Analyzing Exhaled Breath Samples to Quantify Volatile Organic Compound Permeability through Nafion™ Polymer Tubing

Kathleen Hanek[1], Bret Nicholson[1], Mark Woollam[2,3], Fatima Aljamal[2], Eray Schulz[2,3] and Mangilal Agarwal[2,4]

1. Perma Pure LLC, Lakewood, NJ, United States.
2. Integrated Nanosystems Development Institute, Indiana University Indianapolis, IN, United States.
3. Chemistry & Chemical Biology, School of Science, Indiana University Indianapolis, IN, United States.
4. Biomedical Engineering & Informatics, Luddy School of Informatics, Indiana University Indianapolis, IN, United States.

Introduction and Objectives

- Exhaled breath is extremely humid (~100% RH), causing condensation and sensor interference during analysis compromising accuracy and reliability.
- Nafion™ tubing, built on a PTFE backbone with sulfonic acid (–SO_3H) side chains, selectively removes water vapor via diffusion while preserving critical gases like CO_2, O_2, and NO.
- Other studies have shown Nafion™ tubing outperforms PFA tubing in moisture removal allowing for mitigation of condensation risk in breath sampling lines.
- Despite effective drying, some VOCs such as alcohols and amines may be lost or altered by Nafion™ polymer, posing challenges for breath-biomarker diagnostics.
- Emerging nanomaterial-based VOC sensors deliver rapid, sensitive detection, but their accuracy is compromised by high humidity.
- Integrating Nafion™ polymer drying technology with nanosensor platforms could improve accuracy, but a deeper understanding of VOC retention and loss is essential.

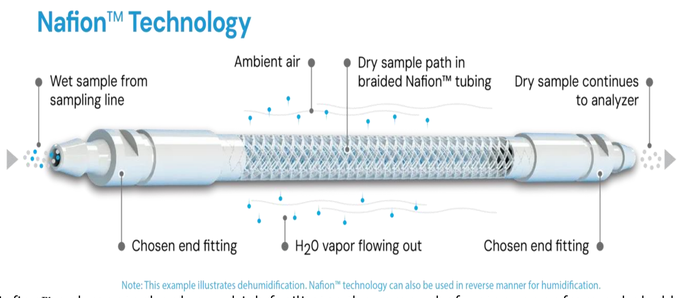

Figure 1. Overview of Nafion™ polymer technology which facilitates the removal of water vapor from exhaled breath[1]

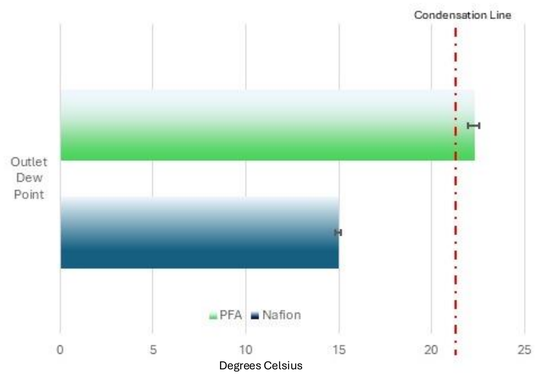

Figure 2. Experimental comparison of outlet dew points for humidified air simulating exhaled breath (37 °C, 99 % RH) passed through Nafion™ polymer tubing versus of PFA tubing. Ambient laboratory conditions: 21.3 °C. The data illustrate that Nafion™ tubing achieves substantially greater removal of water vapor, resulting in a lower dew point and greatly reduced likelihood of condensation

Our work aims to:

- Identify potential contaminants introduced or removed by Nafion™ polymer.
- Compare VOC retention and loss in breath samples between Nafion™ polymer and PFA tubing.

Methodology

- Ambient air samples were collected from the lab and transferred using Nafion™ polymer tubing and perfluoro alkoxy (PFA) tubing (n = 5 each; 10 total)
- Pure nitrogen samples were collected in pre-cleaned Tedlar bags and transferred using Nafion™ polymer and PFA tubing (n = 5 each; 10 total)
- A single volunteer donated breath samples into Tedlar® bags which were transferred using Nafion™ polymer and PFA tubing (n = 5 each; 10 total)
- All samples in Tedlar® bags were transferred to Tenax sorbent tubes at a flow rate of 200 mL/min, collecting 1 L per sample over 5 minutes
- All Tenax tubes were analyzed immediately using thermal desorption coupled to gas chromatography-mass spectrometry (TD GC-MS)

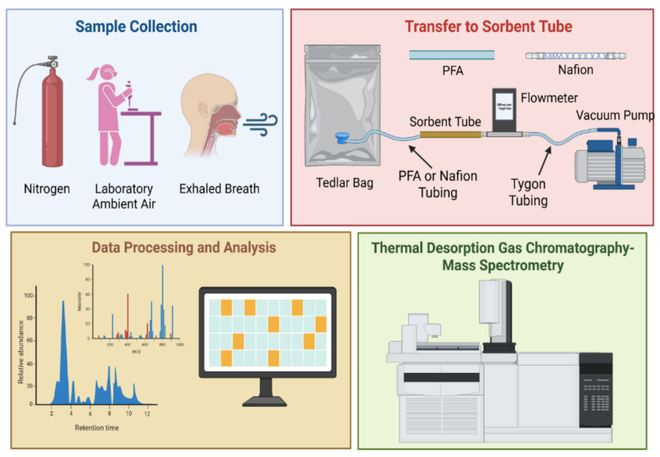

Figure 3. An overview of the experimental procedures undertaken for high-fidelity analysis of VOC profiles in exhaled breath and background ambient air samples. This includes sample collection, transfer process using Nafion™ polymer tubing and PFA, TD GC-MS analysis, as well as data processing and chemometric analysis.

Results – On Breath Analysis

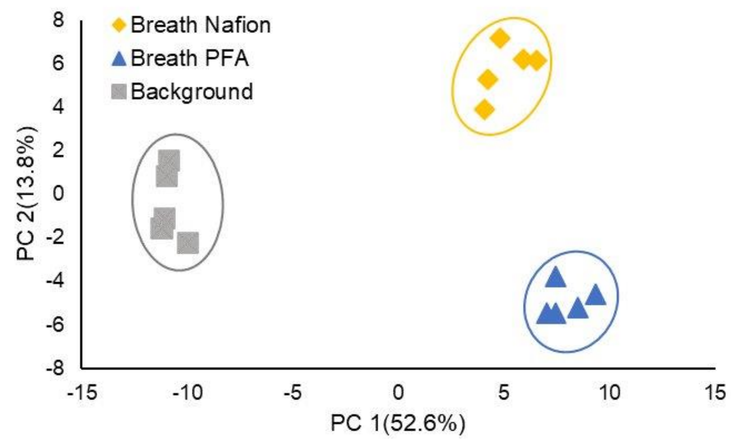

Figure 4. Unsupervised multivariate analysis in the form of principal component analysis (PCA) shows that all exhaled breath samples regardless of tubing type display differences from background ambient air. However, it also shows the stratification of Nafion™ polymer tubing and PFA-collected breath samples, indicating changes to the native profile.

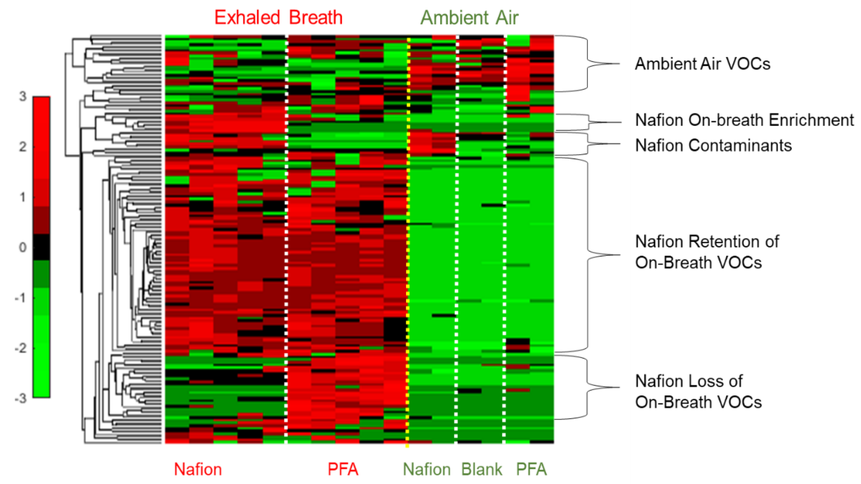

Figure 5. Hierarchical heatmap of 148 VOCs detected in exhaled breath and ambient air samples analyzed by TD GC-MS. Most VOCs (111) were enriched regardless of tubing type. Among them, 84% showed higher or comparable signals with Nafion™ polymer tubing versus PFA, while 16% were significantly depleted in Nafion ($p < 0.05$).

Results: Nafion™ Tubing Interferent and Contaminant Effects

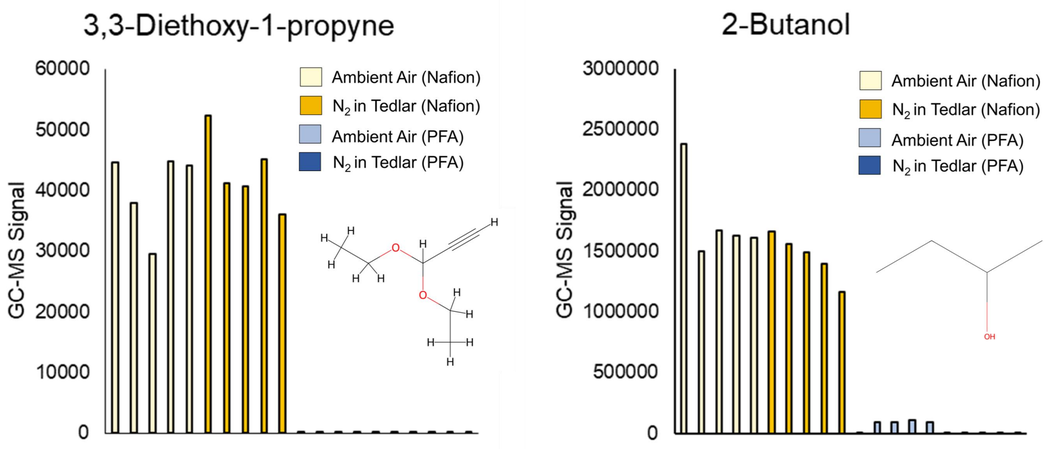

Figure 6. Bar plots for integrated GC-MS signals of VOCs characteristic to Nafion™ polymer tubing in ambient air samples as well as nitrogen samples collected in Tedlar® bags. These compounds were detected at lower abundance in PFA tubing.

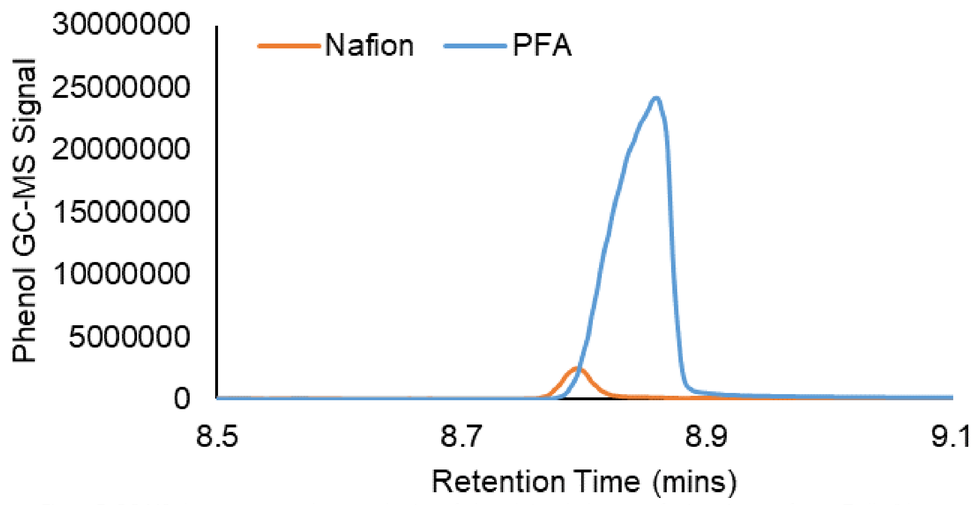

Figure 7. GC-MS chromatograms show a reduction of phenol signal when transferring nitrogen from a Tedlar® bag to a sorbent tube using Nafion™ polymer tubing (relative to PFA).

Conclusions:

- Nafion™ polymer tubing is effective at removing water vapor, reducing condensation risk and protecting sensitive diagnostic sensors.
- Our studies showed that 84% of 111 common on-breath VOCs tested were retained by the Nafion™ polymer tubing, making it a strong candidate for breath analysis workflows especially for nonpolar compounds
- Selective removal of phenol is a major advantage, as it reduces Tedlar® bag-related contamination and clarifies VOC spectra.
- Further studies are needed to:
  - Identify the source of Nafion™ polymer-related contaminants.
  - Further characterize the percentages and consistency of VOC retention/losses across clinically relevant compounds and cohorts.