

Final publishable summary

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2 Objective

The goal of project “MEDIXHALE” is to develop a laser absorption spectroscopy (LAS) sensor to assist physicians in the diagnosis and/or monitoring of patients with cystic fibrosis (CF). To do so, the sensor measures a specific chemical compound in exhaled breath.

3 Introduction

The potential of breath analysis for medical diagnostics has been recognized for centuries [1]. But while traditionally physicians had to rely on their sense of smell, in more recent times a number of highly sensitive and selective analytical techniques have become available. In 1971 about 250 substances (other than nitrogen, oxygen, carbon dioxide, and argon) were discovered in breath [2], and many more were identified in the following years. The concentrations of this large number of compounds varies greatly from person to person, but also for the same person throughout the day, depending on activity and metabolism. Diseases can cause significant changes in the concentrations of one or more substances. The variations in these so-called biomarkers therefore have diagnostic potential. Biomarker concentrations are typically very small, below 1 ppm (part per million).

In a small-scale study conducted in our group [3], acetonitrile (methyl cyanide, CH_3CN , abbreviated here as ACN) was identified as a good discriminator between a group of CF patients and a control group. It is likely that acetonitrile is produced, alongside with hydrogen cyanide (HCN), by bacteria that often infect the lungs and airways of CF patients. The concentrations of acetonitrile in breath are of the order of 10 ppb (parts per billion).

4 Main Results

After examining the infrared absorption spectra of ACN and of water vapour and carbon dioxide (the two main interferent in breath) we selected a small spectral region around a wavelength of $\lambda = 1.654 \mu\text{m}$ ($1/\lambda = 6044.5 \text{ cm}^{-1}$) (Fig. 1). Interference with water is negligible, while carbon dioxide does cause a reduction in sensitivity due to the partial overlap with the ACN absorption feature.

Absorption at the levels expected in breath is very weak, so a very long absorption path length is required to obtain a measurable signal. We built and characterized a cavity-enhanced absorption (CEA) spectrometer consisting of an optical resonator with an effective path length of 7.3 km (Fig. 2). The gaseous sample is held within the cavity tube, where the injected light beam circulates thousands of times between the two highly reflective mirrors. The key property of the CEA spectrometer is the limit of detection with respect to ACN: it was estimated by measuring

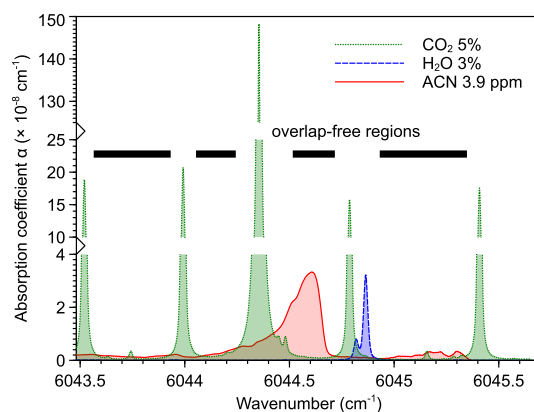


Figure 1: Absorption spectra of acetonitrile (ACN) and of water vapour and carbon dioxide.

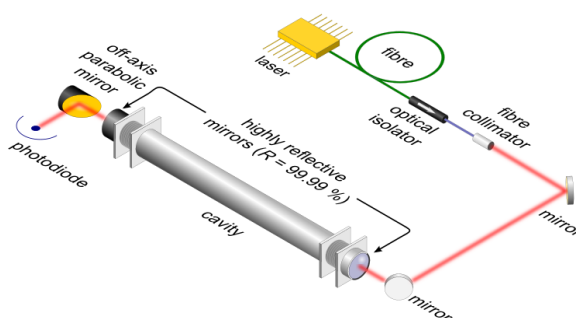


Figure 2: The cavity-enhanced absorption spectrometer.

the error distribution of the concentrations of a series of blanks (Fig. 3). The convention is that the limit of detection is three times the standard deviation of the error distribution, which in this case yields 72 ppb. If large amounts of carbon dioxide are present, as is the case in breath, the limit of detection worsens to 114 ppb. This is not sufficient to measure ACN in breath (~ 10 ppb).

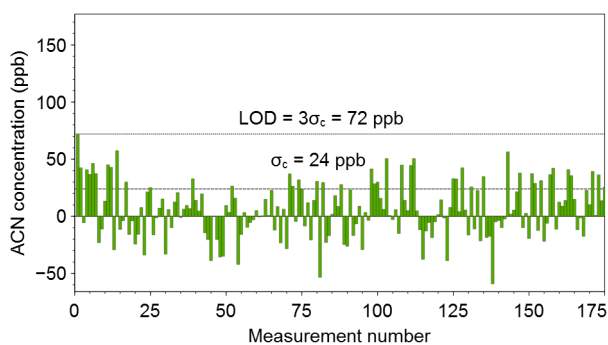


Figure 3: Measured concentration of a series of blank samples.

A preconcentration stage to increase the concentration of ACN and effectively improve the sensitivity of the system was also built and tested (Fig. 4). A carbon molecular sieve (CMS), a substance with similar properties to activated charcoal, is used as a filter to trap all the ACN present in the original sample. At a later time, the CMS is heated quickly to $200\text{ }^{\circ}\text{C}$ and the trapped ACN is released into a small flow of pure nitrogen. The final sample volume is much smaller than the initial one, which leads to an increase of the ACN concentration by a factor of, at most, the initial/final volume ratio. We could routinely obtain a preconcentration factor of 30. Thus, in conjunction with the preconcentration stage, the effective limit of detection of the system decreases to about 4 ppb, which is sufficient to measure ACN in breath.

To test the system under real conditions we took breath samples from two healthy adults, preconcentrated them, and then measured their ACN concentrations (Fig. 5). We obtained 690 ppb and 870 ppb of ACN, which correspond

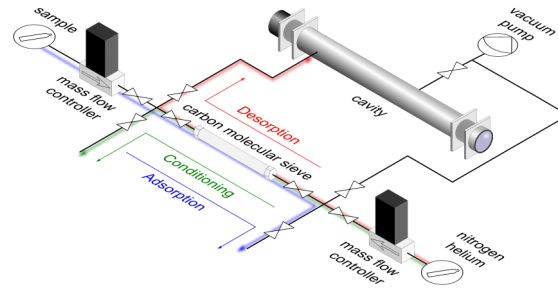


Figure 4: The preconcentration stage used to increase the ACN concentrations.

to 23 ± 3 ppb and 29 ± 3 ppb, respectively, in the original samples (before preconcentration). These values are in line with what we expected to find.

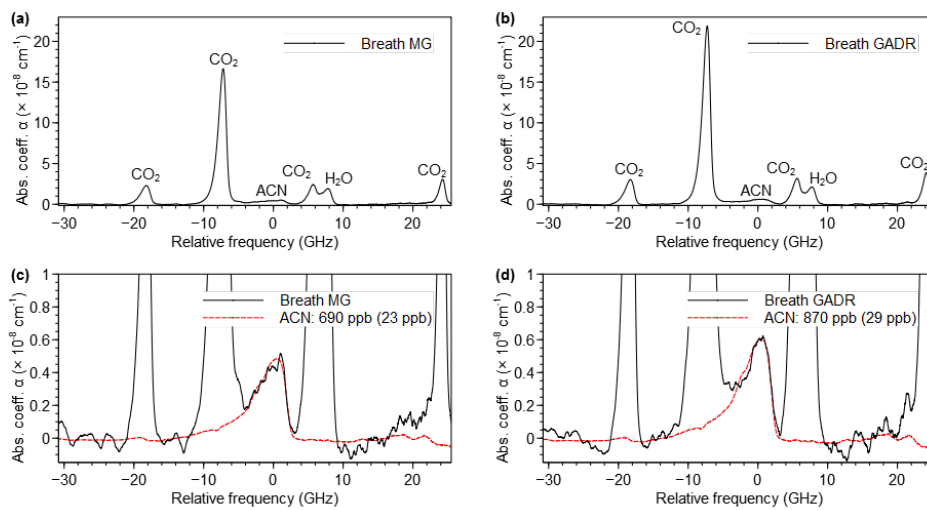


Figure 5: Absorption spectra of preconcentrated breath samples taken from two healthy adults.

5 Conclusions

These proof-of-principle measurements show that quantitative measurements of ACN in human breath are possible. While further work is required to enhance the system's precision (the uncertainties in the ACN concentrations are still relatively large) and to increase the throughput (currently only four measurements can be made per day), the obtained results are very encouraging, and with little technical improvements the system could be made ready for a clinical trial.

We believe that more work is desirable and required to improve the sensor to a point where a clinical trial can be conducted. Such a trial would, among other things, demonstrate whether the measurement of a single biomarker (acetonitrile) yields useful data with respect to the treatment or monitoring of CF patients.

The research carried out in this project might be of interest to professionals involved in the treatment and monitoring of cystic fibrosis patients.

References

- [1] Michael Phillips. Breath Tests in Medicine. *Sci. Am.*, July:74–79, 1992.
- [2] L Pauling, A B Robinson, R Teranishi, and P Cary. Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. *Proc. Nat. Acad. Sci. USA*, 68(10):2374–2376, October 1971.

- [3] L Bennett, Luca Ciaffoni, W Denzer, Gus Hancock, A D Lunn, Rob Peverall, S Praun, and Grant A.D. Ritchie. A chemometric study on human breath mass spectra for biomarker identification in cystic fibrosis. *J. Breath Res.*, 3(4):046002, 2009.