Anton Amann • David Smith
editors

Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring

METABOLITES IN HUMAN BREATH: ION MOBILITY SPECTROMETERS AS DIAGNOSTIC TOOLS FOR LUNG DISEASES

J. I. BAUMBACH, W. VAUTZ, AND V. RUZSANYI

ISAS — Institute for Analytical Sciences,
Department of Metabolomics,
Bunsen–Kirchhoff-Straße 11, D-44139 Dortmund, Germany

L. FREITAG

Lung Hospital Hemer,
Theo-Funccius-Straße 1, D-58675 Hemer, Germany

1. Introduction

The aim of this chapter is to establish a quick and low-cost device for human breath analysis, in addition to investigations of blood and urine, as a non-invasive standard method in hospitals and for medical applications. This procedure is based on miniaturised ion mobility spectrometers supported by mass spectrometric validations. The full procedure, including sampling, pre-separation and identification of metabolites in human exhaled air, will be developed and implemented with a view to future use in hospitals. Metabolic profiling of the breath of healthy individuals and those suffering from different diseases, in particular lung cancer, will be considered at various lung hospitals and point-of-care centres.

It is well recognised in the medical community that people exhale volatile compounds that may carry important information about the health of the individuals. Thus, a successful detection of the products of different metabolic processes is attractive, especially if the detection limits of the spectrometric methods used are sufficiently low and the instruments are available at moderate price levels so that they can be used as standard methods in hospitals. The vision of the authors is to contribute to the development of breath analysis as a diagnostic method for disease in support of blood and urine analysis.

Human breath contains numerous volatile substances derived both from endogenous metabolism and exposure to ambient vapours and gases and their metabolites. Approximately 200 different compounds have been detected in human breath; some are correlated with various common disorders like diabetes, heart disease and possibly lung cancer.[1–20] Generally, the composition of different constituents in expired air is representative for blood concentrations resulting from gas exchange at the blood/breath interface in the lungs.[21] Thus, the presence and concentrations of specific volatile organic compounds, VOCs, in expired air are directly linked to their presence in the blood, which is in contact with diseased tissues and organs. Furthermore, metabolites derived from local bacterial infections in the airways can be also detected directly in breath. Pulmonary infections carry a significant risk for people with week immune systems, especially for long term and post-operative patients.

Different techniques are used for breath analysis. A popular sampling method is the use of Tedlar bags to collect human breath. The components of the exhaled breath sample are collected using a sorbent-trap or a cryo-trap followed by desorption into an analytical instrument such as the often used gas chromatograph with an analytical mass spectrometer, GC-MS.[22] This is a rather time consuming process with numerous steps that may lead to loss of analytes,[2,23] since different analytes may adsorb on the surface of the bag,[24] which is especially troublesome at trace gas levels. The number of compounds detected in exhaled air and their concentrations vary according to the sampling procedure and the analytical method used. The major VOCs found in the breath of healthy persons (with their typical concentrations in parts-per-billion by volume, ppbv) are isoprene (10–600), acetone (1–2000), ethanol (10–1000), methanol (150–200 ppbv). All are products of the standard metabolic processes.[9]

The high moisture of exhaled breath samples is a major problem for most analytical methods, except for SIFT-MS (see the article by Smith and Španěl[25] on page 3 of this book). It can seriously compromise GC-MS analyses and so the water vapour should be removed using a cryo-trap for GC-MS to be used effectively. Because these laboratory instruments are usually large and expensive and require an analysis time of nearly one to two hours, depending on the sample preparation steps necessary, there is a need for instruments that can perform on-line, real time breath analysis.

If the number of sample handling steps could be minimised and no additional carrier gases of high purity are required (like nitrogen or helium, as used in GC-MS), then on-line methods for effective breath analysis proce-

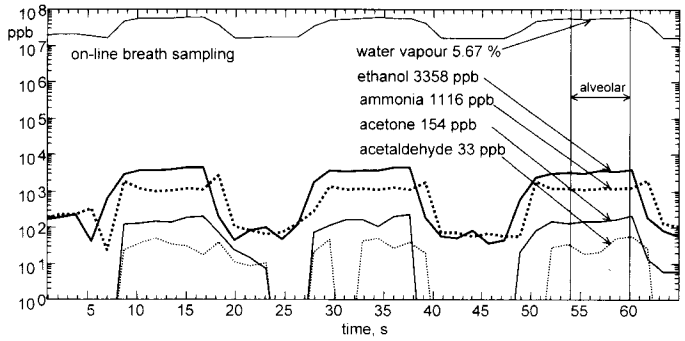

Fig. 3. The concentrations of water vapour and the breath metabolites indicated in three sequential breath exhalations obtained simultaneously using the MIM mode of SIFT-MS analysis. Note the very wide concentration range from that of water vapour down to acetaldehyde. The known breath water vapour concentration acts as a very valuable internal calibration for the analyses. Reproduced from Ref. 70 with permission from IOP publishing, copyright 2002.

are monitored as air or breath displaces the ambient air at the entrance to the sample entry port (see Figure 1). This is achieved by rapidly switching the downstream mass spectrometer between the masses of all the primary ions and the selected product ions and dwelling on each of these masses for a predetermined short time interval (see Figure 3). This real-time monitoring is possible because of the fast time response of SIFT-MS, largely determined by the fast flow rates of the carrier gas along the flow tube and the sample gas along the inlet tube. The response time is about 20 ms. There is no fundamental limit to the number of different ion masses that can be recorded simultaneously using this technique. However, with the values of the precursor ion count rates currently available in SIFT-MS instruments, the practical limit is about fourteen ions at trace gas concentrations in the ppb regime. If larger numbers of ions need to be monitored it is much more convenient to sequentially record several full scan spectra in the time allowed by the sample volume and construct a table of count rates of all ions within the m/z range.[36]

2.4. *Sampling Procedures*

SIFT-MS analysis of ambient air is achieved by simply opening the sampling port to allow the air to enter the carrier gas (via the calibrated capillary; see Figure 1) whilst the detection mass spectrometer is operated in the FS or

Lavoisier reported the first quantitative analysis of carbon dioxide in 1784 and demonstrated conclusively that this breath compound was a product of normal respiration. In the interim there were a number of reports of breath analysis for molecules such as ethanol. The earliest publications of modern day breath analysis appeared in the late 1960s and early 1970s, which was the time of nascence for modern analytical chemistry. Researchers such as Pauling,[1] Larsson,[2] Chen,[3] Cohen,[4] and Phillips[5] reported some of these pioneering studies. Many of these studies were only possible as a result of enhanced separation of gaseous molecules by gas chromatography, increased selectivities of mass or optical spectrometers and improved limits of detection from high parts-per-million to parts-per-billion.

A number of investigators have reported on the analysis of breath in the intervening period and the subsequent discussion is based upon a Library of Medicine computer search (`www.ncbi.nlm.nih.gov`) performed on September 10, 2004. More than 5000 citations to breath and clinical tests were found in this search and the distribution of the studies, based upon the identity of the compound, are shown in Table 2.

Table 2. Number of published clinical uses of breath

Compound	Number of publications
Oxygen	2155
Carbon dioxide	2027
Hydrogen	1722
Ethanol	1053
Hydrocarbons	968
Carbon monoxide	849
Water	679
Nitric oxide	638
Pentane	141
Ethane	128
Acetone	126
Ammonia	95
Acetaldehyde	83
Isoprene	66
Methanol	51
Methylamine	47
Ethylene	38
Methanethiol	29
Carbon disulfide	23
Carbonyl sulfide	5

Table 3. Distribution of published studies

Physiological basis	Number of publications
Pulmonary diseases	2983
Asthma	1178
COPD	417
Emphysema	222
Cystic fibrosis	221
ARDS	104
Infectious diseases	1674
Cardiovascular diseases	1551
Cancer	1334
Liver diseases	730
Renal diseases	230
Organ transplantation	221
Gastrointestinal diseases	81
Dental diseases	47
Normal physiology	4453
Alcohol assessment	1858
Exercise	1322
Exposure assessment	1142
Smoking	1004
Anesthesia	556
Nutrition	445

Only breath molecules whose origins are endogenous (except oxygen) have been included in this tabulation. As expected, this distribution of molecules in terms of number of studies is directly related to their concentrations (compare the concentrations of molecules shown in Table 1 to the ranking contained in Table 2). These same publications have been regrouped in terms of their clinical or physiological uses and Table 3 presents this compilation.

Pulmonary diseases, particularly asthma, and the understanding of normal physiology were the most popular published uses of breath analysis. Two endogenously produced molecules dominate this distribution of published studies: nitric oxide deriving from airway tissues and cells, and carbon dioxide originating from normal and abnormal metabolism. A summary of the biochemical or physiological bases for the remaining endogenous molecules is presented in Table 4.

Inspection of this information shows that metabolism of foods or exogenous substances is the major source of breath molecules and therefore the use of odors of breath for clinical diagnosis can be confounded by the characteristic odors that result from the metabolism of foods containing garlic, onions, fish, spices, mints or ethanol. Also, if the origin of a molecule is metabolism then the composition of breath will have a temporal relationship.

Table 4. Physiological origins of some endogenous breath molecules

Compound	Physiological basis
Acetaldehyde	Ethanol metabolism
Acetone	Decarboxylation of acetoacetate
Ammonia	Protein metabolism
Carbon disulfide	Gut bacteria
Carbon monoxide	Production catalyzed by *heme oxygenase*
Carbonyl sulfide	Gut bacteria
Ethane	Lipid peroxidation
Ethanol	Gut bacteria
Ethylene	Lipid peroxidation
Hydrocarbons	Lipid peroxidation/metabolism
Hydrogen	Gut bacteria
Isoprene	Cholesterol biosynthesis
Methane	Gut bacteria
Methanethiol	Methionine metabolism
Methanol	Metabolism of fruit
Methylamine	Protein metabolism
Nitric oxide	Production catalyzed by *nitric oxide synthase*
Pentane	Lipid peroxidation

THE COMBINED USE OF SIFT-MS AND FA-MS TO INVESTIGATE FIRST-PASS METABOLISM OF ETHANOL

T. S. WANG

Institute of Science and Technology in Medicine, Medical School, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent, ST4 7QB, UK

P. ŠPANĚL

V. Čermák Laboratory, J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejškova 3, CZ-182 23 Prague 8, Czech Republic

D. SMITH

Institute of Science and Technology in Medicine, Medical School, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent, ST4 7QB, UK

1. Introduction

A large amount of research has been carried out into the nature of ethanol in the human body, work driven to a large extent by clinical and legal implications. As is well known, when ethanol (alcohol) is ingested in excess and over the long term it is poisonous and is destructive to the liver. At any dosage, it can affect judgement and reaction time. Above a certain limit, ethanol can be detected in exhaled breath and this phenomenon is exploited by law enforcement agencies at the street side using so-called breathalysers.[1] Legal limits are set for acceptable levels of ethanol in the breath and blood of drivers: currently in the UK this limit in blood is set at 80 mg of ethanol in 100 ml of blood,[2] which is equivalent to a partial pressure in exhaled breath of 180 parts-per-million, ppm[3,4] (this is discussed further in Section 5). This is massively greater than the ethanol level in breath resulting from endogenous processes (principally produced by the action of gut bacteria on carbohydrates),[5] these being typically within the range of 0.01–1.0 ppm for healthy persons.[5,6] Quite sophisticated analytical devices are required to detect breath ethanol at these levels, such as our

selected ion flow tube mass spectrometer, SIFT-MS, instrument which will briefly be described later.

Almost all the studies of ethanol metabolism have been carried out by monitoring venous blood levels with time following either oral or intravenous administration.[7] We have shown recently,[8] and we emphasise below, that using our SIFT-MS analytical method single breath exhalations can be analysed for ethanol, and indeed several other metabolites simultaneously, thus providing data from which accurate decay rates of breath ethanol can be determined. What is very clear from many studies is that (following oral ingestion) the speed of absorption of ethanol from the stomach and gut depends on time of day, drinking pattern, dosage and concentration of ethanol in the drink, and particularly the feeding or fasting state of the individual.[5] It is also clear that at high doses the rate of metabolism is zeroth-order, *i.e.* conforms to saturation kinetics, whereas at low doses this rate is first-order (unsaturated).[7]

There is much interest in so-called first-pass metabolism of ethanol (by the liver via the portal vein and partially also within the gastric system).[7] The contribution of this process is commonly estimated from the difference in the area under the ethanol blood concentration time curves obtained following oral and intravenous administration of ethanol, but it is not fully appreciated that this technique provides an accurate indicator of first-pass metabolism only when the clearance of the compound under consideration obeys first-order (unsaturated) kinetics throughout the range of blood concentrations observed.[7] Thus, following the comments in the previous paragraph, it follows that studies of first-pass metabolism should ideally be carried out at low ethanol concentrations. Better still, it is desirable that first-order kinetics should be seen to be occurring during the experiment. This can be checked using SIFT-MS breath analyses, as we show later. However, first-pass metabolism remains difficult to assess because of the dose and time factors mentioned above. Ethanol is transported by the bloodstream to all parts of the body and the rate of equilibration within the body is governed by the blood flow rate and the total body water (TBW) in which it readily dissolves; note that ethanol has low solubility in lipids and does not bind to plasma proteins.[9] It has been shown that TBW is very varied amongst individuals and is correlated with gender and age (greater in men than women; greater in the old).[10] These findings have been confirmed by experiments carried out using the recently developed flowing afterglow mass spectrometry, FA-MS, technique, which allows on-line, real time determination of TBW using non-invasive breath analysis[11,12] (see also Ref. 14,

ously, including acetaldehyde, ammonia and acetone, the last two compounds being indicators of nutritional status.[20]

A standard approach was also taken to the FA-MS measurements in that measured quantities of D_2O in proportion to the body weight, typically at a dose level of 0.3 g/kg body weight, were diluted in about 200 mL of drinking water before drinking. The deuterium content of the breath water vapour in single breath exhalations was determined using FA-MS, again over a period of some two hours. In both the SIFT-MS and FA-MS experiments, a few measurements were taken just before the ingestion of the ethanol and the D_2O, establishing the so-called base line or predose levels of ethanol and HDO.

4. Sample Results

In this short report chapter we present the results obtained for a single individual (male, body weight 60 kg, BMI 22) simply to demonstrate how a coordinated SIFT-MS and FA-MS study of ethanol dispersal/metabolism and water dispersal throughout the body allows the fraction of the ingested ethanol that is first-pass metabolised to be obtained.

4.1. *Ethanol Dispersal and Metabolism*

The typical time variations of the breath ethanol, acetaldehyde and ammonia after ingestion of 5 mL of ethanol are shown in Fig. 1. Also shown are the predose breath levels of these species. The breath ethanol level is relatively high immediately after ingestion due to unavoidable mouth/saliva contamination during drinking. This contamination then diminishes and a clear minimum in the breath ethanol is reached following which it increase towards a maxima value as the ethanol passes from the stomach, into the small intestine and then into the blood stream when it is immediately seen in the exhaled breath. Finally, the blood/ breath ethanol decreases due to the combination of dispersal and dilution into the TBW and metabolism. Actually, these processes occur together as the breath ethanol maximum is approached and is passed, but at later times metabolism dominates the loss of ethanol from the blood/breath and a near-exponential decay results with a time constant, τ, of 20.7 minutes in this example (see Fig. 1). This indicates that first-order (unsaturated) kinetics dominates, which means that first pass metabolism can be properly investigated at these low ethanol doses. This phenomenon will be examined later after the TBW (FA-MS) data have been discussed. Note that the previous SIFT-MS experiments[8]

showed at an ethanol dose some 3 times greater that ethanol loss is better described by zero-order (saturated) kinetics. (At an ethanol dose about 3 times smaller, the ethanol was very rapidly removed, which we now understand — following the present study — is due to first-pass metabolism effectively removing the entire amount of ethanol). It can be seen in Fig. 1 that the physiological (pre-ingestion) ethanol level is re-established after about 2 hours. Note, also, that the acetaldehyde level tracks the ethanol level, as expected, with a short time lag.[21,22]

A further observation that demonstrates the extraordinary value of the SIFT-MS technique is the simultaneous observation of breath ammonia, as is also shown in Fig. 1. Ammonia is present in the breath of all healthy individuals, typically in the partial pressure range from 200 to 2000 ppb.[6] SIFT-MS experiments have shown that breath ammonia levels "dip" immediately after the ingestion of protein and carbohydrate meals following overnight starvation.[20] This phenomenon also occurs when the ethanol aqueous solution used in these experiments is ingested, as can be seen in Fig. 1. This is surely due to the stimulation of the portal blood flow and the enhanced removal of ammonia by the liver. Note that the minimum in the

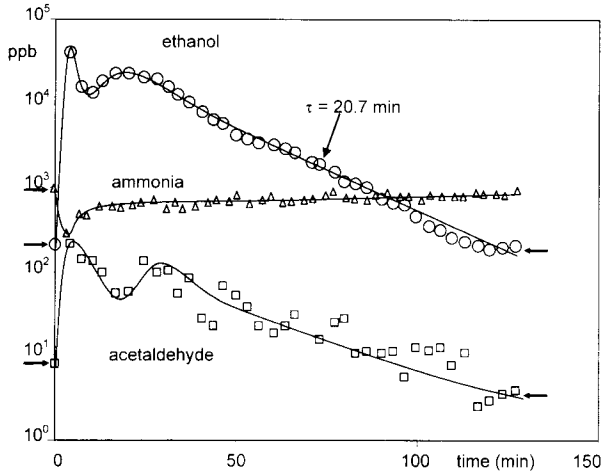

Fig. 1. Time variations of the breath levels in parts-per-billion, ppb, of ethanol, acetaldehyde and ammonia after ingestion of the ethanol/water solution. The amount of ethanol ingested was 5 mL (0.067g/kg body weight). The time constant for the rate of ethanol metabolism, τ, at the later times is 20.7 min. The physiological (predose ethanol) levels of these compounds are indicated by the continuous arrows both at the start and end of the measurement period.

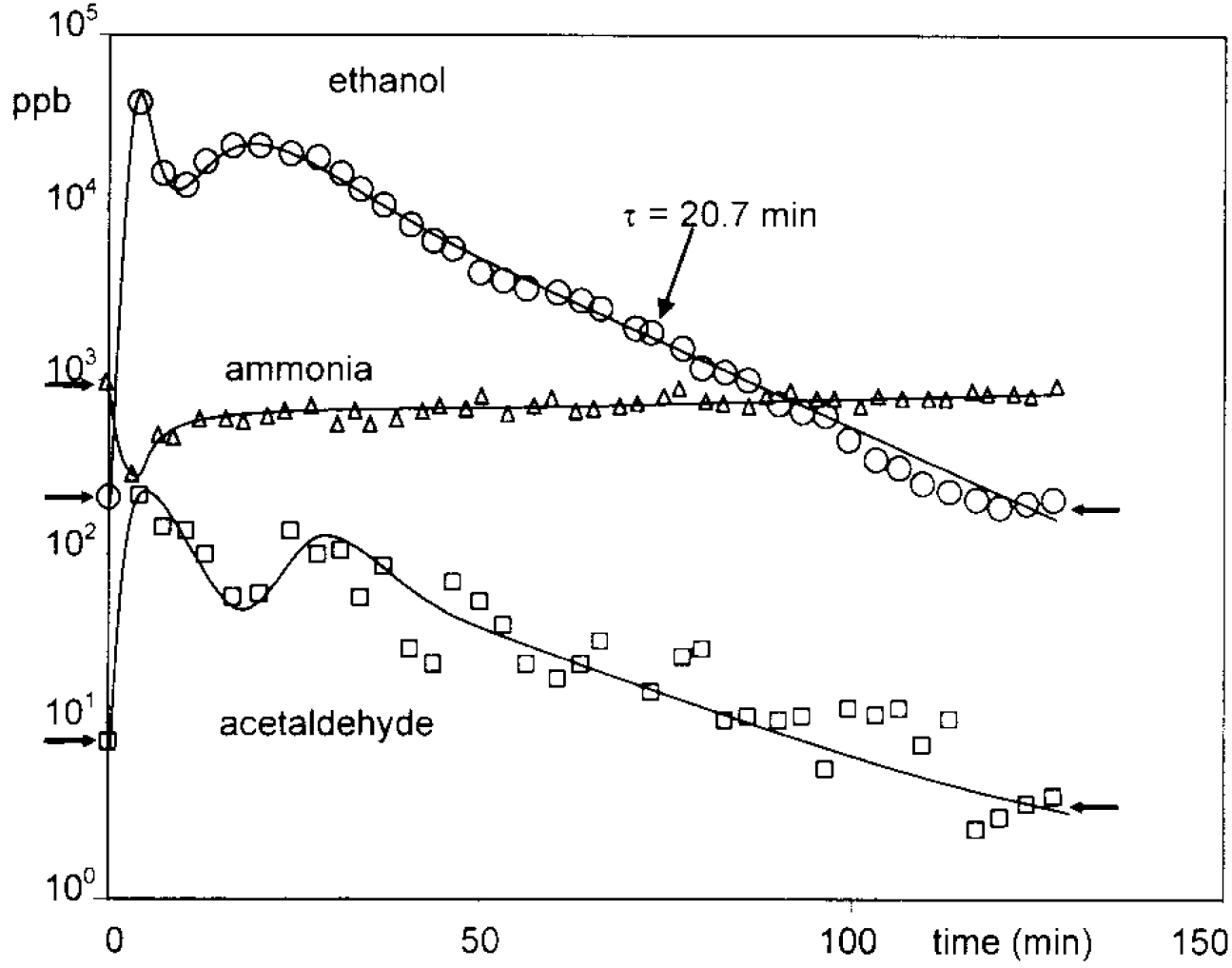

Fig. 1. Time variations of the breath levels in parts-per-billion, ppb, of ethanol, acetaldehyde and ammonia after ingestion of the ethanol/water solution. The amount of ethanol ingested was 5 mL (0.067g/kg body weight). The time constant for the rate of ethanol metabolism, τ, at the later times is 20.7 min. The physiological (predose ethanol) levels of these compounds are indicated by the continuous arrows both at the start and end of the measurement period.