Your breath is a mixture of nitrogen, oxygen, carbon dioxide, water vapour, inert gases, and trace amounts — parts per million (ppm) by volume to parts per trillion (ppt) by volume — of volatile organic compounds (VOCs). These VOCs are the result of endogenous metabolic processes, which are transported by the blood to the lungs, where they are exhaled.

Research conducted in the late 1990s, using gas chromatography and mass spectrometry, identified over 1000 endogenous VOCs — alkanes, alkenes, arenes, oxygen-containing compounds, such as ketones, aldehydes and alcohols, and a number of nitrogen, sulfur and...
Taking breath

halogen-containing compounds — in the breath of 50 healthy people. The average breath sample contained over a thousand VOCs with only 27 VOCs common to all 50 individuals, suggesting that these resulted from ‘core’ metabolic processes in the body. There was also considerable variation in the presence and abundance of VOCs from person to person.

Since then other research has confirmed that the type and composition of the VOCs in a person’s breath can be an indicator of various disease states.2 The sweet smell of acetone in the breath of diabetic patients has, for example, been known for many years. Thus the analysis of exhaled breath has the potential to be a non-invasive diagnostic or prognostic tool in clinical medicine.

Analytical challenges

While breath is both simple and quick to collect, making it ideal for dealing with large numbers of patients, its analysis is not straightforward. By its very nature breath is transient, humid and the VOCs are present at trace levels. There are also questions of reproducibility and standardisation of breath sampling.3 Further, breath is not a homogenous sample. The first few cm³ of exhaled breath is the ‘dead space’, which sits in the airways and does not participate in

In brief

- Breath contains trace amounts of volatile organic compounds (VOCs), the mixture of which differs from person to person.
- Research finds that different VOCs can be linked to various diseases of the lung and liver, for example.
- Exhaled breath could therefore provide an non-invasive way of assessing a person’s health.
- Two variants of mass spectrometry (MS) — proton transfer MS and selected ion flow MS — are proving insightful in the real-time analysis of breath as an online health check.
gas exchange in the lungs. Beyond this region in the lungs is the ‘alveolar’ air, which is in equilibrium with the blood and where gas exchange takes place. The alveolar air contains the highest concentration of blood-based VOCs, and is therefore the main target of VOC breath analyses.

Ideally, any instrument used to analyse trace constituents in exhaled breath should be able to:

- determine the absolute trace gas concentrations;
- have detection limits of parts per billion (ppb) to ppt by volume;
- be able to measure a target analyte without interference from other components;
- have high temporal resolution of at least 1s timescale for real time analysis; and
- be unaffected by humidity.

To date there has been a range of instrumentation used to analyse VOCs in alveolar breath samples, with various degrees of success. These include traditional gas chromatography-based methods, spectroscopic methods, electronic noses, ion-mobility and mass spectrometry. Many of these methods are ‘off-line methods’ that require sample collection or concentration, some lack chemical specificity or can only measure one compound at a time.

The current focus of R&D is on real-time sampling of breath using two variants of direct mass spectrometry, namely proton-transfer reaction mass spectrometry (PTR–MS)4 and selected ion flow tube mass spectrometry (SIFT–MS).5 Real-time sampling has the potential for high-throughput screening, which as well as being able to monitor patients in a critical condition, reduces the probability of contamination of samples inherent in off-line techniques. These advantages, however, have to be traded off against the ability to separate individual compounds in real time.

PTR–MS was introduced in 1994 by Australian physicist, Werner Lindinger and his coworkers. The technique involves chemical ionisation of the sample VOC by proton transfer using H2O+ ions inside a drift tube.6

\[ \text{H}_2\text{O}^+ + \text{VOC} \rightarrow \text{VOC}^+ + \text{H}_2\text{O} \]

The H2O+ ions do not react with the other constituents of clean air, and other complications from fragmentation are minimal since proton transfer is a relatively soft ionisation technique. A typical PTR–MS instrument consists of an ion source where proton donors are chemical ionisation of the sample VOC by proton transfer using H2O+ ions inside a drift tube.5

\[ \text{H}_3\text{O}^+ + \text{VOC} \rightarrow \text{VOC}^+ + \text{H}_2\text{O} \]

The SIFT technique was developed over 30 years ago by David Smith and Nigel Adams at the University of Birmingham, UK, to study reactions between ions and uncharged neutral molecules, which occur in, for example, space.5 Since then the technique has been exploited for real-time measurement of trace gases and volatile compounds in exhaled breath among other applications. In this method, trace gas molecules in breath samples are ionised using precursor ions which are generated by an ion source (Figure 2). Examples of precursor ions include H3O+, NO+, and O3.6 As in PTR–MS, these ions react with VOCs but not with the major components of air. A quadrupole mass filter selects the ions from the carrier gas contained in the flow tube where they react with the VOC sample. The precursor and product ions are then separated by their mass-to-charge ratios (m/z) by a second quadrupole mass filter and counted by an electron multiplier. Reactions between the precursor ions and trace gas molecules proceed for a defined time, and absolute concentrations of trace gases can be derived down to ppb levels.

VOCs linked to disease

The identity and nature of VOCs in exhaled breath have been investigated for a wide range of illnesses, including liver disease, lung disease, oxidative stress and metabolic disorders among many others.2 The main objective of this research is to develop a robust non-invasive diagnostic method that can be implemented in routine clinical practice. The presence of VOCs in breath can be influenced by endogenous factors – gender, age, weight, genetic background – and exogenous factors, such as ambient air, diet, medication etc.3 The Holy Grail is to find biomarkers or sets of biomarkers that are linked to diseases but are independent of these factors.

While routine clinical breath analysis is still limited, there are a number of approved breath tests in clinical use including, for example, ones for:

- carbon dioxide monitoring during anaesthesia or intensive care;
- carbon monoxide as a test for neonatal jaundice;
- ethanol as a test for blood ethanol;
- hydrogen as a test for disaccharidase deficiency;
- urea as a test for H. pylori infection;
- nitric oxide as a test for asthma therapy, which is FDA approved; and
- hydrocarbons as a test for potential heart transplant rejection, which has been approved by the FDA.3

Liver disease causes incomplete

References

The metabolism of sulfur-containing amino acids, thus higher levels of compounds such as dimethyl sulfide, hydrogen sulfide and mercaptans would be expected in the breath of liver disease patients. Recent studies confirm this and have identified other VOCs as biomarkers for liver disease, such as 2-pentanone and 2-butanone.8

Researchers in Italy have identified heptanal and hexanal as breath biomarkers for lung cancer. Others have identified alkanes and methylated alkanes, not only in lung disease but also in patients with breast cancer.9 The most commonly reported volatiles for breast cancer are 2-propanol, 2,3-dihydro-1-phenyl-4(1H)-quinazolinone, 1-phenyl-ethanone and heptanal. The sensitivity and specificity of these biomarkers were 93.8% and 84.6%, respectively.10 While the analysis of trace gases and peroxides, which can damage cells. Hydrogen peroxide (H2O2), for example, has been identified as a biomarker for oxidative stress in lung diseases, including cancer.11 Hydrogen peroxide is produced by inflammatory cells in the upper and lower airways.11 Similar studies on patients with cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), bronchiectasis and adult respiratory distress syndrome (ARDS) confirm H2O2 as a biomarker in diagnosing oxidative stress in lung diseases. In a separate study, carbonyl sulfide (COS) has been shown to be a biomarker in the breath of patients who are most likely to reject a lung transplant.12

The future of breath analysis
Exciting developments are afoot to combine breath analyses with other non-invasive diagnostics for a holistic measure of a patient’s well-being. Recently, a new sick-bay was launched by the University of Leicester in the A&E department of Leicester’s Royal Infirmary (C&I, 2011, 20, 12). Here breath analysis is being coupled to imaging and non-invasive cardiovascular measurements to make quicker and patient-specific diagnoses.

While the analysis of trace gases on breath offers a new approach to diagnostic medicine, there are still challenges to overcome. For example, the origin and distribution of VOC biomarkers need to be understood, as do the exhalation kinetics.

Further, sampling methodology is critical and more work needs to be done to find out how different breathing patterns that result from various nose and mouth organisms could change the distribution of VOCs. There are also the significant technical challenges as we move from ‘discovery technology’ to bedside tests in a clinical environment, such as the miniaturisation of equipment. Nevertheless, with the development of real-time analysis of breath and the ability to measure multiple components simultaneously, we are now a step closer to being able to have ‘fingerprints’ of breath VOCs linked to a variety of illnesses.