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A microfabricated preconcentration device for breath analysis

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ABSTRACT

Breath analysis promises to be a noninvasive method for diagnosis of lung cancer in its early stages. Certain ketones and aldehydes in exhaled breath have been identified as indicators of lung cancer. We report a preconcentration device or preconcentrator with thousands of micropillars fabricated from a silicon wafer that have been engineered to selectively trap trace gaseous ketones and aldehydes in exhaled breath. The micropillar surfaces were functionalized with a quaternary ammonium aminooxy salt, 2-(aminooxy)ethyl-*N*,*N*,*N*-trimethylammonium iodide (ATM), for capturing trace carbonyl compounds flowing through the preconcentrator by means of an oximation reaction. The unreacted ATM and reacted ATM adducts were eluted out of the preconcentrator with methanol and were directly analyzed by Fourier transform-ion cyclotron resonance mass spectrometry (FTICR-MS). The preconcentration results indicate that the capture percentages of acetone depend on the molar ratio of ATM/acetone. The analysis of exhaled breath demonstrates that the preconcentrator is suitable for quantitative analysis of ketones and aldehydes in exhaled breath.

1. Introduction

The analysis of human exhaled breath has attracted much attention because of its potentially wide applications in health diagnosis, metabolite bioinformatics, and drug discovery [1–4]. Lung cancer continues to be a leading cause of mortality among all cancer patients in the world. The crucial factor in the fatality rate of lung cancer is the lack of early detection method, a major key for improving survival rates of lung cancer and other cancer patients alike [5]. Some volatile organic compounds (VOCs) in exhaled breath have been found to be related to metabolic output of lung cancer [1,2]. The analysis of VOCs in exhaled breath could be used for developing a noninvasive and inexpensive diagnostic method for detection of lung cancer in its early stages [6–11]. Since there are hundreds of trace VOCs mixed in exhaled breath, a preconcentration process is generally required to concentrate VOCs before they can be analyzed by most current analytical instruments [1–4]. There are several preconcentration methods including physical adsorption using carbon-based adsorbents [12–15], solid-phase microextraction (SPME) [6,16,17] and polymers [18–21]. Gas chromatography (GC) coupled with a mass spectrometer (MS) is widely used for analysis of VOCs in breath as indicated in recent review papers [1–4]. Other techniques including proton transfer reaction mass spectrometry (PTR-MS) [22–24] and selected ion flow tube mass spectrometry (SIFT-MS) [25–28]; electronic nose [29–32] and differential mobility spectrometer [33–35] are also used for analysis of VOCs in exhaled breath.

In recent years, several studies indicate that certain ketones and aldehydes in exhaled breath could be used as metabolic markers of lung cancer for noninvasive diagnosis of lung cancer [6–11]. Volatile carbonyl compounds are invariably produced in biochemical pathways as intermediates due to their reactive nature. Some of these compounds are unique to a given metabolic pathway. Ketones and aldehydes in exhaled breath can be detected by PTR-MS [22–24] and SIFT-MS [25–28] without any preconcentration process. The challenge for PTR-MS and SIFT-MS is to identify compounds with certainty since several compounds may overlap on a particular mass-to-charge ratio [6]. Solid phase microextraction (SPME) with adsorbed *O*-2,3,4,5,6-(pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) has been used for analysis of aldehydes in exhaled breath [9,10]. SPME is a popular preconcentration method introduced a decade ago as a rapid extraction technique for analysis of volatile compounds from a variety of matrices [36,37]. Preconcentrators fabricated on silicon wafers using microelectromechanical system (MEMS) technology typically consist of a microhotplate and an adsorbent placed adjacent to the heating element [19,20,38–41]. Physical adsorption for preconcentration of trace VOCs and thermal desorption to release the adsorbed VOCs procedures are commonly used for MEMS preconcentrators. MEMS-based preconcentrators have low physical

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adsorption efficiency and poor selectivity issues [38]. Currently, there is no established protocol for analysis of ketones and aldehydes in exhaled breath. Development of such a protocol may lead to a simple noninvasive method for early lung cancer detection.

In this paper, we report a fabricated preconcentrator with custom-engineered surface functionality for preconcentrating ketones and aldehydes in exhaled breath as cationic derivatives through oximation reaction instead of physical adsorption. The custom-engineered surface functionality was realized by adsorbing quaternary ammonium aminooxy salt, 2-(aminooxy)ethyl-*N*,*N*,*N*-trimethylammonium iodide (ATM), on the surfaces of micropillars in the preconcentrator. The entire trapped analytes can be eluted by flowing methanol through the preconcentrator instead of using thermal desorption and directly analyzed by Fourier-transform ion cyclotron resonance mass spectrometry (FTICR-MS). Furthermore, the cationic derivatives enhance the sensitivity of FTICR-MS. Although FTICR-MS is used for analysis of VOCs with better resolution in this work, other mass spectrometry methods can also be used.

2. Materials and methods

2.1. Materials

All reagents and solvents, including deuterated acetone (acetone-d_6) (99.9%), acetone (99%), 3-pentanone (99%), n-hexanal (98%), n-octanal (99%) and methanol (99.9%) were purchased from Sigma–Aldrich. The aminooxy-based reactive coating, 2-(aminooxy)ethyl-*N*,*N*,*N*-trimethylammonium iodide (ATM) was synthesized according to a published method [42].

2.2. Design and fabrication of preconcentrators

The computational fluid dynamics (CFD) modulus of CoventorWare package was used to simulate gas flow in the preconcentrator and guide the design of the preconcentrator structure in order to achieve uniform gas flow distribution in the preconcentrator. For all simulations, the gas inlet pressure was set to 25 psi and the gas flow rate was varied in a range from 1 ml/min to 5 ml/min. A combination of the inlet and outlet structures, micropillar size and array pattern, and distances among micropillars were simulated using the CoventorWare software. Fig. 1 shows an optimized 2D structure of the preconcentrator with square micropillars in the channel. The simulated preconcentrator dimensions were scaled down accordingly in order to save computer memory and accelerate the simulation process. The preconcentrator was designed with more than five thousand micropillars in the flow channel in order to provide a larger surface area and to uniformly distribute gas flow

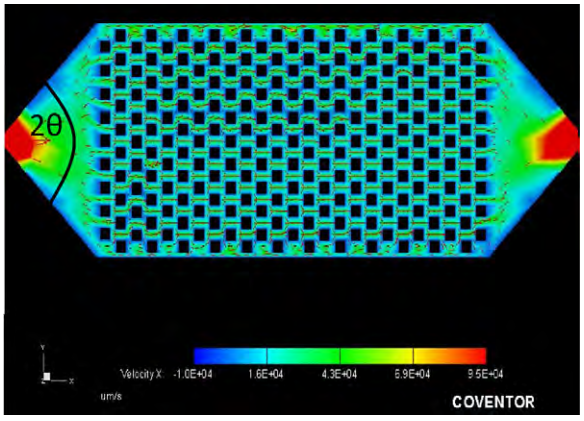

Fig. 1. CFD simulation of a preconcentration device with micropillars in the channel.

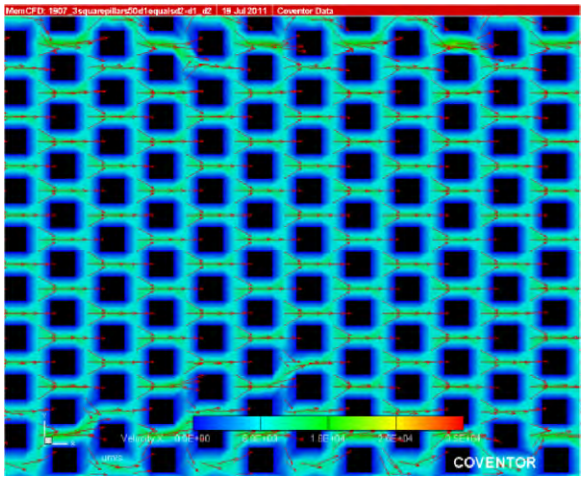

Fig. 2. CFD simulated flow pattern in the center area of the preconcentrator.

so that trace gas molecules have higher probability to collide with the aminooxy group of ATM molecules on the micropillar surfaces for oximation reaction. The CFD simulation results indicate that the inlet angle affects the flow pattern and flow velocity near the inlet. For the preconcentrator with a triangular shape inlet and outlet and the micropillar array pattern as shown in Fig. 1, a uniform gas flow velocity distribution is achieved when the inlet and outlet triangular angles (2θ) are smaller than 120°. Fig. 2 shows an enlarged flow pattern in the middle section of the preconcentrator. The gas flow velocity near the surfaces of micropillars is smaller than that in the centers between two micropillars in cross-sectional lines. Gas flow velocity distribution is uniform in the cross-sectional areas. The preconcentrators with optimized structure were fabricated and tested for preconcentration of carbonyl compounds. The fabrication procedures are commonly used for microelectromechanical systems (MEMS) device fabrication [38–41]. Fig. 3 shows the fabrication process flow. The whole fabrication process requires only one photo mask which makes fabrication simple. Starting from Fig. 3a, the surface of the silicon wafer is thermally oxidized to form a 0.5 μm SiO_2 thin film. Then, a positive photo resist Microposit S1813 is coated on the wafer. After photolithography, the thermal oxide in the preconcentrator area is patterned as deep reactive ion etching (DRIE) mask by wet etching in a buffered oxide etching

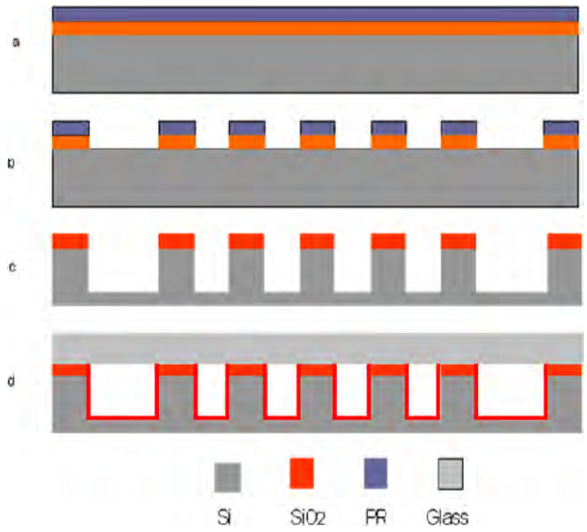

Fig. 3. Fabrication process flow in cross-sectional view.

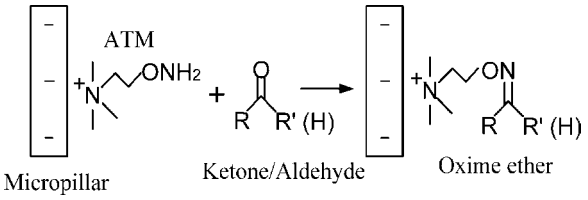

Fig. 4. Schematic illustration of the oximation reaction of ATM with ketones and aldehydes, ATM ions adsorbed on the silicon dioxide surfaces of the micropillars.

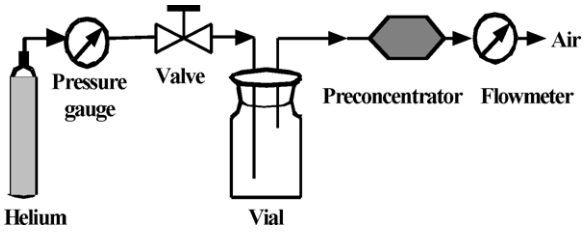

Fig. 5. Schematic diagram of the preconcentration setup.

solution (BOE) as shown in Fig. 3b. The micropillars in the preconcentrator channel are defined by DRIE using a STS silicon DRIE machine (Fig. 3c). Next, the wafer is oxidized to form a 50 nm SiO_2 layer on the micropillar surfaces in a "wet" O_2 and H_2O atmosphere in a thermal oxidation furnace. Then, the wafer is sealed by anodic bonding with a glass wafer (Fig. 3d). Subsequently, the wafer is diced and the connection ports are opened. The surface coating of the micropillars using ATM ions was done by injecting a known amount of synthesized ATM-iodide salt in methanol into the preconcentrator channel through one connection port, followed by evaporation of methanol in a vacuum oven. The slightly negative charge of silicon oxide on the micropillars enforces the close association of ATM with the surfaces of the micropillars. Fig. 4 shows the schematic illustration of ATM adsorbed on micropillar and oximation reaction with carbonyl compounds. Finally, the inlet and outlet of the preconcentrator were connected with 190 μm O.D., 100 μm I.D. deactivated fused silica tubes using a silica-based bonding agent.

2.3. Preconcentration of carbonyl compounds

The fabricated preconcentrators were characterized by preconcentration of trace ketones and aldehydes in a carrier gas, helium (He), using the setup shown in Fig. 5. A preconcentrator is connected to an ultra high purity He gas cylinder by the attached fused silica tubes, septa and stainless steel tube. A mechanical needle-valve made by the Hoke Company is used to adjust the He flow rate, while a flow meter is used to measure the He flow rate. There is no detectable leakage of He in the setup verified by a He gas leakage detector. After the He flow rate is stabilized and the setup is flushed for at least 10 min, a known amount of acetone-d_6 or ketone and aldehyde mixture diluted in methanol is injected into the vial with He flowing through the preconcentrator as shown in Fig. 5. After flowing 20 min, the flow is stopped. The flow rate and flow time are recorded after the injection of ketone and aldehyde. Then the preconcentrator is disconnected from the setup. The reacted ATM adduct and unreacted ATM are eluted out of the preconcentrator by flowing methanol from one slightly pressurized vial through the preconcentrator, then into an empty collecting sample vial. The collected solutions were directly used for FTICR-MS analysis without any further process.

To study the effect of ATM/acetone molar ratios on the capture efficiency, a constant amount of 3.65×10^{-7} mole ATM was loaded into each preconcentrator, while the amount of acetone added to the He flow stream was varied. After preconcentration, unreacted ATM and reacted ATM adducts were eluted out of the preconcentrator. Then, a 5 μl solution containing 1.14×10^{-8} mole acetone-d_6 and 1.17×10^{-7} mole ATM in methanol was added into each eluted methanol solution as the internal reference for FTICR-MS analysis. The reason for high ATM/acetone-d_6 molar ratio is to ensure complete reaction of acetone-d_6 as the internal reference. The amount of captured ketones and aldehydes was determined by comparing the signal abundance of ATM–acetone-d_6 with that of ATM–ketone and ATM–aldehyde adducts of FTICR-MS spectra.

2.4. FTICR-MS instrumentation

The samples of eluted methanol solutions were analyzed on a hybrid linear ion trap–FTICR-MS (Finnigan LTQ FT, Thermo Electron, Bremen, Germany) equipped with a TriVersa NanoMate ion source (Advion BioSciences, Ithaca, NY) with an electrospray chip (nozzle inner diameter 5.5 μm). The TriVersa NanoMate was operated in positive ion mode by applying 2.0 kV with no head pressure. Initially, low resolution MS scans were acquired for 1 min to ensure the stability of ionization, after which high mass accuracy data were collected using the FTICR analyzer where MS scans were acquired for 8.5 min and at the target mass resolution of 100,000 at 800 m/z. Mass spectra were exported as exact mass lists into a spreadsheet file using QualBrowser 2.0 (Thermo Electron), typically exporting all of the observed peaks. ATM and ATM derivative species were assigned based on their accurate mass by first applying a small (typically <0.0005) linear correction based on the observed mass of the internal standard. Detailed FTICR-MS information can be found in a published paper [43].

3. Results and discussion

The fabrication processes are commonly used in MEMS device fabrication. Fig. 6a shows an optical micrograph of the fabricated preconcentrator. The preconcentrator has a micropillar array area of 7 mm × 5 mm. The total empty space volume in the preconcentrator is about 5 μl. Fig. 6b shows a SEM micrograph of the micropillars. The micropillars have high-aspect-ratio with dimensions of 50 μm × 50 μm × 250 μm. The distances from center to center of the micropillars are 150 μm. There are more than five thousand square micropillars within the microreactor corresponding to a total micropillar surface area of about 260 mm^2. ATM molecules adsorbed on the surfaces of the micropillars react with carbonyl compounds through an oximation reaction as shown in Fig. 4.

The preconcentration setup as shown in Fig. 5 was tested by preconcentration of added trace acetone-d_6 in He flowing through the preconcentrator. Acetone-d_6 was used to distinguish any trace carbonyl compound contamination from ambient air. 3.65×10^{-7} mole to 3.65×10^{-11} mole of acetone-d_6 in 10 μl methanol was injected into the vial as shown in Fig. 5 and evaporated into He. The flow rate of He was adjusted to 5 ml/min and the flow time was 20–30 min. The flow time was recorded immediately after injection of acetone-d_6. For even the lowest amount (3.65×10^{-11} mole) of added acetone-d_6 in the He, acetone-d_6 was trapped and detected by FTICR-MS. More than 98% loaded reacted and unreacted ATM was eluted with 10 μl methanol [44]. Trace acetone contamination from ambient air was also detected. The loaded 3.65×10^{-7} mole of ATM was eluted out of the microreactor with about 40 μl methanol.

A series of experiments for preconcentrating acetone at different ATM/acetone molar ratios was performed to study the effect of ATM/acetone ratio on the capture percentages of acetone. In this series of experiments, 3.65×10^{-7} mole to 3.65×10^{-11} mole acetone in 10 μl methanol was added into the He flowing through

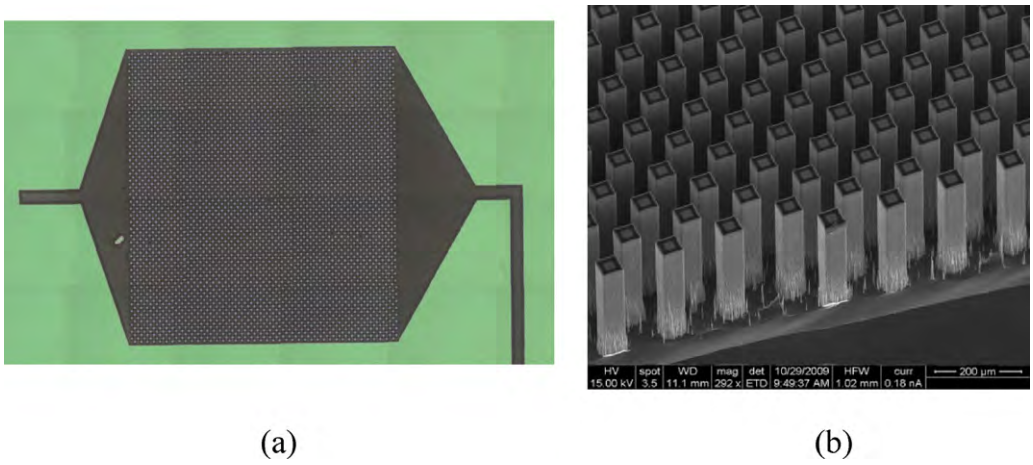

Fig. 6. (a) Optical micrograph of a fabricated preconcentrator and (b) SEM micrograph of the micropillars.

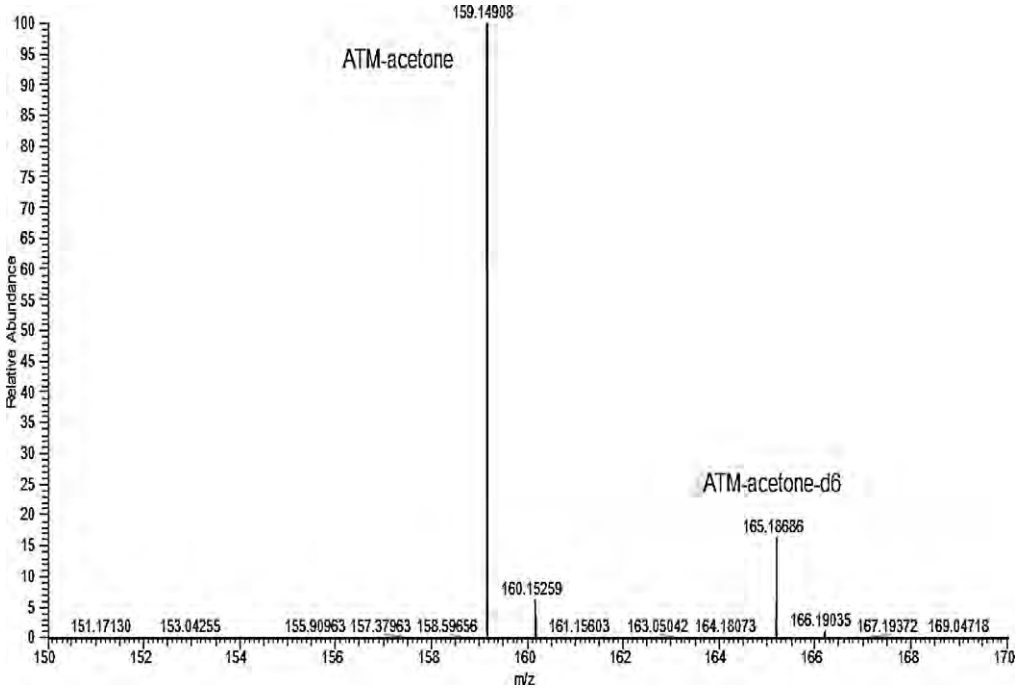

Fig. 7. FTICR-MS spectrum of preconcentrated acetone and ATM–acetone-d_6 was added to the eluted solution as the internal reference.

the preconcentrator for preconcentration of the added acetone. A known amount of acetone-d_6 completely reacted with ATM in methanol was added into each eluted solution as the internal reference for FTICR-MS analysis. Fig. 7 shows a typical FTICR-MS spectrum of an eluted ATM adduct solution. The spectral region depicts the oximation product of ATM with acetone-d_6 (165.18686 ion) as the internal reference. Fig. 7 also shows the captured acetone reacted with ATM (159.14908). The amount of captured acetone can be determined by comparing the relative abundance of ATM–acetone-d_6 with that of ATM–acetone. The capture percentage of acetone was calculated by dividing the captured amount of acetone by the added amount of acetone. Fig. 8 shows the capture percentage of acetone by the preconcentrator. The capture efficiency is affected by the ATM to acetone molar ratio. At the ATM/acetone molar ratio of 1:1, about 83% of acetone flowing through the preconcentrator can be captured. As the ATM/acetone molar ratio increased to larger than 5:1, the capture efficiency achieves 99% under the same operation condition. This result indicates that the designed preconcentrator based on the simulation

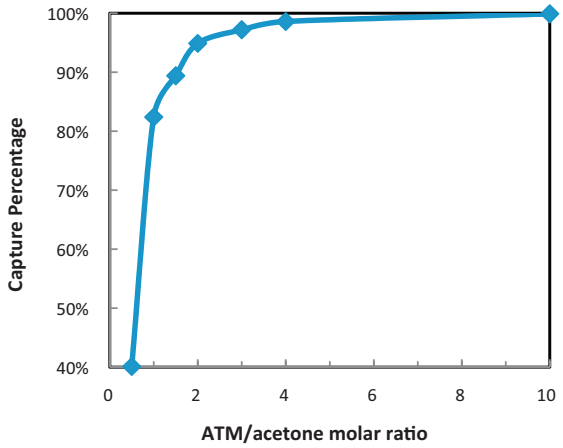

Fig. 8. The relationship between capture percentage of acetone and the ATM/acetone molar ratio.

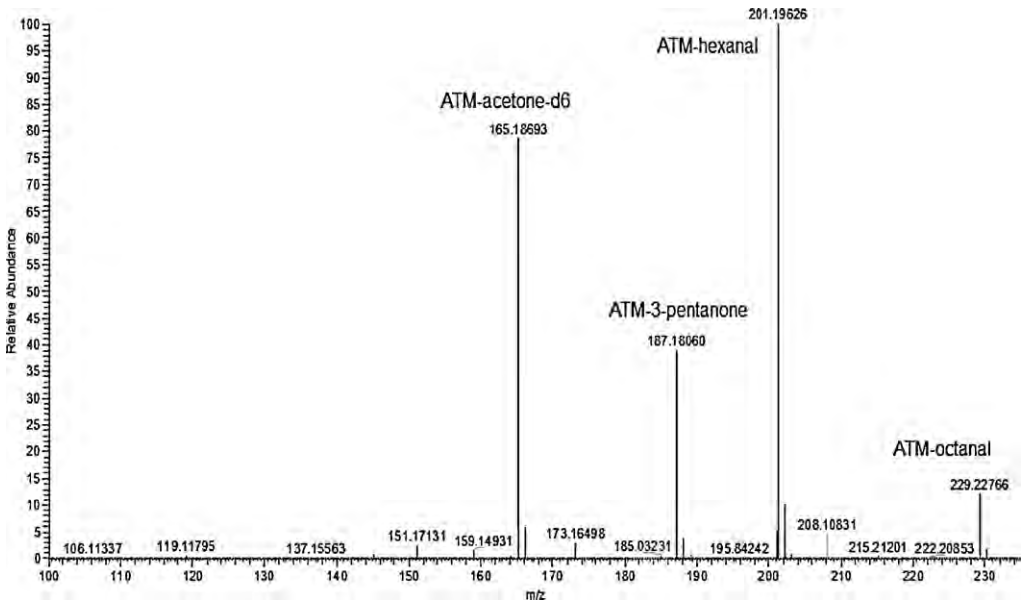

Fig. 9. FTICR-MS spectrum of preconcentrated carbonyl compound by the preconcentrator.

provides uniform gas flow distribution and high probability for ketones and aldehydes to react with ATM. Since ketone and aldehyde in exhaled breath are in trace level normally from ppbv to pptv range [9–11], it is easy to achieve an ATM/ketone or aldehyde molar ratio higher than 10:1 by increasing ATM load in the preconcentrators in order to obtain 99% capture efficiency. For the lowest amount of acetone (3.65×10^{-11} mole) evaporated in the He flow stream at a flow rate of 5 ml/min for 20 min, the equivalent concentration is 3.65×10^{-10} mol/L (~8.9 ppbv). The FTICR-MS signal of ATM–acetone adduct for the sample of 3.65×10^{-11} mole acetone was still very strong. The reported ketone and aldehyde concentrations in exhaled breath of healthy human beings are in ppbv to pptv range [9–11,44–46]. Therefore, this method is well suitable for preconcentration and analysis of exhaled breath.

In order to characterize the capture efficiencies for individual carbonyl compound in gas mixtures, a solution containing 3.65×10^{-8} mole of acetone-d_6, 3-pentanone, n-hexanal and n-octanal in methanol was injected into the vial with He flowing through the preconcentrator as shown in Fig. 5. 15 μl of 3.65×10^{-7} mole of ATM–iodide in methanol solution was injected into the preconcentrator to functionalize the surfaces of the micropillars for preconcentration of ketones and aldehydes. Fig. 9 shows a FTICR-MS spectrum of the preconcentrated ketones and aldehydes. The preconcentrator successfully captured all aldehydes and ketones. The capture efficiency of acetone-d_6 is higher than that of 3-pentanone, while the capture efficiency of n-hexanal is higher than that of n-octanal. This result may indicate the reactivity difference between ketones and aldehydes with different alkyl chains due to increased steric hindrance in oximation reaction. Therefore, the reactivity of ATM with ketones and aldehydes affects the capture efficiency.

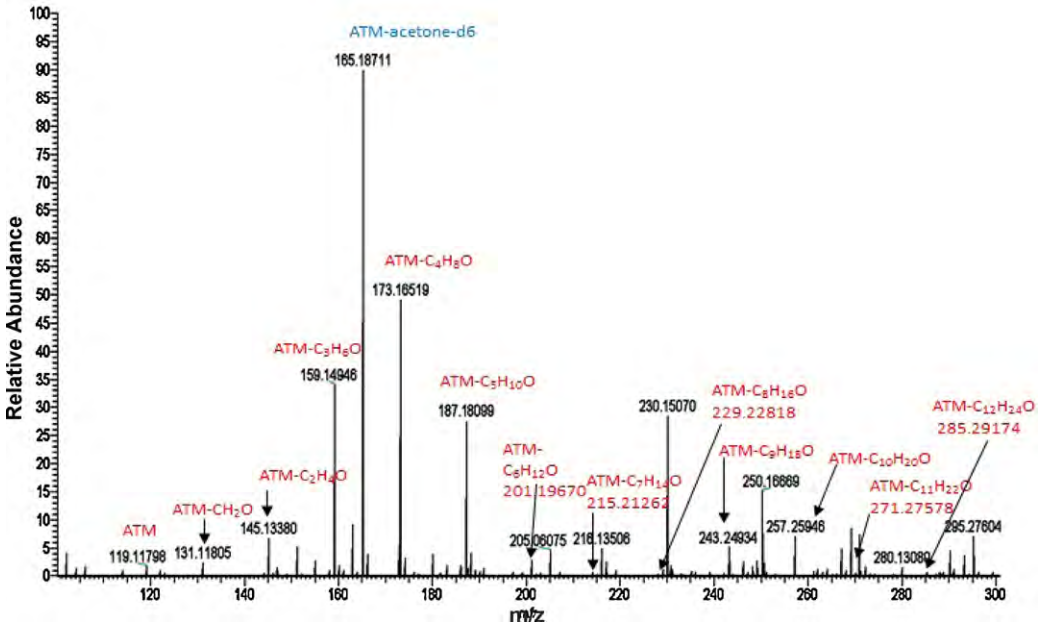

Fig. 10. FTICR-MS spectrum of exhaled breath preconcentrated by the preconcentrator.

We have tested the preconcentrator for preconcentrating ketones and aldehydes in exhaled breath [44]. The collection of exhaled breath samples was approved by Internal Review Board (IRB). Tedlar's bags with 1 Liter size were used to collect mixed alveolar breath. The breath gas in the Tedlar's bag directly flew through the preconcentrators by applied vacuum using an oil free diagram vacuum pump. Fig. 10 shows a typical FTICR-MS spectrum of exhaled breath samples. Ketones and aldehydes with alkyl chains from C1 to C12 were detected. The preliminary results indicated that by using acetone-d_6 reacted with ATM as the internal reference, the concentrations of all detected ketones and aldehydes in exhaled breath were determined [44]. This result demonstrates the preconcentrator is suitable for preconcentration of carbonyl compounds in exhaled breath. Future work on the relationship between ATM reactivity the capture efficiency and identification of the captured carbonyl compounds in exhaled breath is required for quantitative analysis of ketones and aldehydes in exhaled breath.

4. Conclusions

The results of this work show that the silicon-based preconcentrator can capture trace acetone with an efficiency of 99% or higher. The unique microstructure of the preconcentrator provide uniform gas flow and good oximation reaction probability between ATM on the surfaces of micropillars and gaseous carbonyl compounds. The oximation reaction between the functional cationic aminooxy compounds coated on the surfaces of micropillars and gaseous carbonyl species is the key for the selective capture of ketones and aldehydes. The unique combination of aminooxy and quaternary ammonium groups also enables direct positive mode analysis by mass spectrometry, such as demonstrated here using FTICR-MS. Furthermore, the preconcentration efficiency is improved by avoiding the current practices of physical adsorption and thermal desorption for analyte detection. The use of solvent elution is effective for recovery of more than 98% of the oxime ether adducts. The combination of preconcentrator design and cationic aminooxy chemistry make the present preconcentrator approach attractive for analysis of ketones and aldehydes in exhaled breath.

Acknowledgments

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