As long ago as 1972, the diagnostic potential of human exhaled breath was pointed out by no less than Linus Pauling, founder of the theory of the nature of the chemical bond and two times Nobel prize laureate (1.). Since that time, a number of research groups have been working in this field and several instruments already exist which use human breath for the determination of certain parameters in man. Of these the most well-known are alcohol, hydrogen and the relative ratio of C-13 in gastric Helicobacter pylori infection. Indeed, exhaled breath can be described as an information carrier of processes occurring in the body and this technique has a wide range of potential uses in modern medicine. These include medical diagnostics, monitoring of pharmaceuticals and drug analysis, either in the testing laboratory or in the workplace. The great advantage of the analysis of exhaled breath is that this is one of the least invasive methods for monitoring human parameters.

The analysis of exhaled breath can be performed with many kinds of analytical instruments, however, each technique has its own advantages and limitations. Although gas chromatography is both rapid and sensitive, its range of applications depends on the nature of the column used, which, for any particular set of conditions, can only analyse a particular set of compounds. Likewise, an infra-red analyser can be affected by variations depending on the nature of the composition of the carrier gas matrix. One other technique, Mass spectrometry (MS), can in theory overcome such technical difficulties.

Mass spectrometry

Although there are many variations, all mass spectrometry involves a sequence of individual steps which, when taken together, provide an overall powerful analytical process. These steps are:

- Introduction of matter into a high vacuum region;
- Transformation to gas phase molecules;
- Formation of ions by energy transfer processes;
- Mass separation by electromagnetic fields;
- Particle detection by ion counters.

Mass spectrometers are widely used and well-established in a great variety of purely chemical analysis fields. They are also increasingly being applied to medical diagnostic applications.

One disadvantage of the technique is the fragmentation of molecules due to the high energy (typically 70 eV) involved in the ionisation process. Fragmentation results in the loss of the molecular structure, which makes it difficult to distinguish between different compounds. For example, homologues of alkanes, alcohols, aldehydes, and also higher substituted aromatics, can have the same mass fragments. Despite this, the recent rapid evolution of MS, together with the development of sophisticated software, now enables the routine, detailed analysis of even complicated macromolecules such as proteins and nucleic acids.

Soft ionisation

Soft ionisation adds selectivity to the principle of mole-
cular weight detection through well defined ionisation processes involving charge transfer reactions from an ion beam to the sample gas molecules. This allows complex gas mixtures to be differentiated without any need for pre-selection prior to the MS.

Completely different molecules may have identical molecular weights. For example, nitrogen and carbon monoxide, formaldehyde and nitric oxide, carbon dioxide and laughing gas. MS differentiates between the different molecules by using different ionisation levels. Every molecule has an individual energy (i.e. ionisation potential) that is necessary to remove an electron, thus converting it into an ion. For example, a krypton ion beam with 13.9 eV energy separates nitrogen (14.2 eV) and carbon monoxide (13.7 eV), which both have a mass of 28. Ethene (C₂H₄), which also has a mass 28, can be ionised with mercury ions, whereas krypton ions generate C₂H₃⁺ ions from ethene. The mass identity of oxygen (O₂) and methanol (CH₃OH), which both have a mass of 32, is resolved by ionisation with xenon (12.2 eV). This generates an O₂ ion of mass 32 and a CH₃O⁺ ion with a mass of 31. Larger hydrocarbons need ionisation energies in the range of 10.4 eV, which is achieved using a mercury ion beam.

The soft ionisation technique has been used in many applications over the past 15 years. These include ambient air monitoring where online detection at ppt levels of sensitivity is required, fast multicomponent gas analysis in 10 msec range for dynamic transitions in catalyst research, and remote, fully automated quality assurance monitoring of carbon dioxide gas for the food industry.

As the source of the gas to be analysed by MS is not important, this technique has been extended to the analysis of exhaled breath. Many medical specialists have long been looking for a non-invasive analytical tool which can provide additional information to the more invasive techniques currently in use. Breath analysis fulfils these criteria.

Breath analysis
Breath analysis is a very complex process, whether one is testing for a few or many parameters. Any technique used for this purpose must therefore satisfy a wide range of specifications:

- The instrument should be able to measure concentrations as low as 0.001 ppm, as well as those as high as percentage concentrations. All important organic and inorganic compounds and compound classes should be detectable, i.e. all compounds existing in the gaseous phase or dissolved in the water vapour of exhaled breath.
- It should also be possible to use the system in either an online or offline mode. The V&F-Airsense analyser is an example of a modern instrument which fulfils these specifications (figures 1 and 2). The online analysis time using this system is just 300 msec per compound, while the offline analysis time is >5 seconds per compound. In both cases, no prior preparation of samples is required.

Offline analysis
The advantages of offline analysis are apparent when analysing large sample numbers, for example during clinical studies. To sample low molecular weight compounds, the patient exhales into a vial which is then sealed. A second vial is placed in the surrounding environment and this is used as a control sample. The samples are placed in the V&F-autosampler and then subsequently analysed in the mass spectrometer. Up to 200 different masses may be detected.

Exhaled breath carries a number of low molecular weight compounds at sub-ppm concentrations, in addition to nitrogen, oxygen, carbon dioxide and water. The intensity and distribution of these sub-ppm compounds can give an indication of how certain body processes are functioning. Some 60 different molecular compounds found in exhaled breath in a concentration range from 2 ppb to 20 ppm have been linked to individual organ functions (2). These compounds therefore have the potential to be indicators of any malfunction of these organs.

Important compounds in human exhaled breath
In a study designed to determine the concentration ranges of the relevant volatile compounds detected in exhaled breath, samples were taken from 797 individuals during a three day medical check-up (3). 114 volatile compounds were measured in all the samples by the V&F-Airsense. The variation in concentrations of 41 of the most significant compounds are shown in figure 3. It can be seen that compounds such as acetaldehyde, acetone, acetonitrile, benzene, methane, acetic acid and isoprene showed variations of up to a factor of eight. Other components, such as ammonia and ethanol, varied by up to a factor of 130.

The sensitivity of the technique is such that many of the peaks detected correspond to molecules which have not yet been associated directly with specific underlying pathologies. In other cases, it is well known that the compounds measured are associated with medical conditions. For example, a higher nitric oxide level is associated with inflammation and asthma, while changes in nitric oxide and nitrogen dioxide are frequent characteristics of gastrointestinal diseases. The breath of smokers may contain a higher concentration of 2,5-dimethylfuran and higher acetone levels are well documented in patients with diabetes. In addition, higher ammonia levels correlate with liver problems, higher methane concentrations are associated with certain bacteria in the gastrointestinal tract and, of course, ethanol due to the consumption of alcohol is probably the most well known of all compounds in human exhaled breath.
Online analysis

Online analysis is used for near-patient testing when a quick turnaround time of results is required. Possible applications include aerobic and anaerobic metabolism studies in sports training or drug monitoring. Any compounds of interest can be monitored immediately using a mobile analysis system.

Breath analysis in pharmacokinetics

In certain cases of acute or chronic bronchitis, treatment involves the administration of thymol in the form of tablets. A study was made by quantitative online gas analysis of both thymol and ethanol in the exhaled breath of a 63 kg man after he had ingested a 300 mg tablet of thymol. Measurement of the breath analytes was carried out over a period of 90 minutes in the V&F mass spectrometer using a four second cycle type.

Figure 4 shows the concentration of thymol in the breath of the subject. It can be seen that a maximum of 3ppb of thymol is detected 36 minutes after ingestion of the tablet. Thereafter the concentration slowly declines, reflecting the metabolism/excretion of thymol. It can also be seen that the level of ethanol begins to increase 30 minutes after ingestion of the tablet, from an initial concentration of 0.5 ppm to a maximum level of 7 ppm after 90 minutes. The pharmacological properties of these drugs are well established, for example bronchospasmyotic or antibacterial activity. However, the direct determination of these compounds using MS of exhaled breath enables more precise tailoring of the dosage to the individual requirements of the patient.

Further studies

Further studies to investigate possible applications for breath analysis using the V&F-Airsense are currently under way. These include the evaluation of various liver diseases (differentiation between a healthy liver, non alcoholic steato hepatitis, alcoholic steato hepatitis and liver cirrhosis) (4) and malabsorption of fructose and lactose. Lung transplant rejection is also a field of active research using this technique (5), as is the diagnosis of cystic fibrosis and asthma. Drug metabolism measurements may also be performed and drugs of abuse are also detectable. Moreover, this technique is being used to measure the absorption and clearance of organic vapours to which certain workers are exposed. The evaluation of aerobic and anaerobic metabolism in sports training and the correlation to blood lactate concentration is also an active field of research utilising this technique.

Conclusion

Mass spectrometry, both online and offline, is a fast, simple but systematic technique for the analysis of volatile compounds, such as ammonia, methane, acetone, isoprene, alcohols, aldehydes, ketones, carboxylic acids, amines, etc. in human exhaled breath. Studies have shown that the technique can provide a non-invasive way of establishing a diagnosis, or a method for monitoring a disease. The information generated by mass spectrometry can be used as a screening method in itself, or as a source of additional information to supplement other, well-established clinical diagnostic parameters.

References


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