooden stick incense, a mosquito repellant candle, and a normal unscented candle to analyze the effect of exposure to household pollutants on exhaled breath. In addition, the composition of breath samples obtained after exposure to air freshener spray, fragrance mists, and smoking of cannabis was also investigated. The volunteers were asked to refrain from eating at least 3 h and

to wash their mouth with water prior to exposure. In addition, the sampling tubes were cleaned with nitrogen gas (Fig. S2a), and a control sample was obtained via SPME to assess breath composition pre-exposure. Each volunteer's breath was obtained once pre-exposure to study the breath composition resulting from exogenous sources.

The exposure environment was created by lighting an incense stick or a candle. A distance of ~ 50 cm was maintained between the source of the smoke and the volunteer's nose, and the volunteer was instructed to breathe normally. Each test used an exposure time of 1 h, as this was the time required to completely burn one incense stick. Once the exposure time had elapsed, the incense/candle smoke was removed from the environment, and the breath samples were obtained and analyzed. For sampling after smoking, the breath sample was obtained after the smoking of cannabis in routine conditions.

Exposure to the fragrance mist and air freshener was conducted by releasing five spritzes of the aerosol at a distance of ~ 25 cm from the face of a volunteer who was breathing normally. For these tests, breath samples were obtained and analyzed following an exposure of 5 min. During some of the samplings, to study the effect of breath droplets, the mouthpiece was equipped with a filter during breath sample collection after exposure, in order to prevent breath droplets from reaching the sampling tube. Sampling was repeated 1 h after exposure for some of the volunteers. The sampling tube and extraction experiments are shown in Figs. S2b, c. During all experiments (except cannabis smoking), DVB/CAR SPME devices were also positioned close to the volunteer's nose to determine the concentration of air pollutants in the inhaled air. Every sample was quantified in SIM mode, but one run per sample was performed in TIC mode to detect any other potential components.

Results

Filtration efficiency

The filtration efficiency of the devices was analyzed using the SMPS, with the results being shown in Fig. S4. As can be seen, the NTD provided a filtration efficiency of > 99%. Since the droplets under study had a very small size range (between 5 and 225 nm) with theoretically minimum filtration efficiency [41], it can be expected that similar or better filtration efficiency can be obtained in a sample matrix.

BTV investigations

To find the BTV, the sampling volume was increased to 250 mL, and a secondary needle was monitored for signals from the analytes. The results of these tests showed that the BTV was not reached until 250 mL (which covers the sample volume = 125 mL), as desorption of the secondary NTD did not show any peaks associated with the VOCs under study prior to this level. Based on the obtained results, it was concluded that BTV was not reached during breath sampling (sampling volume = 100 mL).

Extraction time: gas/droplet stability and SPME equilibrium time

1. The stability of the gas mixture in the tube was assessed by extracting the sample via SPME (1 min) immediately after injection and every 5 min for a period of 30 min. The results are shown in Fig. 4.

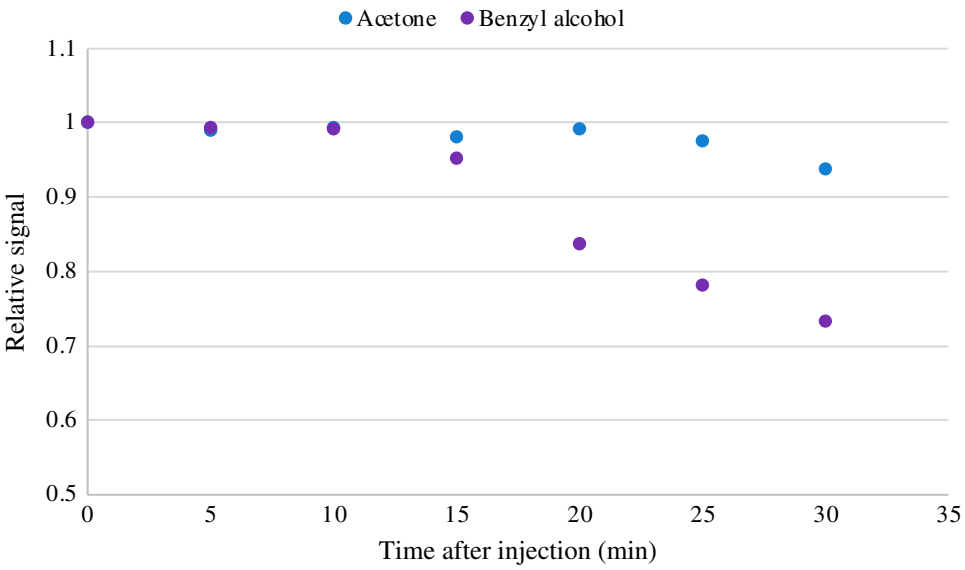

Fig. 4 Relative signal of acetone and benzyl alcohol in gas mixture over a 30-min period after injection into the sampling tube

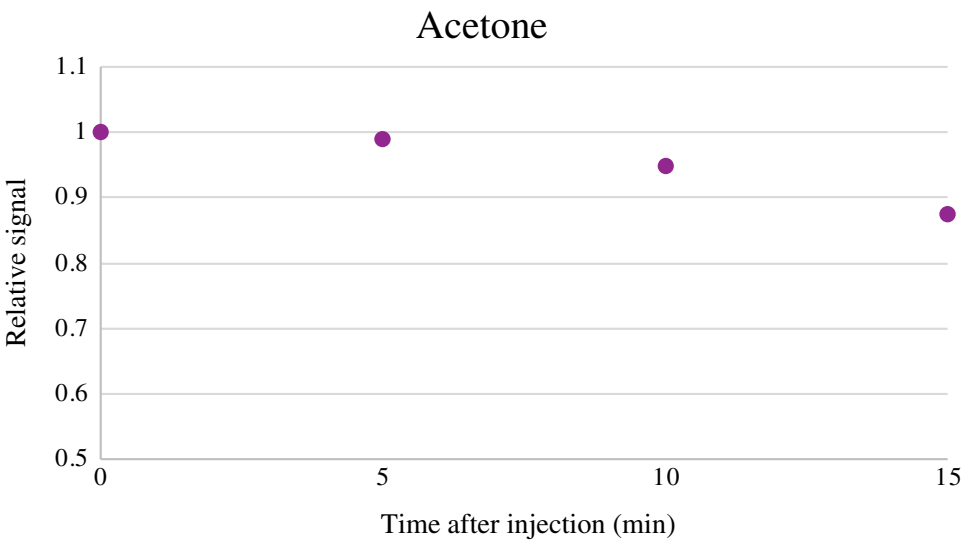

Fig. 5 Relative signal of acetone in breath samples detected via NTD over 15 min following sampling

As the data suggests, volatiles and non-volatiles remain stable in the gas mixture up to the 15-min mark, but this stability begins to diminish beyond this point. This loss of stability may be the result of the compounds settling in the walls of the sampling tube or escaping from the device through connections or valves. Since heavier compounds were found to diminish more rapidly, it can be concluded that this instability is primarily attributable to the settlement of compounds in the walls of the tube.

The gas-phase study (Fig. 4) showed that acetone remains quite stable for up to 20 min in the sampling tube. Significantly, acetone's polar structure allows it to also be present inside breath droplets, which is why it was selected as a marker for monitoring the stability of droplets inside the sampling tube.

2. Next, a breath sample containing acetone was obtained from a volunteer, with subsequent extractions being performed using the NTD. The concentration reported via the NTD consisted of both gas-phase and droplet-bound acetone. Based on these explanations, and considering the stability of acetone in gas phase (Fig. 4), it can be assumed that any decrease in the concentration detected via the NTD during this time range can be attributed to the settlement of droplets in the sampling tube. The stability of acetone (relative signal) is reported in Fig. 5. Based on these data, breath droplets can be considered stable for up to 10 min after sampling.
3. To find the best extraction time, it was also important to study the equilibrium time of analytes extracted using SPME. As the equilibrium time profile reveals (Fig. 6), equilibrium is achieved at around 15 min for most of the compounds; however, more hydrophobic characterized by higher distribution constant components required 30 min to reach equilibrium.

As explained earlier, both sample stability and equilibrium time can be considered as limiting factors in finding the optimum extraction time. As the equilibrium time data shows, a 30-min extraction time is required for full

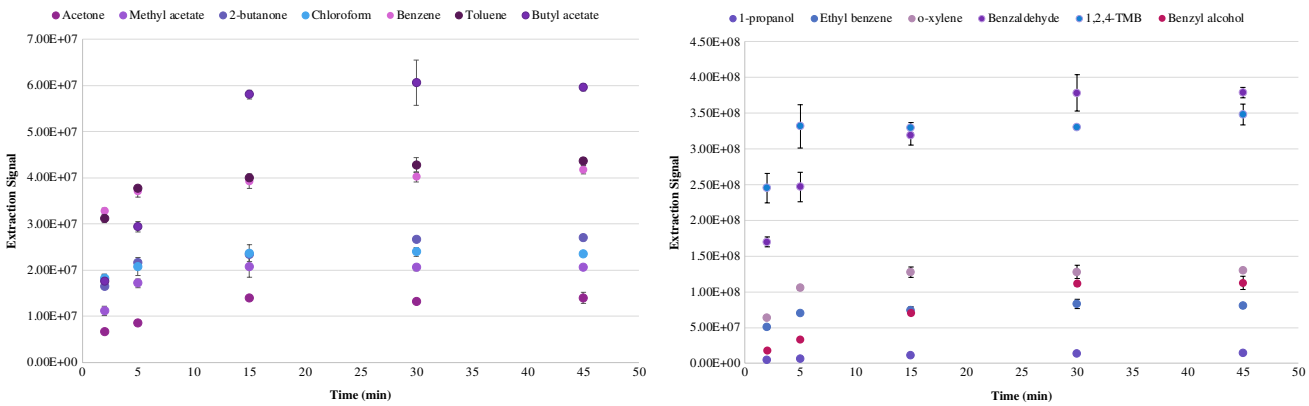

Fig. 6 Equilibrium time profile of VOCs using DVB/CAR SPME

equilibrium to be reached between the analytes and the SPME fiber coating; however, gaseous analytes and droplets can remain stable inside the prepared sampling device for up to 10 min. To ensure the reproducibility and stability of the sampling method, and based on the discussed results, a pre-equilibrium condition with a 5-min extraction time was selected as optimum for this study. That is, extractions were performed by leaving the DVB/CAR SPME fiber inside the tube for 5 min; similarly, sampling was conducted with the DVB/CAR NTD for a period of 5 min (flow rate = 20 mL min^{-1}).

Method validation

The figures of merit, including LODs, LOQs, and LDR, were studied with the DVB/CAR SPME fiber and the DVB/CAR NTD using different concentrations of gaseous mixture in the glass bulb. The inter-day and intra-day RSD can be found in Table S2. The results of these tests are provided in and were capable of meeting the concentration limits set forth by health agencies.

Table 1. As the data suggests, the method's sensitivity regarding the detection and quantification of the analytes under study was satisfactory, considering the pre-equilibrium condition of the study and the low sample volume. Indeed, the observed sensitivities were similar to or better than those reported in previous breath studies using NTD or SPME [23, 42–45] and were capable of meeting the concentration limits set forth by health agencies.

Real sample results

Some compounds such as acetone were detected before exposure, but are not reported as they were considered to be "endogenous," not the result of exposure. The analytes are reported in Table 2 and considered "exogenous," only when they were not detected pre-exposure. Additionally, after each sampling and cleaning of the sampling tube, the cleanness of the tube was tested and no compound was detected. The concentrations of the compounds detected and determined

Table 2 Concentration of compounds determined via DVB/CAR SPME and DVB/CAR NTD in ambient air and exhaled breath following exposure (ND = Not Detected)

	Breath		Air
Incense smoke	SPME	NTD	SPME
1,2,4-TMB	5.6 ± 0.7	6.6 ± 1.2	79.3 ± 5.1
o-Xylene	ND	ND	38.5 ± 6.3
Mosquito-repellant candle	Breath		Air
	SPME	NTD	SPME
Benzene	ND	ND	15.4 ± 1.2
Ethyl benzene	4.1 ± 0.5	3.8 ± 0.6	28.3 ± 1.9
Candle with wood smell	Breath		Air
	SPME	NTD	SPME
Benzene	ND	ND	8.9 ± 1.5
Cannabis smoke	Breath		Air
	SPME	NTD	SPME
o-Xylene	14.3 ± 1.4	15.3 ± 1.7	–
Spray #1	Breath		Air
	SPME	NTD	SPME
Acetone	2.4 ± 0.4	5.3 ± 0.4	87 ± 2.4
Benzaldehyde	11.7 ± 1.5	14.4 ± 2.7	184 ± 12
Spray #2	Breath		Air
	SPME	NTD	SPME
Benzyl alcohol	5.6 ± 0.6	6.4 ± 1.1	215 ± 23
Spray #3 (sampled through mouth filter)	Breath		Air
	SPME	NTD	SPME
Acetone	4.1 ± 0.5	3.5 ± 0.3	85 ± 8
Benzyl alcohol	7.9 ± 1.1	7.6 ± 0.6	193 ± 16

Table 1 Figures of merit for the study of analytes using the DVB/CAR SPME fiber and DVB/CAR NTD using standard gas with humidity

	LOD (ng mL^{-1})		LOQ (ng mL^{-1})		LDR (ng mL^{-1})	
Analyte	NTD	SPME	NTD	SPME	NTD	SPME
Acetone	0.25	0.3	0.8	1	1.3–316	1.3–316
Methyl acetate	0.2	0.3	0.7	1	0.7–374	1.5–374
1-Propanol	0.2	0.22	0.7	0.8	1.3–322	1.3–322
2-Butanone	0.15	0.2	0.5	0.7	0.6–322	1.3–322
Chloroform	0.15	0.24	0.5	0.8	1.2–591	1.2–591
Benzene	0.09	0.12	0.3	0.4	0.7–352	0.7–352
Toluene	0.09	0.1	0.3	0.3	0.7–348	0.7–348
Butyl acetate	0.14	0.2	0.5	0.7	0.7–352	0.7–352
Ethyl benzene	0.05	0.08	0.2	0.3	0.7–348	0.7–348
o-Xylene	0.06	0.07	0.2	0.2	0.7–352	0.7–352
Benzaldehyde	0.1	0.15	0.3	0.5	0.8–418	0.8–418
1,2,4-TMB	0.05	0.09	0.2	0.3	0.7–352	0.7–352
Benzyl alcohol	0.09	0.12	0.3	0.4	0.8–416	0.8–416

in exhaled breath samples with the DVB/CAR SPME fiber and the DVB/CAR NTD are provided in Table 2, and the chromatogram in SIM mode for the study of spray #1 is shown in Fig. S3. In addition to studying exhaled breath, air inhaled by the volunteers was also studied to determine the correlation between their respective compositions. As expected, higher concentrations of pollutants were detected in the air samples. However, one notable finding relates to the difference between the concentrations reported with NTD and SPME: whereas both NTD and SPME reported similar concentrations for non-polar pollutants, NTD generally reported higher concentrations for polar compounds (acetone, benzaldehyde, and benzyl alcohol), with the largest difference being observed for acetone. This difference can be attributed to the NTD's ability to trap breath droplets, which enables it to report the total concentration of compounds (both in exhaled breath vapor and in exhaled breath aerosol). Non-polar components prefer the vapor phase, while polar and less-volatile analytes tend to remain inside the droplets. This claim is supported by the data for spray #3, which was obtained through a filter that prevented aerosol droplets from reaching the sampling tube. In this case, only exhaled breath vapor was available for extraction and, as a result, similar concentrations for acetone and benzyl alcohol were determined by the NTD and SPME fiber (unlike spray #1). These data clearly suggest that the differences observed for these methods between samples are due to the NTD's ability to trap droplets. Similar concentrations of these air pollutants were reported previously [46, 47].

In some of the cases, the sampling was repeated 1 h after exposure. In most cases, the compound was undetectable after 1 h. In the case of incense smoke, 1,2,4-TMB was detected (below LOQ) even after 1 h from exposure. This was a significant finding, revealing how the long-term exposure to these household air pollutants can introduce a large concentration of hydrocarbons into the human body. It also shows that polar compounds can be removed faster, because in some cases, the concentration of polar compounds detected in breath was higher; however, they were eliminated from the body faster and became undetectable earlier than the non-polar compounds. This finding is attributed to the elimination of polar compounds through the kidneys, while non-polar compounds are generally removed via breath [12, 17, 48].

Data in TIC mode: As mentioned previously, all air and breath samples were analyzed once in TIC mode to identify any other components that may be present. Overall, the following compounds were detected in the air samples: pyrene, anthracene, para-ethyl styrene, isopropyl benzene, pinane, limonene, pyridine, limonene, linalool, 1,3,5-trimethyl naphthalene, benzofuran, benzyl benzoate, isoeugenol, diethyl phthalate, citronellol, geraniol, cinnamaldehyde, and carvone. The compounds detected via breath analysis in TIC mode included cinnamaldehyde, pyridine, limonene, and isoeugenol. It should be mentioned that there were some other tested candles and sprays; however, they are not reported here as there was no compound detected in their associated breath sample after exposure.

Discussion

The term "air pollution" can be misleading, as mostly people generally think of car exhaust and factory smoke when they hear this term. However, studies conducted by the World Health Organization (WHO) have found that "8 million people die every year globally because of air pollution. Among these, 4.3 million die because of air pollution from household sources." Some of the main sources of household air pollution include cooking-related smoke, smoking, perfume and deodorants, and building materials. While these types of pollution may seem negligible based on type and amount, long-term exposure has proven to be problematic and, in the worst cases, deadly [49]. Some of these pollutants such as acetone are harmless up to high levels; others, including chloroform, can be toxic if inhaled.

Breath analysis is one of the best options for studying exogenous compounds and monitoring exposure patterns, as it is non-invasive and fast, and enables real-time monitoring; other options are urine and blood analyses. The main challenge associated with this form of analysis is that exhaled breath is aerosol in nature. This is problematic, as breath studies that are limited to analysis of the gas phase will not be able to detect polar compounds hidden inside droplets. Thus, the NTD developed in this work is an important contribution to this area of study, as it enables the gas-phase and droplet-bound components in breath samples to be studied simultaneously.

A comparison of the results obtained with the developed NTD and fiber format of SPME confirmed the NTD's superior performance, especially for polar components. The NTD allows breath droplets, including polar components, to be trapped, desorbed, and studied, while SPME is only capable of studying exhaled breath gas. The superiority of the values obtained via NTD compared to SPME was demonstrated through an experiment designed to control for the effect of droplets in the other studies. In this experiment, samples were obtained through a mouthpiece equipped with a filter to remove all droplets from the sample. With the droplets removed, both methods produced similar values for polar compounds.

The compounds detected in the breath samples, as well as the identification of other chemicals in TIC mode, revealed the extent of the types of air pollution that are voluntarily produced inside people's houses. While the concentrations of detected components in breath are low and are removed

quickly from the body, long-term exposure to smokes and sprays can be problematic and initiators of respiratory diseases and allergies.

In addition, this study also introduced a new device for acquiring breath samples. This device consisted of a sampling tube equipped with valves at either end and a hole (covered with green septum) in the middle to enable sampling with the SPME fiber and NTD. Furthermore, the device's use of discard bags made it possible to completely eliminate pre-existing mouth air and enable reproducible alveolar breath sampling. The one-way valves situated on either end of the sampling tube facilitated dynamic breath sampling over time, or time-weighted averaging studies, by allowing the previous sample to be replaced with freshly exhaled breath. Moreover, the sampling tubes were re-usable; this was enabled by passing clean air or nitrogen gas through them after each application.

The designed breath sampling device has a limited volume, leading to the instability of breath aerosol. It means that if the device is applied for on-site sampling, the obtained sample should be extracted with designed devices immediately. However, after extraction, the devices can be stored in low temperature for up to a few days before transferring into the lab for analysis.

Conclusion

This study investigated the potential of using a filter-incorporated NTD for the analysis of breath composition and exposure patterns. The simultaneous application of NTD and SPME provided a comprehensive view of the sample by distinguishing the free and droplet-bound components. The results obtained with developed devices confirmed their tremendous potential for the investigation of polar components in breath samples, which are often lost due to their affinity for attaching to droplets. Furthermore, the re-usable sampling tubes designed for this research are cheap and enable the possibility of real-time dynamic sampling, and they can also be applied for time-weighted averaging studies wherein sampling is repeated at different time points to find the average concentration of desired compounds in breath samples. The combined use of the designed sampling devices provides a fast and green method for studying breath composition and the effects of inhaled air on expired breath. Some chemicals were detected both in the air samples close to sources of pollution (smokes and sprays) and the acquired breath samples, revealing the potential dangers of exposure to routine household air pollutants. While the analyzed breath samples contained low concentrations of air pollutants, long-term exposure to these chemicals can be hazardous. In this study, only direct products of sprays and smokes were studied; it is possible to extend this study to the metabolites of these compounds after entering the body. Untargeted determination via GC × GC would enhance the determination of the impact of the exposure as it facilitates monitoring the change in breath of the endogenous compounds, which might indicate the subject's health status. The developed devices are simple and can be conveniently adopted to common use. Characterization of compounds carried by aerosol particles and dissolved in gas might has significance leading to correct medical diagnosis.

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Declarations

Ethical approval The protocol for breath sampling was based on the ethical clearance approved by University of Waterloo #42853.

Conflict of interest None.

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