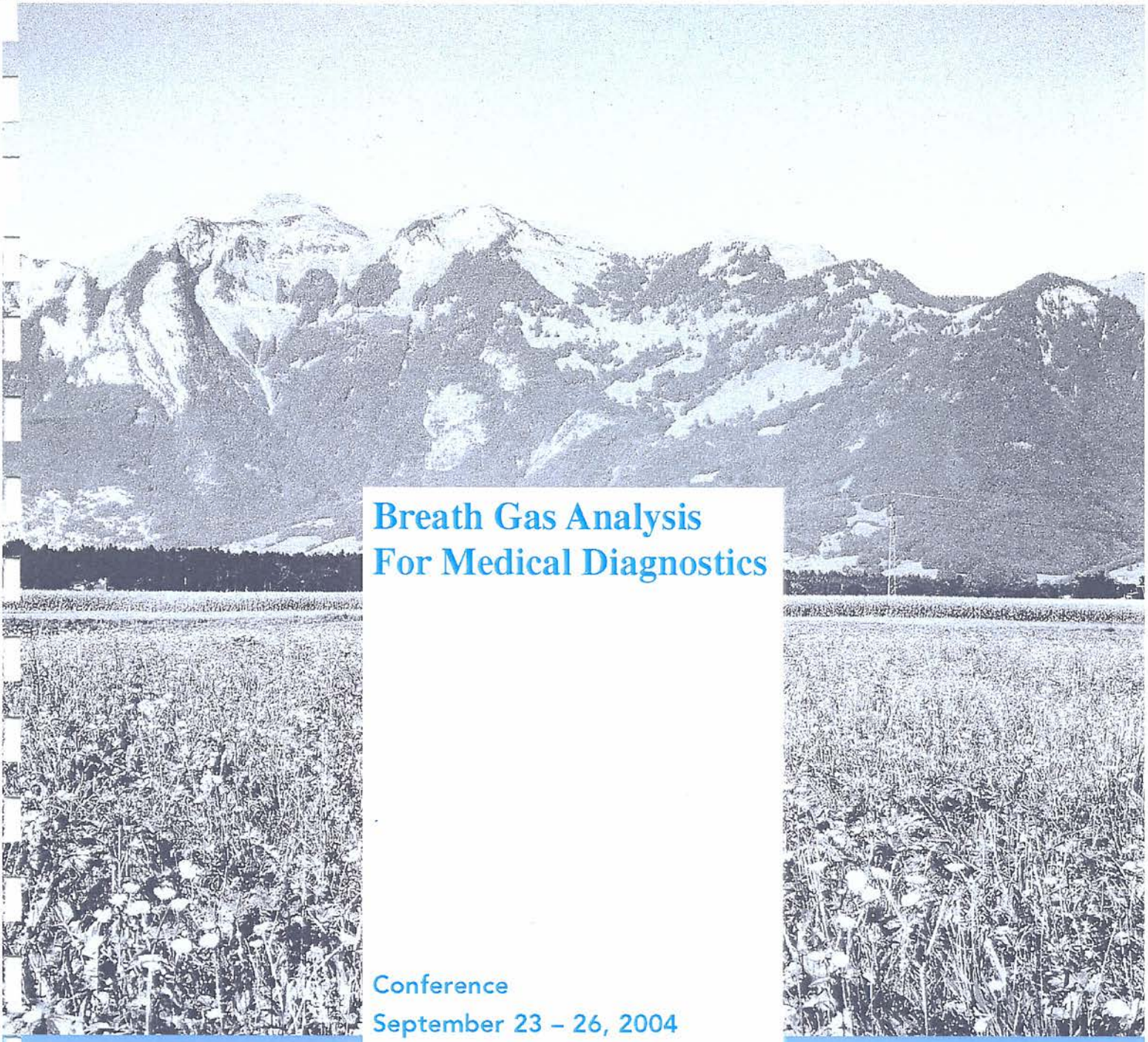

Breath Gas Analysis For Medical Diagnostics

Conference
September 23 – 26, 2004

Vorarlberg University of
Applied Sciences
Dornbirn, AUSTRIA

Chairman: Anton Amann, Innsbruck
Secretary: Marco Freek

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Augustine Choi, Pittsburgh ||| Heinz Drexel, Feldkirch ||| Hartmann Hinterhuber, Medical University of Innsbruck ||| Bernhard Lang, Lochau ||| Tilmann Märk, Vice Dean of the University of Innsbruck ||| Oskar Müller, Dean of the Vorarlberg University of Applied Sciences ||| Nandor Marczin, London ||| Ulrich Müller-Herold, ETH-Zürich ||| Michael Phillips, New York and New Jersey |||Bernd Saletu, Vienna ||| Jochen Schubert, Rostock ||| David Smith, Keele University (GB)

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Fachhochschule Vorarlberg
University of Applied Sciences

Welcome to Vorarlberg University of Applied Sciences

As Rector of Vorarlberg University of Applied Sciences, it is a great pleasure for me to welcome you to our University and the conference "Breath Gas Analysis for Medical Diagnostics", which we are delighted to host.

The fast increasing international interest in biomedical research has motivated us to found a research group dealing with biomathematical signal analysis, modelling and simulation. Our research focuses particularly on ECG during ventricular fibrillation, fibrillation detection and scoring algorithms, EEG, sleep stage detection and online time series analysis of breath gas concentration. In the near future, we anticipate expanding our research to include such areas as lung mechanics and the simulation and modelling of hemodynamics.

This new research focus at our University is managed in cooperation with several partners, among them the University of Innsbruck (Univ.-Prof. Dr. Anton Amann) and the University of Vienna. Together with our partners, we are chiefly interested in medically important research with a strong mathematical and software/hardware background that is potentially promising for clinical applications. We plan to extend our research and would be pleased to have you as partners in future projects.

I wish you a successful conference and an enjoyable stay in Dornbirn.

Prof. (FH) Dr. Oskar Müller, Rector

Foreword

We are very pleased to welcome you to the University of Applied Sciences in Dornbirn and to this conference on "Breath Gas Analysis for Medical Diagnostics". Breath gas analysis is an emerging scientific field, which has great potential for clinical diagnosis and therapeutic monitoring, but which, as yet, is not widely utilized in clinical practice. This conference will cover several aspects of breath analysis ranging from developments in sampling and analytical methodologies to basic scientific investigations and pilot case studies that have already been shown to be potentially valuable in clinical practice. Possible applications of exhaled breath analysis include lung and breast cancer screening, studies of asthma, on-line renal dialysis, liver failure, cardiovascular disease, metabolic diseases such as diabetes, on-line quantification of metabolic processes during sleep and basic physiological studies.

The major analytical techniques used in the field of breath gas analysis are gas chromatography mass spectrometry (GC-MS), proton transfer reaction mass spectrometry (PTR-MS), selected ion flow tube mass spectrometry (SIFT-MS) and near infrared laser absorption spectroscopy (NIR). These different analytical methods and their applications, together with their associated sampling techniques and the sources of exogenous compounds that can distort the analyses, will be addressed in several talks. Modelling of the metabolism and excretion of inhaled VOCs after human exposure to polluted environments will be also discussed. A new technique - flowing afterglow mass spectrometry, FA-MS, - for deuterium analysis of water vapour in single breath exhalations and above aqueous liquids (e.g. dialysate fluid) will be described, which allows total body water to be determined and the transport kinetics of the peritoneal membrane to be studied.

GC-MS provides very detailed information on the compounds in breath, but generally requires sample collection onto traps and is time consuming. PTR-MS, SIFT-MS and NIR often give less detailed information, but allow on-line breath analysis, even of single exhalations. The detailed discussions of these various techniques and the results of the associated studies will show how they complement each other and how they may be jointly exploited to maximum effect for breath analysis.

We hope that this conference will be a forum for discussion of the wider aspects of trace gas analysis and new areas of application that will spawn similar meetings in the future. We also hope that it will foster important collaborations in this exciting field so that the emerging analytical methods for trace gas analysis can be properly assessed and utilised to greatest effect.

We thank the Government of Vorarlberg for its generous help with funding for this conference. The help of many people at the University of Applied Sciences, in particular Prof. Karl Unterkofler, Prof. Thomas Breuer, Claudius Caba, Isabella Natter, Monja Domig, Susanne Doppelmayer and the Rector, Prof. Dr. Oskar Müller, has been invaluable in creating an excellent ambiance for this conference. We also thank Marco Freek, Christian Lorünser and our Scientific Board colleagues for their expert and invaluable assistance in the organization and planning of this conference.

Anton Amann and David Smith

Helpdesk

If you need logistic help *(transfer to and from hotel, internet connection, powerpoint presentation, etc)* during the conference, please contact the following helpful persons *(wearing a red-colored name badge)*:

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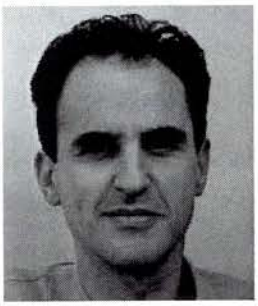

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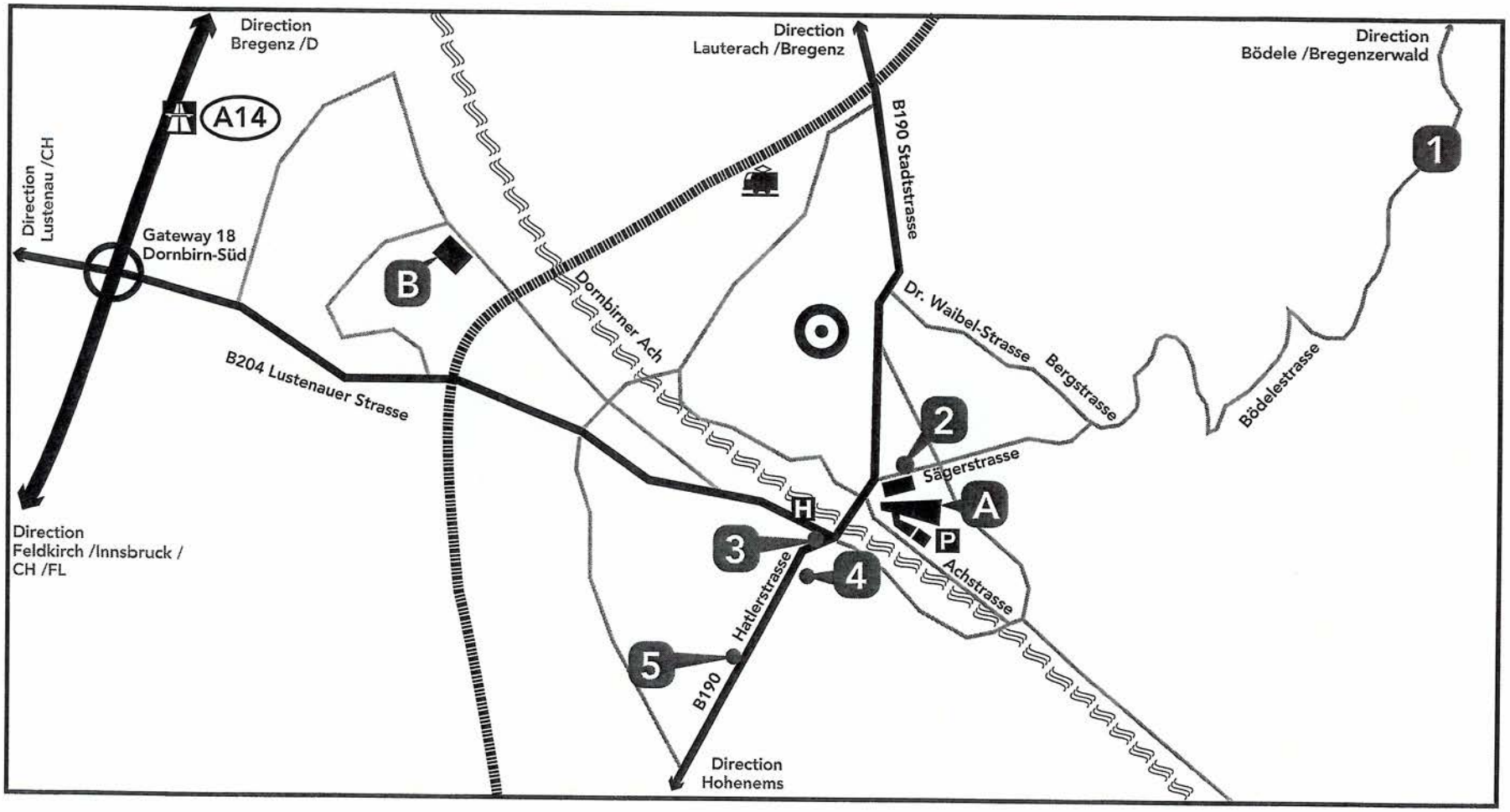

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- General Hospital Dornbirn
- Center of Dornbirn
- Train Station Dornbirn
- Parking FH Vorarlberg
- Train
- River
- Highway
- Mainstreet
- Sideroad

- A Fachhochschule Vorarlberg Building Achstrasse and Sägerstrasse
- B Fachhochschule Vorarlberg Building Höchsterstrasse
- 1 Hotel Rickatschwende Bödelestrasse
- 2 Pension Sonne Sägerstrasse 8
- 3 Hotel Krone Hatlerstrasse 2
- 4 Hotel Bischof Hatlerstrasse 7
- 5 Hotel Rose Hatlerstrasse 31

Programme of the conference

Programme of the Conference

"Breath Gas Analysis for Medical Diagnostics"

Vorarlberg University of Applied Sciences, Dornbirn

September 23rd to 26th, 2004

Thursday, Sept 23rd

8:00	**Registration**
8:45	**Welcome address** by Oskar Müller and Anton Amann
	Mass spectrometric techniques for breath gas analysis *Chairman:* Tilmann Märk
9:00	Michael Phillips: *"Methods in breath testing"*
9:30	Armin Hansel and Armin Wisthaler: *"PTR-MS; a new tool for the rapid detection and quantification of VOCs in air at ultra-trace levels"*
10:00	Patrik Španěl: *"Selected ion flow tube mass spectrometry, SIFT-MS: accurate absolute quantification of metabolites in breath"*
10:30	Ingo Baumbach: *"Detection of Metabolites in Human Breath: Ion Mobility Spectrometers as Diagnostic Tools for Lung Diseases"*
11:00	*Coffee break*
	Spectroscopic and other techniques for breath gas analysis *Chairman:* Lars Gustafsson
11:30	Manfred Mürtz: *"Laserspectroscopic online monitoring of exhaled trace gases"*
12:00	Gerard Wysocki: *"Exhaled human breath analysis by means of quantum cascade laser based gas sensors"*

New techniques for breath gas analysis (two parallel sessions)

	Chairman: Michael Phillips	*Chairman:* Ingo Baumbach
12:30	Jörg Lauenstein: *"Isotope selective detection of nitric oxide in human exhalation"*	Claudio Perret: *"Validation of a new portable telemetric-ergospirometric device during exercise"*

12:50	Andrew Ellis: *"PTR time-of-flight mass spectrometry: a good prospect for diagnostic breath analysis?"*	Wolfgang Miekisch: *"Out of breath – analysis of volatile organic breath markers -pitfalls, tips and tricks"*

13:20 *Lunch*

14.20 **Panel discussion:** *Is it time for establishing an "International Society for Breath Diagnostic Research"?*

Chairman and discussion moderator: Ulrich Müller-Herold
Participants: A Amann, R Dweik, L Gustafsson, A Lindstrom, N Marczin, J Lundberg, W Miekisch, M Mürtz, M Phillips, J Pleil, T Risby, G Rolla, J Schubert, D Smith

Some points and questions for this panel discussion:

Are there (potentially) enough interested people to keep such a society running?

What contacts would there be to other societies, like ORCA (European organisation for Caries Research), European Thoracic Society, International Society for Breath Odor Research, etc.

How could one optimize the benefits of cross-fertilisation of ideas from related areas?

Could breath tests (say in the next two decades) become an integral part of medicine and dentistry (as well as other fields), in a broadly analogous way to blood tests in hospitals?

Should such a society concentrate on breath, OR would it be more appropriate to include blood and urine headspace also?

Who are the persons who could be sufficiently involved to give the society a flying start and maintain the momentum when the society is formed? Should a working group be formed to gauge interest? (it would be disastrous if interest was lost soon after its formation)

Who could potentially sponsor such a society?

15.30 *Coffee break*

Breath gas analysis and physiology

Chairman: Raed A. Dweik

16:00 Lars Gustafsson: *"Exhaled NO: how and why we know it is important"*

16:30 Jon Lundberg: *"Diagnostic use of a nasal NO test"*

17:00 Andrew Lindstrom: *"The use of exhaled breath analysis in human exposure assessment studies"*

17:30 Joach Pleil: *"The unique value of breath biomarkers for estimating pharmacokinetic rate constants and body burden from random/intermittent dose"*

Poster session A

Poster organizer: Manfred Mürtz

18:00-19:00

T Birken, B Brock, JK Schubert: *"A standardized CO_2 controlled alveolar breath sampling technique"*

U Janovsky, S Scholl-Bürgi, DB Skladal, G Poupart, A Schmid, A Amann: *"Breath gas analysis in paediatric patients with propionic acidaemia"*

N Lalic, A Schmid: *"Sampling of breath gas using Tedlar® bags"*

MJ McEwan, Jennifer M Scotter, VS Langford, PF Wilson and ST Chambers: *"Real-time detection of common microbial volatile organic compounds (VOCs) from medically important fungi by SIFT-MS"*

R Bloor, TS Wang, P Španěl, D Smith: *"Applications of selected ion flow tube mass spectrometry (SIFT-MS) in addiction research"*

A Cathcart, CA Wyse, PS Yam, SA Ward, MJ Padgett and KS Skeldon: *"The effect of maximal exercise on exhaled CO and ethane in human and animal athletes"*

M Basanta, T Koimtsiz and CL Paul Thomas: *"Protocol for producing an ion mobility spectrometry library of VOC in human breath"*

V Gufler and M Ledochowski: *"Breath gas analysis in patients with malabsorption syndroms"*

V Gufler and M Ledochowski: *"Breath tests in gastroenterological practice – carbohydrate malabsorption"*

Mona Lärstad: *"Are ethane, pentane and isoprene produced locally in the airways? – a methodological study"*

GV Kamarchuk, OP Pospyelov, AV Yeremenko, EG Kushch, LV Kamarchuk: *"Selectivity of sensor response to the breath gas components as a method of noninvasive diagnostics of human diseases"*

OP Pospyelov, GV Kamarchuk, YL Alexandrov, AV Kravchenko, E Faulques: *"TCNQ derivatives-based sensors for exhaled breath analysis"*

Dinner at Hotel Krone at 19.30
Remark: beverages are not included

Friday, Sept 24th

Breath gas analysis for diagnostics and therapy

Chairman: David Smith

8:00 Jochen Schubert: *"The background dilemma - influence of inhaled substance on the results of breath analysis"*

8:30 Keary Cope: *"Lung cancer breath biomarkers"*

9:00 Stefan Ryter and Augustine Choi: *"Can inhaled CO be a therapeutic modality in human diseases"*

Poster session B

Poster organizers: Giovanni Rolla and Joach Pleil

9:30-10.30

A Malinovschi, C Janson, T Holmkvist, P Meriläinen, M Högman: *"Exhaled nitric oxide levels in relation to allergic sensitisation"*

JB Mei, GA Reineccius, WB Knighton, EP Grimsrud, and T Krick: *"Comparison of linear response in the detection of aroma compounds by APCI-MS and PTR-MS"*

H Oser, S E Young, and MJ Coggiola: *"Breath analysis using photoionization mass spectrometry"*

S Prettin, K Roecker and S Sorichter: *"Automated and intra-breath shape analyses of expirograms on a breath-by-breath-basis - potential for diagnostic applications"*

SM Cristescu, BWM Moeskops , AKY Ngai, FJM Harren: *"Sensitive monitoring of the UV-induced lipid peroxidation in human breath"*

T Hemmingsson, D Linnarsson, R Gambert: *"Novel hand-held device for exhaled NO-analysis in research and clinical applications"*

A Grundmann, J Nolte: *"Breath analysis by on-line TDS/GC/MS"*

JI Baumbach, V Ruzsanyi, S Sielemann, A Tappe: *"Rapid 3D - spectra classification, identification and quantification of gaseous metabolites in human breath using ion mobility spectrometers"*

M. Rothe, A. Richter, G. Becher, R. Siemeres: *"Interleukin 6 and Total Protein in Exhaled Breath Condensate of CF-Patients - methodical examinations"*

C Gessner, S Hammerschmidt, L Engelmann, H Kuhn, U Sack, H Wirtz: *"Exhaled breath condensate nitrite as a marker of overdistension of the lung parenchyma"*

KD Skeldon, L McMillan, C Wyse, C Longbottom, G Gibson and MJ Padgett: *"Real-time sub-ppb ethane measurement using a field-robsut laser spectrometer"*

I Gorev: *"Air volatile chelates preconcentration and determination by graphite furnace of atomic absorption spectrometer"*

10:30 *Coffee break*

Current topics in breath gas analysis (two parallel sessions)

	Chairman: Patrik Španěl	*Chairman:* Andrew Lindstrom
11:00	Cathy Wyse: *"Potential application of breath analysis in veterinary medicine"*	Gunther Becher: *"Exhaled Breath Condensate (EBC) alternative or additional diagnostic procedure?"*
11:20	Petra Reinhold: *"Potential for and limitations of exhaled breath analysis in large animal models"*	Hans-Jürgen Smith: *"Capnography in the evaluation of pulmonary emphysema"*
11:40	Marianne Högman: *"Extended NO analysis in a random sample population"*	Caroline Penault: "*Detection of H.pylori infection by breath ammonia following urea ingestion"*
12:00	Tianshu Wang: *"A comparative study of breath ethanol and HDO using SIFT-MS and FA-MS: ethanol metabolism and total body water"*	Johannes Villinger: *"Occupational exposure assessment through analysis of human breath and ambient air using mass spectrometry"*
12:20	Norman Ratcliffe: *"Rapid diagnosis of gastrointestinal conditions from faecal volatiles"*	Khaled Ismail: *"Increase of acetone in urine headspace; a potential indicator of ovulation"*
12:40	Susanne Teschl: *"A model of the human cardiovascular-respiratory control system"*	Bart Knols: *"Breath gas analysis and vector-borne disease diagnosis"*
13:00	Anil Modak and Yasuhisa Kurogi: *"Detection of $^{13}CO_2$ for diagnostic non-invasive breath tests with stable isotopes"*	Claire Turner: *"Analysis of breath using SIFT-MS: a comparison of breath profiles of healthy volunteers and seriously ill ICU patients"*
13:20	*Lunch*	
14:20	*Visit to Rolls-Royce Museum and High Tea there at 15:30*	

Breath gas analysis in real time (at Rolls-Royce museum)

Chairman: Jochen Schubert

16:00	David Smith: *"Applications of SIFT-MS in physiology, clinical diagnostics and therapeutic monitoring"*
16:30	Anton Amann: *"Breath gas analysis as biochemical probe in sleeping individuals"*
17:00	Terence Risby: *"Effects of ventilation on the collection of exhaled breath in humans"*

17:30 **Presentation (at Rolls-Royce museum)**
John Vaughan: *"Practical applications for exhaled breath condensate pH"*

Buffet at Rolls-Royce Museum at 18:15

Saturday, Sept 25th

	Clinical breath gas analysis ***Chairman:*** Heinz Drexel
9:00	Terence Risby: *"In vivo assessments of injury and disease based on breath"*
9:45	Caterina Bucca: *"Exhaled nitric oxide and pulmonary complications after allogeneic stem cell transplantation"*
10:15	Michael Phillips: *"Breath volatiles - making sense of the data: Screening for cancer and other diseases"*
11:00	Maximilian Ledochowski: *"Breath gas analysis in patients with malabsorption syndrome"*
11:45	***Bernhard Lang Award Ceremony for the best poster and best short oral presentation***
12:00	*Lunch*
	Clinical breath gas analysis (continued) *Chairman:* Anton Amann
13:30	Jochen Schubert: *"VOC breath markers in critically ill patients - potential and limitations"*
14:30	Raed A. Dweik: *"Nitric oxide in exhaled breath: a window on lung physiology and pulmonary disease"*
15:20	*Coffee break*
16:00	Nándor Marczin: *"Diagnostic aspects of exhaled nitric oxide and carbon monoxide in cardiothoracic anaesthesia"*
17:00	Giovanni Rolla: *"Exhaled nitric oxide in hepatopulmonary syndrome"*

Important notice. There is a bus transfer to the conference banquet venue: please gather at the designated meeting point at 19:15! The conference banquet starts at 20:00 at the restaurants "Altes Gericht" and "Torggel" in Sulz.

Sunday, Sept 26th

9:35	**Please gather at the designated meeting point at 9:35** Bus transfer from Dornbirn to **Andelsbuch** (Bregenzerwald)
	Lectures at the "Gemeindesaal Andelsbuch"
	Novel approaches to breath gas analysis *Chairman:* Nándor Marczin
10:30	Frank Kühnemann: *"A breath test for plants: photoacoustic trace gas analysis and its application in plant science"*
11:00	Patrik Španěl and David Smith: *Flowing afterglow mass spectrometry, FA-MS, measurements of deuterium abundance in the water vapour in exhaled breath and above aqueous liquids; determination of total body water*
11:30	Jochen Schubert: *"New approaches to the analysis of small hydrocarbons in exhaled breath: Breath test for the monitoring of ischemia-reperfusion injuries "*
12:00	*Final Remarks:* Anton Amann and David Smith
12:15	Invited lunch
13:30	Walk from Andelsbuch to Bersbuch
14:35	Nostalgic train trip from Bersbuch to Bezau
15:30	Farewell and transfer to Dornbirn arriving at 16.15

Abstracts of talks

Methods in breath testing

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A sample of normal human breath contains approximately 200 different VOCs, mostly in picomolar concentrations (10^{-12} mol/l). Progress in breath research has been delayed by the technical difficulties of sample collection and analysis.

Technical problem 1: capturing breath VOCs

a. Resistance to expiration: It is obviously difficult to breathe out against resistance, especially for the elderly and those with respiratory illness. Tubing should be wide in bore (at least 2-3 cm diameter) and contain little or no obstruction to the free flow of breath.

b. Infection control: A breath donor suffering from pulmonary tuberculosis could contaminate the device with infectious organisms, and potentially infect a subsequent breath donor. The device must employ a combination of disposable components and careful design to prevent cross infection.

c. Water condensation: Breath is saturated with water, which promptly condenses within the tubing of the capture system. Breath VOCs may then partition from the gas phase into the aqueous phase. Since water is very abundant and breath VOCs are present in very low concentrations, this partitioning process may deplete the gas phase of VOCs and result in inaccurate low readings in the subsequent assay

d. Chemical contamination: Breath VOCs are present in picomolar concentrations, and the highly sensitive assays required to detect them may be readily contaminated by VOCs from other sources. These may include volatile adhesives and plasticizers in disposable components. The breath collection apparatus must therefore be constructed from components which are least likely to contribute VOCs e.g. stainless steel and inactive plastics e.g. Teflon.

e. Dead space air dilution: Breath is not a homogeneous gas. At rest, an adult expires approximately 500 ml with each breath, of which the first 150 ml is dead space air from the upper airways and nasopharynx, and the subsequent 350 ml is alveolar breath from within the lungs. For analytical purposes, only alveolar breath is of value.

f. Breath container artifact: Sample collection into a plastic bag (e.g. Tedlar or Teflon) or a partially evacuated metal cylinder or sphere entails a risk of artifactual loss of sample, due to adsorption of VOCs to the walls of the container.

Technical problem 2: concentrating the VOCs in breath

a. Cold trapping: Typical a donor blows through a U-tube which is immersed in a cryogenic fluid (e.g. liquid nitrogen or acetone chilled with dry ice). The U-tube may be packed with glass beads which provide a large surface area for the breath VOCs to condense upon. The sample is then heated, and the volatilized concentrated VOCs are then analyzed by conventional laboratory methods e.g. gas chromatography (GC) possibly with mass spectroscopy (MS).

b. Condensate trapping: This method is a variant of cold trapping in which the sample is cooled only moderately. A similar system to cold trapping is employed, but the cooling fluid is usually ice water at 0^0C. VOCs partition into the water which condenses from the breath and this sample of condensed water is then analyzed by conventional laboratory methods e.g. high performance liquid chromatography (HPLC), or GC/MS.

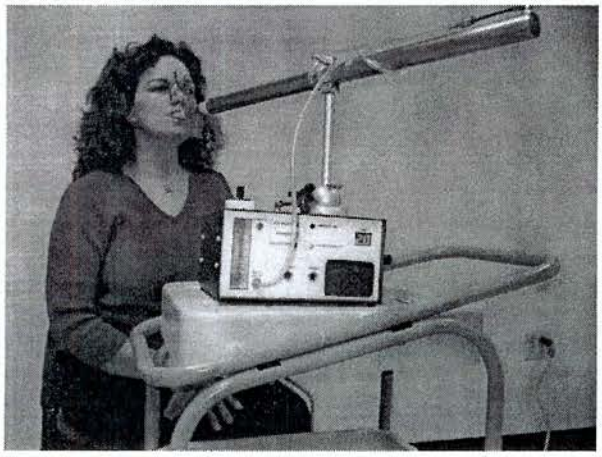
c. Chemical trapping: Breath is bubbled through a solution which chemically interacts with the analyte of interest. These methods are of great antiquity, dating from the 18^{th} and 19^{th} century, and have largely been displaced. However, modern variants have been described for the

detection of carbon disulfide and mercury in breath, as well as for the capture of radiolabeled analytes in load tests for GI diseases such as H. pylori infection.
d. Sorbent trapping: In this method, breath is passed through a bed of a material such as activated carbon or a specialized resin (e.g. Tenax) which captures the VOCs. The process is reversible, so that the captured VOCs may be subsequently eluted by heating or by chemical stripping with a solvent.

Technical problem 3: analysis of concentrated breath VOCs
a. Portable hand-held instruments: The most familiar of these are "breathalyzers" for ethanol which have been employed by police forces for several years. These devices generally employ a fuel cell which oxidizes ethanol in breath to acetaldehyde, and the resulting electrical current (which varies with the concentration of ethanol in breath and blood) is displayed digitally. In recent years, newer devices have become available for analysis of other vocatives in breath such as nitric oxide, sulfur derivatives and carbon monoxide.
b. Conventional laboratory instruments: The instrument of choice in most breath research laboratories is currently GC/MS.

Technical problem 4: compensation for VOCs in background air
As assays of concentrated breath became increasingly sensitive, researchers learned that a sample of normal room air also contains most of the VOCs which are present in the breath. Petane can be detected in room air in concentrations not very different from those in breath. It is now known that VOCs are manufactured and cleared in the body, and the composition of inspired air is different from expired breath.

An improved method for breath collection is shown in this figure. The breath collection apparatus (BCA) compensates for all of the technical problems described above. Samples of breath and air are analyzed by GC/MS, and a subtraction chromatogram (VOCs in breath minus VOCs in room air) is constructed.



The breath collection apparatus (BCA) in use. The patient wears a nose clip and breathes in and out through a disposable mouthpiece into the BCA for 2.0 min. Through the valved mouthpiece, the patient inspires room air and expires into a breath reservoir which is open to room air at its distal end. There is virtually no resistance in the system, and breath samples may be readily collected even from elderly patients and those with respiratory disease. The breath reservoir separates alveolar from dead space breath, and alveolar breath is pumped from the reservoir through a sorbent trap, a stainless steel tube packed with two grades of activated carbon which capture the VOCs in 1.0 l breath. A 1.0 l sample of room is also collected onto a second trap.

PTR-MS: a new tool for the rapid detection of VOCs in air at ultra-trace levels

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The development of new methods capable of rapid, sensitive, and selective analysis of volatile organic compounds (VOCs) in complex matrices such as human breath may have important medical applications including non-invasive clinical diagnosis or therapeutic monitoring. The utility of VOC analysis in human breath has been appreciated for some time (Pauling et al., 1971) but extensive use has been inhibited by the lack of simple and reliable analytical equipment. Proton-transfer-reaction mass spectrometry (PTR-MS) is a new and emerging technique for the real-time monitoring of VOCs at low concentrations in gaseous samples.

The PTR-MS technique was developed at the University of Innsbruck (Hansel et al., 1995). A detailed description of the principles of operation of the commercially-available PTR-MS apparatus (Ionicon GmbH, Innsbruck, Austria) is given elsewhere (Lindinger et al., 1998a,b); therefore only the salient features will be presented here. PTR-MS is a chemical ionization mass spectrometry technique based on proton transfer reactions from H_3O^+ ions to gaseous organic analytes. H_3O^+ primary ions are produced in a hollow cathode ion source and injected into a flow drift tube which is continuously flushed with analyte air. No time-consuming preconcentration procedures that may alter the nature of the sample are needed. On each collision between a H_3O^+ primary ion and an organic molecule the proton H^+ is transferred thus charging the reagent. Primary and product ions are mass analyzed in a quadrupole mass spectrometer and detected by a secondary electron multiplier/pulse counting system. PTR-MS detects VOCs with a higher proton affinity than water. This includes the large majority of polyatomic volatile organic molecules with the exception of small aliphatic and cyclic hydrocarbons (e.g. alkanes<C_8, ethylene, acetylene, cyclohexane, a.o.). The detection limit (S/N=2) is in the 10-to-100 pptV range for a 1 sec signal integration time per mass. An accuracy of 5 % has been obtained using calibrated VOC mixtures, uncalibrated accuracy is on the order of 30 %. A major drawback of the PTR-MS method is limited selectivity due to isobaric interferences.

At the Institute of Ion Physics, PTR-MS has been successfully implemented not only for environmental and food research, but also for breath analysis. Applications include the

detection of acetonitrile and benzene in the breath of smokers (Jordan et al., 1995), the monitoring of volatile sulfur compounds after the ingestion of garlic (Lindinger et al., 1998b), and the detection of isoprene (Taucher et al., 1997) and methanol in human breath (Taucher et al., 1995). Future research will be focused at Ionimed Analytik GmbH, a spin-off company of the University of Innsbruck.

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Selected ion flow tube mass spectrometry, SIFT-MS: reliable, real time quantification of metabolites in air, exhaled breath and liquid headspace.

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A description of selected ion flow tube mass spectrometry, SIFT-MS, will be presented. SIFT-MS is a unique, novel technique that allows on-line, real time quantitative analysis of the trace gases in humid air, single breath exhalations and the headspace above aqueous liquids (such as urine) down to the parts per billion (ppb) level in times of the order of seconds. Direct sampling of exhaled breath into the instrument is a special feature, obviating either the collection of samples into bags or the adsorption of metabolites onto traps, thus providing analyses of the alveolar portions of the exhaled breath. Thus, when used in the clinical environment for non-invasive breath analysis, the results are immediately available to the clinician.

The SIFT-MS technique involves the ionization by selected precursor ions, in a well-defined time, of the trace gases in an air/breath sample that is introduced into a helium-buffered fast flow tube. A schematic that illustrates the major features of the method is given in Figure 1, together with indications of the various

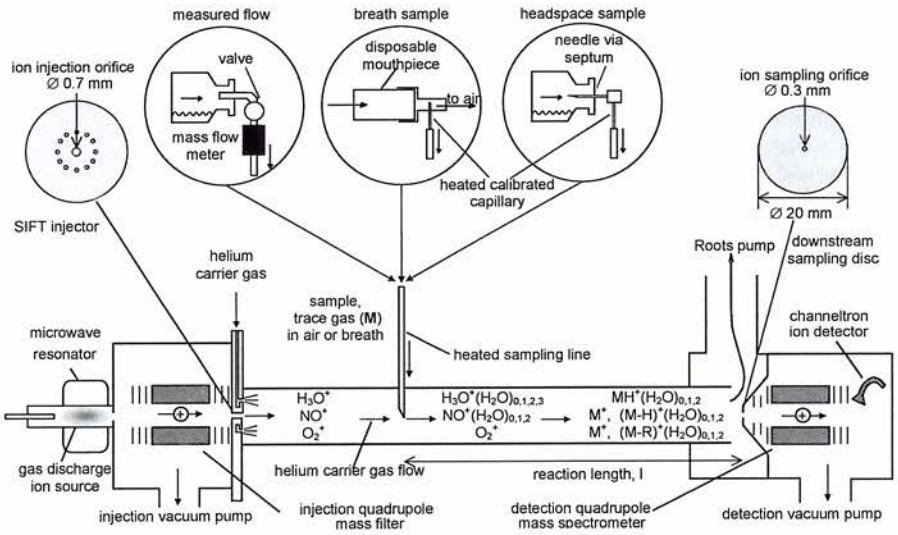

Figure 1 Schematic diagram of a Selected Ion Flow Tube-Mass Spectrometry instrument.

sampling methodologies employed. The available precursor ions are H_3O^+, NO^+ and O_2^+, because these react only slowly with the major air/breath components (N_2, O_2, CO_2, Ar, H_2O) and react very rapidly with most other molecular species. Each of the three precursor ions can be selected on demand allowing all three to sequentially analyse a given air/breath sample. This is one of the great strengths of SIFT-MS. Thus, a wide variety of volatile organic compounds (VOCs) and some inorganic species in a sample can be identified and quantified that cannot be fully analysed using just one precursor ion species. H_3O^+ is a very versatile precursor ion in that it can be used to analyse a wide variety of VOCs, but it cannot, for example, be used to analyse NO and NO_2 for which O_2^+ is well suited. NO^+ is also valuable for the analysis of a wide range of VOCs notably ketones and those compounds with low ionization energies. When isomeric forms of some molecules are present or if doubt exists on the assignment of a neutral trace gas from a product ion, then two or three of the precursor ion species in combination can be used to assist analysis by virtue of the different ion chemistries involved. An essential requirement is an extensive kinetics database of the rate coefficients and product ions of the precursor ion reactions with likely trace gases in samples to be analysed. This database has been built from numerous selected ion flow tube, SIFT, studies of ion molecule reactions at thermal energies.

SIFT-MS instruments can be operated in two distinct modes. The full scan mode (FSM) involves the scanning of the analytical (downstream) quadrupole mass spectrometer over a predetermined m/z range in order to determine the count rates of all the reactant ions, i.e. variously $H_3O^+(H_2O)_{1,2,3}$, $NO^+(H_2O)_{1,2}$, O_2^+, and all the product ions of their reactions with the trace gases, M, in the air/breath sample, e.g. MH^+, $(M-H)^+$, NO^+M, M^+. Thus, the FSM is largely used to identify the trace gases from the nature of the product ions. The multi-ion mode (MIM) involves the rapid switching of the analytical mass spectrometer between the appropriate precursor ions and selected product ions, determining the count rates of each ion species and from the rate coefficients of identifies reactions providing more accurate analyses of samples. Thus, real time analyses of time-varying samples such as exhaled breath can be obtained. Examples of FSM and MIM data are shown in Figure 2.

The physics involved in SIFT-MS will be outlined, in particular the flow dynamics an understanding of which allows the reaction time to be defined, as will the ion chemistry in order to illustrate the value of each precursor ion species in the analysis of particular compounds. It will be shown how the presence of water vapour, always present at relatively high concentrations in exhaled breath and urine headspace samples, is accounted for in a refined SIFT-MS analytical procedure. Significantly, this procedure also allows the humidity of the sample to be obtained, which acts as an internal validation of the analysis when the sample humidity is know, as it is for exhaled breath. Experiments will be described by which the quantitative accuracy of SIFT-MS analyses has been validated down to the parts per billion level of partial pressure. Throughout the talk, sample data will be presented to illustrate the essential features of the SIFT-MS technique and how it can be used to great effect in the clinical environment.

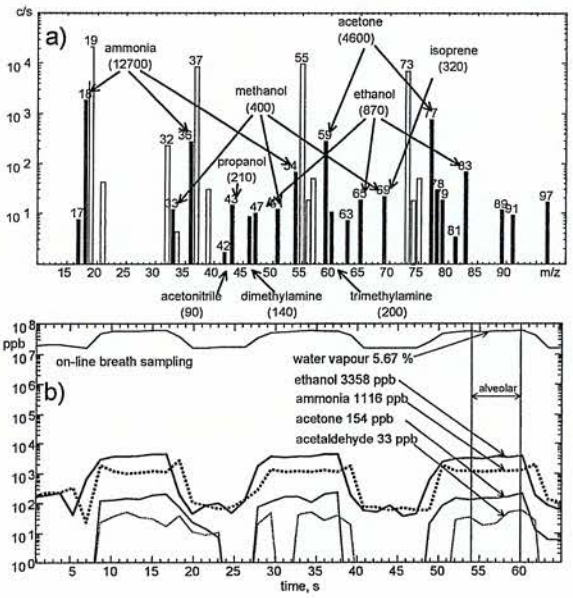

Figure 2 a) Full Scan Mode (FSM) SIFT-MS spectrum of breath of a patient with total renal failure. **b)** Concentrations of compounds in breath calculated from multiple ion monitoring (MIM) data.

Further reading

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Detection of Metabolites in Human Breath: Ion Mobility Spectrometers as Diagnostic Tools for Lung Diseases

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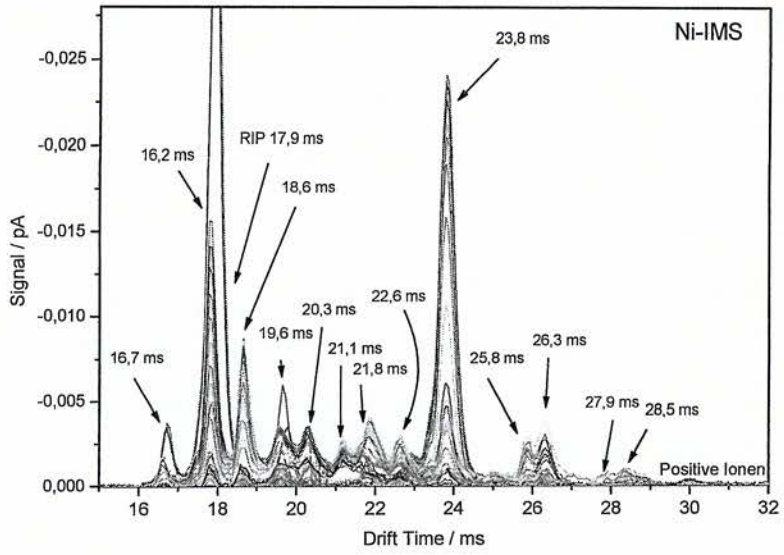
It is well known, that an increased concentration of one or more components in human breath is correlated directly to different diseases. Thus, acetone is a marker for diabetes, nitric acid for asthma, ammonia for hepatitis and alkanes and the ratio of different benzene derivates for lung cancer.

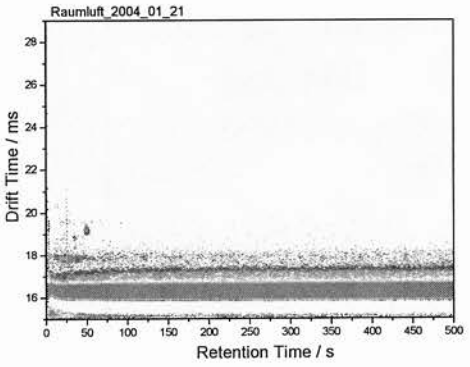
In the present study, an ion mobility spectrometer (IMS) coupled to a multi-capillary-column (MCC) is used for the identification and quantification of volatile organic compounds occurring in human breath down to the ng/L- and pg/L-range. In co-operation with a German lung hospital the pattern of analytes and concentration dependencies were investigated with respect to different typical lung diseases like Bronchitis, Pseudomonas, Tracheitis, Tonsillitis, Purulent Sputum and Lung Cancer. The influence of administered drugs and medicine was considered also.

The aim of the studies is to introduce the investigation of metabolites in human breath as method for early recognition of selected diseases on the bases of ion mobility spectrometry data.

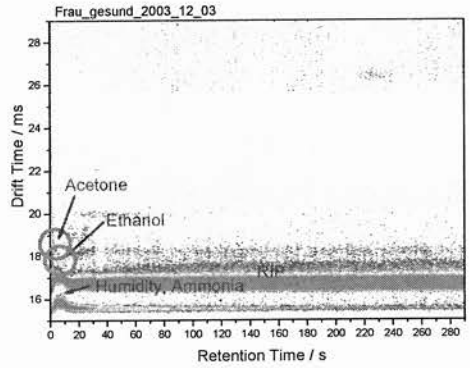
New challenges occur not only with respect to the instrumentation, like miniaturisation, but also for rapid classification of analytes. Fast identification and quantification of unknown analytes requires also new calibration procedures as well as new concepts in the field of operation of the spectrometer. Peak-height-diagrams and more dimensional data presentations will be completed by fast classification procedures like cluster analysis and multidimensional calibration methods. The advantages of the method with respect to the operation of the instrument, spectra classification, identification and quantification of gaseous metabolites including examples of relevant and successful applications will be discussed in detail.



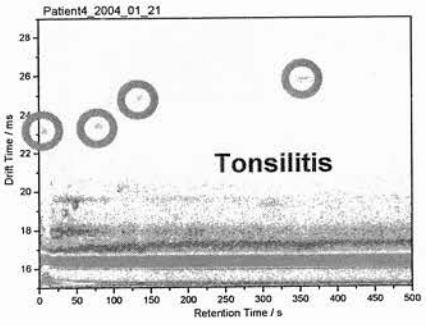
Metabolites of standard lung cancer cells in air - ion mobility spectra of positives ions



Indoor air

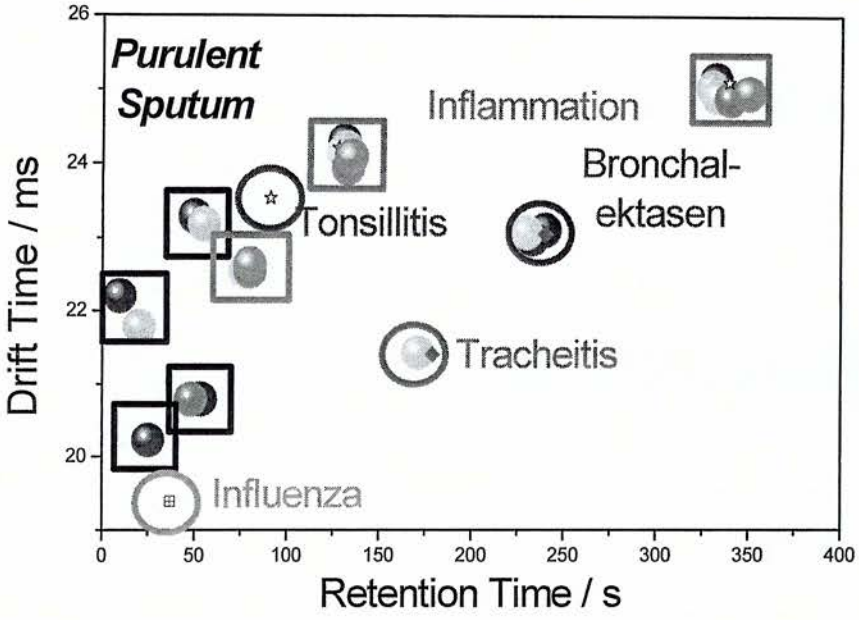


"Healty" person

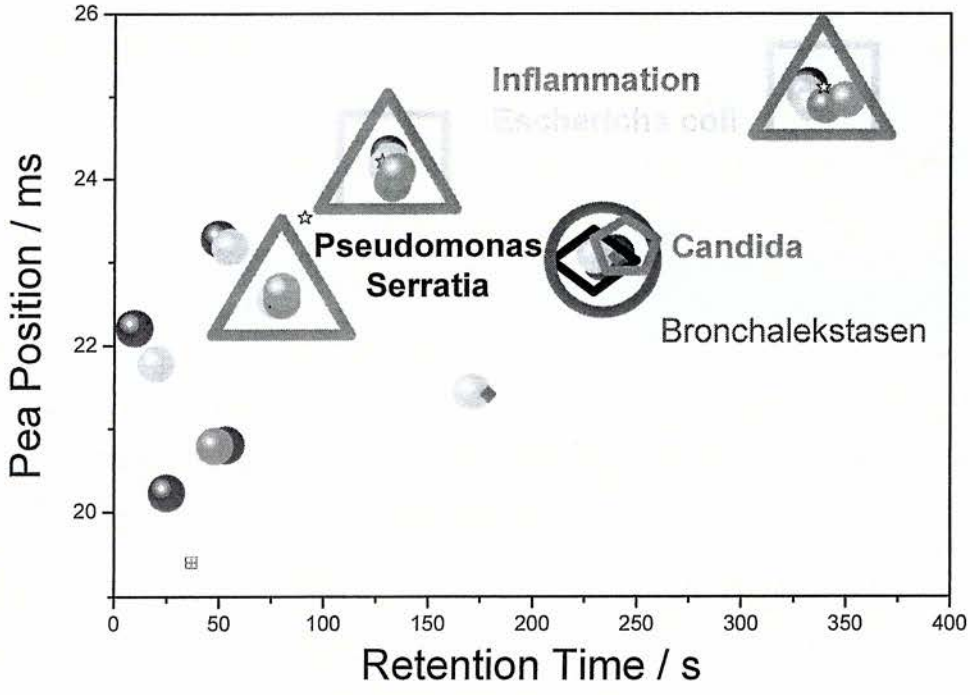


Tonsillitis

IMS-Chromatograms of indoor air, human breath of a "healty" person and a patient with Tonsillitis



Peaks occuring at infectious patients exclusively



Correlation between metabolites arising from human breath and different bacteria

Laser spectroscopic online monitoring of exhaled trace gases

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The analysis of hydrocarbons, such as ethane, in exhaled human breath is generally performed via gas chromatography (GC). Due to the insufficient sensitivity of the GC technique, the breath sample must undergo a trap-and-purge process before analysis. This is time-consuming and does not enable the time-resolved analysis of single exhalations.

In this talk we report on our advances in extremely sensitive and specific online analysis of exhaled trace gases by means of infrared laser absorption spectroscopy. This optical method shows great advantages for sensitive and specific trace gas analysis since most relevant trace compounds exhibit a characteristic fingerprint spectrum in the mid-infrared. Our measurements are mainly carried out by infrared cavity leak-out spectroscopy (CALOS) which proved to be an unique and universal tool for rapid and precise medical breath testing [1]. CALOS is an ultra-sensitive laser absorption spectroscopy method which allows for rapid analysis of various VOCs as wells as NO, CO, OCS, etc. on the parts-per-trillion level. Currently we are exploring this technique for the quantitative online detection of ethane which is considered as the most important volatile marker of free-radical induced lipid peroxidation and cell damage in the human body.

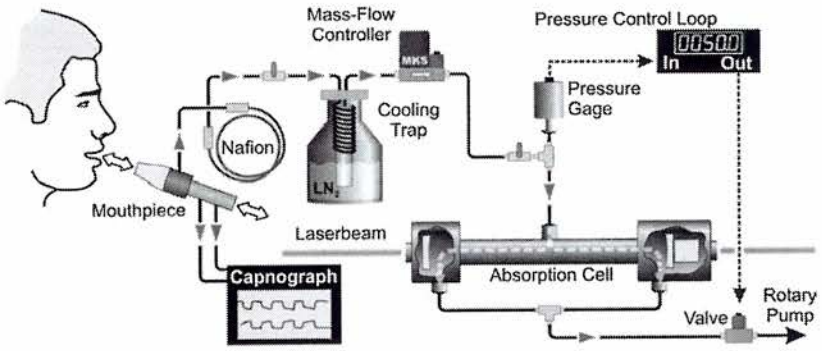

Figure 1: Schematic of the gas setup. [1]

Fig. 1 shows a schematic of the breath sampling system. A part of the exhaled breath sample is directed into the absorption cell after cleaning the gas flow in a Nafion tube and a cooling trap. The O_2 and CO_2 concentrations as well as the breath flow are monitored via a capnograph. Fig. 2 is a diagram of the optical setup of the CALOS analyser. We use wavelength tunable lasers (CO laser, difference frequency laser, or OPO) which operate in the mid-infrared wavelength region (3 – 8 µm). The laser light is injected into the absorption cell which is a two-mirror optical cavity and the ethane concentration is determined via measurement of the decay rate of the radiation confined in the cavity after turning off the

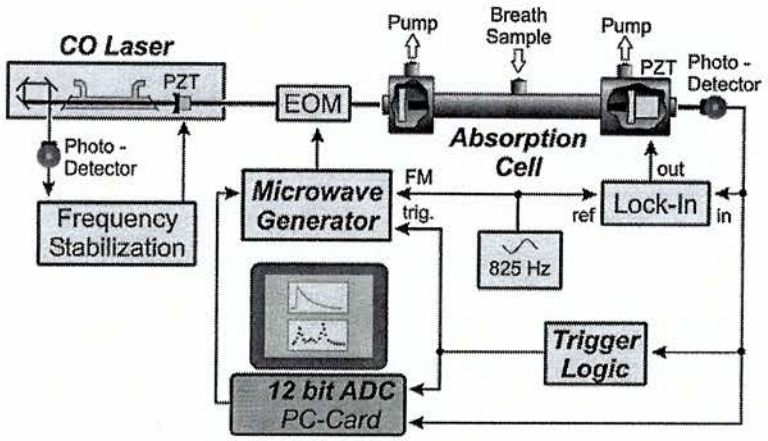

Figure 2: Schematic of the CALOS analyzer. The CO laser may be replaced by a difference frequency laser or an OPO. EOM: electro-optic modulator, PZT: piezoceramic transducer, ADC: analog-to-digital converter, FM: frequency modulation. [1]

laser. We achieve an ethane detection limit of 400 ppt or 0.4 nl/l and a time resolution of better than 0.8 s which is sufficient for the time-resolved online measurements of single exhalations.

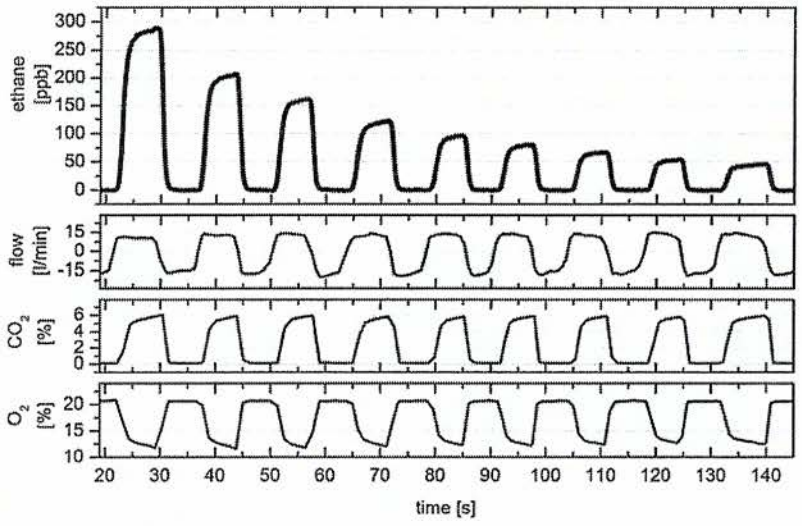

Figure 3: Representative example of an online recording of ethane, CO_2, and O_2. The subjects performed predefined breathing maneuvers. [1]

Fig. 3 shows a representative example of an online recording of ethane, flow, CO_2, and O_2. The subject performed a predefined breathing maneuver. Each single expiration is analysed separately which enables, e.g., investigations of the sloping alveolar plateau .

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Exhaled human breath analysis by means of quantum cascade laser based gas sensors

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Abstract: Mid infrared laser absorption spectroscopy (MIRLAS) allows selective measurements of low part per billion (ppb) concentrations of trace gases, which is of great importance for clinical breath analysis. Application of a pulsed quantum cascade lasers (QCL) allows design of sensitive, compact, user-friendly, and liquid-nitrogen free spectroscopic trace gas sensors for rapid, *in situ*, detection of target molecules in expired breath. Simultaneous exhaled carbonyl sulfide (OCS) and carbon dioxide (CO_2) concentration measurements in human breath have been demonstrated. A noise equivalent sensitivity (1σ) of 1.2 ppb was achieved by measuring a well isolated OCS P(11) absorption line in the ν_3 band at 2057.6 cm^{-1} using an astigmatic Herriott cell of 36 m optical path length and a 0.4 sec. acquisition time.

SUMMARY:

The low-ppb concentration range of many volatile molecular species in human breath presents a challenge for clinical breath analysis applications, which require rapid, *in situ* detection of trace gases. High molecular selectivity and sensitivity can be achieved using MIRLAS based gas sensors. This technique does not require the sample preparation or pre-concentration techniques associated with gas chromatography, which is the most frequently used method for trace detection in various applications. MIRLAS in combination with pulsed QCLs, which are efficient, robust and reliable mid-infrared sources operating within thermoelectric cooling ranges with minimal component requirements[1], permit the design of selective, sensitive, compact and liquid-nitrogen free trace-gas sensors suitable for field applications. In this work the development and evaluation of an advanced design of a QCL-based sensor for simultaneous concentration measurement of OCS and CO_2 will be reported. Detection and analysis of OCS is of importance in a number of applications that include atmospheric chemistry and more recently in medical diagnostics. Elevated OCS concentrations in exhaled breath have been reported in lung transplants recipients suffering from acute rejection[2] as well as patients with liver disease[3]. In contrast to the currently used invasive diagnostic methods (e.g. bronchoscopic lung biopsies to assess lung transplant acute rejection), rapid analysis of expired breath using MIRLAS is a desirable non-invasive alternative. The thermoelectrically cooled QC laser used in this work operates in a pulsed mode at 4.85 μm and can access a number of strong absorption lines (line intensities > 1×10^{-18} cm^{-1} /molecule · cm^{-2}) in the P branch of the OCS fundamental rotational-vibrational spectrum. The availability of a neighboring CO_2 line within the tuning range of the QCL allows ventilation monitoring simultaneously with an OCS measurement and can be used to normalize the resulting OCS concentrations, and to standardize measurement conditions. To minimize pulse-to-pulse fluctuations of the laser radiation, the reference and sample beam signals are measured by means of a single photovoltaic HgCdTe detector followed by time resolved data acquisition system. Feedback electronics is applied for stabilization of laser power fluctuations resulting from the wavelength tuning process. The absorption spectrum is digitized and recorded using a fast data acquisition card. A minimum detection limit (1σ), of 1.2 ppb (for 100 averaged 400 point frequency scans acquired within ~ 0.4 sec) of the OCS trace gas sensor platform using a compact 36 m multipass absorption cell was demonstrated[4]. A digital signal processing (DSP) platform for biogenic trace gas sensors is in a development stage to provide fast data acquisition (>1 MHz), standalone data processing functions, increased reliability, and enhanced sensor portability. Such a sensor will be able to improve sensor data acquisition, while simultaneously satisfying space and safety constraints related to a medical setting.

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Isotope Selective Detection of Nitric Oxide in Human Exhalation

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We detect nitric oxide in human exhalation using one photon laser induced fluorescence (LIF). An ultraviolet beam excites the NO molecule electronically via the (A ← X) gamma band around 226 nm. The beam is generated by a dye laser, that is pumped by a XeCl Excimer laser at 308 nm. The dye laser is operated by Coumarine 47 to generate radiation between 440 nm and 484 nm. This laser beam is frequency doubled by a second harmonic generation to obtain the required wavelength. We detect the following red shifted fluorescence between 245 nm and 248 nm with a photomultiplier. Interference from scattered laser light is removed by a band pass filter. The experimental setup is schematically shown in figure 1. The advantages of this method is the extremely high sensitivity and the excellent selectivity. Furthermore, the technique is remarkably rapid. Theoretically, it is possible to detect 0.01 ppt (parts per trillion) of nitric oxide in real time. In our experiments we successfully monitored NO concentrations from the ppm range down to sub-ppt range. Therefore we are able to measure the $^{14}N^{16}O$ and $^{15}N^{16}O$ content without concentrating the sample by cryo-trapping. This allows us to perform tracer experiments: a test person takes in several hundred milligrams of ^{15}N endowed arginine. Then we measure the time dependent $^{15}N^{16}O$ concentration in his exhaled breath. Typical measurements from selected test persons and calibration data of the setup will be presented. For the future we plan to realize online measurements of $^{14}N^{16}O$ and $^{15}N^{16}O$. With our experimental setup it is also possible to investigate the NO emissions from human skin, animals and plants.

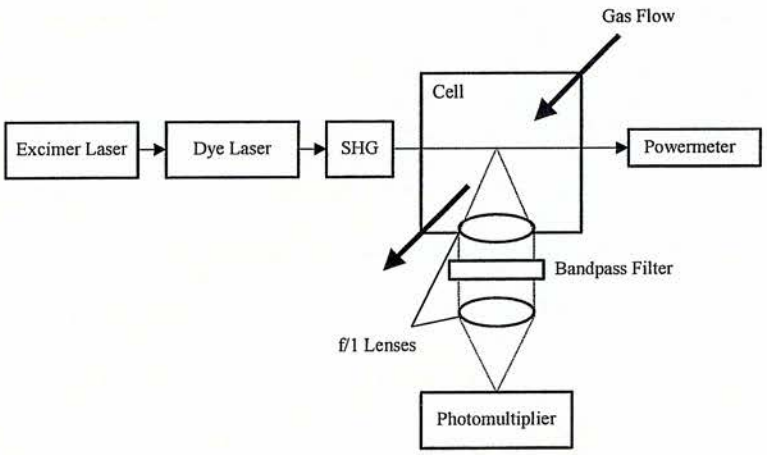

figure 1: experimental setup

Validation of a new portable telemetric-ergospirometric device during exercise

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Introduction:
Portable ergospirometric devices allow to measure gas exchange and ventilatory variables during field tests under sport specific conditions and therefore to obtain important information about metabolism and energy costs of exercise. Further, comparisons with data from laboratory measurements were possible, if data are reliable. The aim of the present study was to validate a new portable spirometric-telemetric device (Oxycon® Mobile) over a wide range of exercise intensities against a well known accurate stationary apparatus (Oxycon® Pro).

Methods:
Fifteen endurance-trained subjects (VO_{2peak}: 58.8 ± 5.2 $ml*min^{-1}*kg^{-1}$) performed an incremental exercise test to volitional exhaustion. Gas exchange and ventilatory variables were measured by the two ergospirometric devices simultaneously. Fur this purpose, a special face mask was designed which allowed respiratory gas sampling with both devices at exactly the same time.

Results:
Compared to the Oxycon® Mobile (OM), the Oxycon® Pro (OP) showed significant higher values for oxygen uptake (VO_2) at 200 and 250 W during the incremental exercise test (Fig. 1). For carbon dioxide release (VCO_2) no significant differences were found although the OM seemed to measure systematically higher values. With regard to the respiratory exchange ratio (RER) the OM showed significantly higher values at all exercise intensities (Fig. 2).

Fig. 1

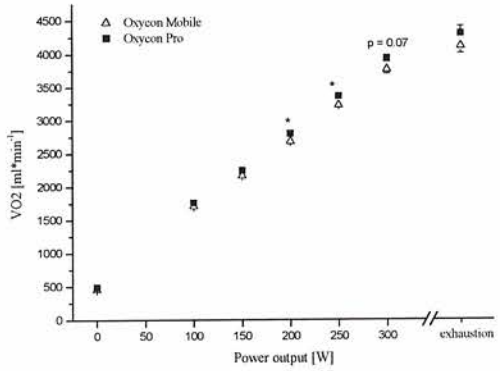
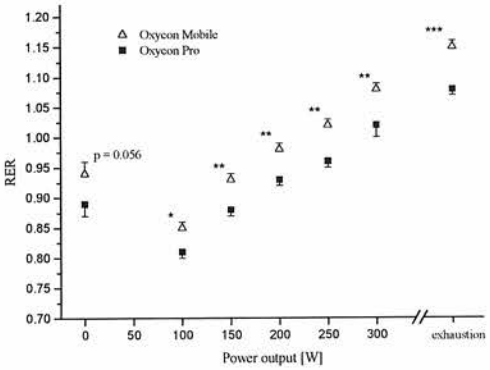


Fig. 2



Conclusion:
Compared to the OP as an accurate reference system, the OM significantly underestimates VO_2 at high workloads above 200 W and overestimates RER at all workloads tested during exercise. This has to be considered if the data measured by the OM are used for metabolic calculations like energy costs of exercise.

PTR time-of-flight mass spectrometry: a good prospect for diagnostic breath analysis?

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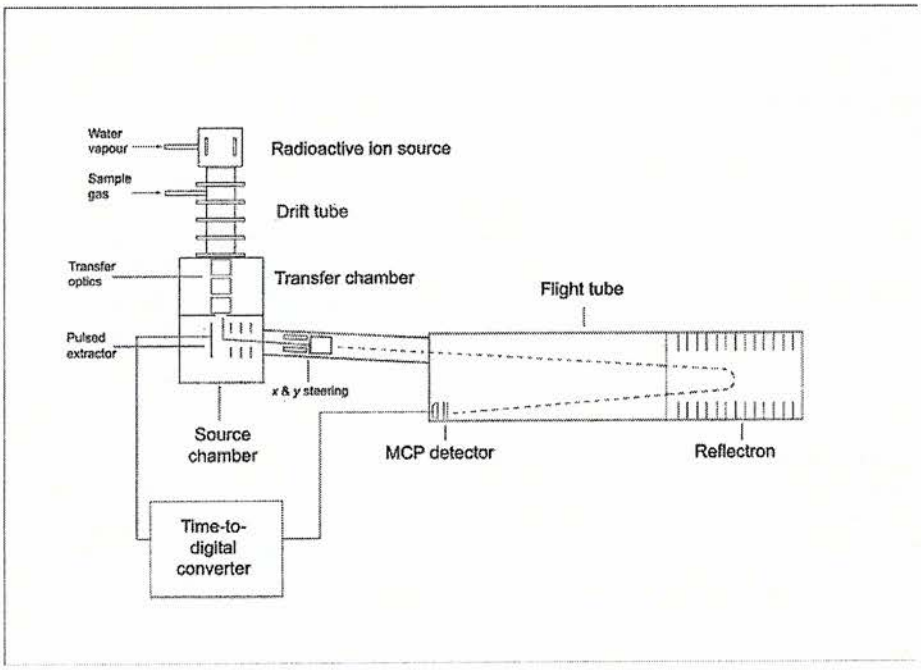
Proton transfer reaction (PTR) mass spectrometry utilising a time-of-flight mass spectrometer TOFMS) will be described.[1] A homemade ion source and drift tube have been coupled with a purpose-built reflectron TOFMS. Both radioactive and electrical discharge sources have been successfully employed to generate H_3O^+ ions. These ions are then mixed with an air sample at the top of a drift tube, whereupon proton transfer to trace organic molecules in the air commences. VOC concentrations are determined from the ratio of the ion count rates of RH^+ versus H_3O^+. A diagrammatic representation of the instrument is shown below.



Multichannel detection in TOFMS allows capture of data in all mass channels simultaneously, with the added bonus of good resolution and a virtually unlimited mass range. Data of this type extracted from breath samples may be suitable for 'fingerprinting' certain medical conditions. Individual VOCs at ppbv concentration levels can be detected in less than one minute of data accumulation using the current instrument, but improvements under development will offer much greater sensitivity. A status report on these developments will be included in the presentation.

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Out of breath – analysis of volatile organic breath markers -

Pitfalls, tips and tricks

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Despite a number of very promising results revealing interesting diagnostic properties of different markers, analysis of volatile organic compounds in breath has not yet been introduced into clinical practice. The main obstacles are technical problems such as sampling, preconcentration and analysis as well as basic methodological issues such as normalisation and expression of data.

Far more than 500 different compounds have been described in human breath. Due to the very low concentration of some of this markers (nmol/l- pmol/l) accuracy and reliability of results will depend on the analytical method and the skill of the analytical chemist in charge.

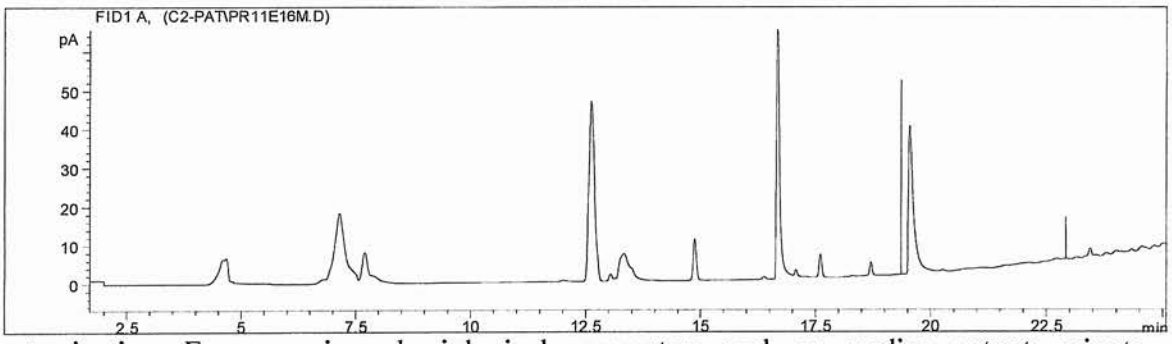
The first challenge is the collection of gaseous samples. Depending on the mode of sampling (mixed expiratory or alveolar) substance concentrations may vary by a factor of two or three. Considerable loss of (polar) substances may occur if samples are stored in bags or canisters. Preconcentration represents the next problem. Adsorbent properties, flow dependent break through volumes and effect of humidity have to be taken into account. Unfortunately some compounds have tendency not to be captured at all, others will never come off the adsorbent again. For this reason, violent conditions of desorption have sometimes been used. High energies applied in this way, however, may generate ("new") substances from the adsorbent materials.

GC analysis is the method commonly used for analysis of breath VOC´s. Normal GC – columns did not always realize sufficient separation of all compounds and thus confusion was caused through coelution of substances (e.g. isoprene - pentane). Due to the large number of compounds and due to effects of humidity GC-retention time is not sufficient for substance identification. Any unknown substance has to be unequivocally characterized by mass spectrometry. Only if the separation is perfect and substances are identification is obvious (e.g. by MS) easier and cheaper detector systems like FID and NPD can be used.

Using the available analytical armamentarium in an intelligent way most of these problems can be solved. CO_2 controlled alveolar sampling ensures maximum sensitivity and minimum contamination from dead space air. Preconcentration on multibed sorbents avoids loss of

analytes or memory effects even if very volatile and polar substances are present at the same time. Tender desorption and reversal of flow direction enables complete recovery of all substances. In the same way, adsorbent materials can be conditioned for reuse using slightly higher energies. High performance GC-columns (e.g. PLOT) and MS confirmed identification will provide excellent separation and unambiguous recognition. Ongoing technical developments such as micro extraction techniques, selective membranes, multi-dimensional GC and new detector system like ion mobility, laser spectroscopy and selected ion flow tube may further expand the potential of breath analysis.

Beyond these technical aspects some basic methodological difficulties are left we have to surmount. In order to be comparable data have to be normalized and expressed in a physiologically reasonable way. If for any reason alveolar sampling was not done or not possible data should be normalized by expired or end tidal CO_2 to account for dead space



contamination. Encompassing physiological parameters such as cardiac output, minute ventilation, body weight or surface area may help to transfer data from one study to the other. Meticulous attention has to be paid to the effects of inspired substance concentration since these may seriously affect results of breath analysis.

Figure1: Chromatogram of exhaled air from a mechanically ventilated pig; Peak at 7,6:Ethane, 16,8: Isoprene, 17,6: Pentane. (multibed sorbent 3, TDSA, cryofocusation, Porabond Q, FID)

Bearing that in mind it will be possible to produce breath taking results instead of getting out of breath!

Exhaled NO: how and why we know it is important.

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The publication of the presence of NO in exhaled breath in 1991 has lead to an intensive development in exhaled breath analysis. The main goal has been to explore the utility of exhaled NO, especially in the field of asthma research. Also other markers have been studied with the aim of improving diagnosis of pulmonary disease. Only modest attention has been paid to the criteria for identification of exhaled markers. Furthermore, when a method for an exhaled marker has been identified it should be realised that the instruments or methods used are still experimental. Only when methods or instruments fully validated for clinical use are at hand can the necessary clinical trials be performed that will determine if a marker gives useful clinical information in a regular clinical setting. Today, this has only been achieved for exhaled NO as an indicator of inflammation in asthma. Thus NO should not be regarded as a diagnostic criterion, but rather as an aid or complement in monitoring and diagnosis, respectively.

It has to be realised that rather rigid criteria have to be applied for a marker of airway disease, discriminating it from other body gases or from exhaled gases of environmental origin. Furthermore, the marker method has to offer advantages compared with presently used methods. Some optimal and necessary marker criteria or characteristics may be suggested for an exhaled marker of airway disease:

Optimal marker characteristics:
- Relation to (but not identity with) other more difficult markers/methods
- Clinical value (correlation to disease/treatment efficacy)
- Easy on patient ="noninvasive"
- Rapid method
- Immediate result

Necessary marker characteristics:
- Defined molecule & formation
- Identity in breath by 2 indep methods
- Airway specific
- "Airway peak conc" (dead space>alveolar conc)
- Not excreted from distant organs
- ("gut/liver"problem)
- Not excreted from environmental contamination

Correspondingly the necessary characteristics may be changed for a marker of alveolar or otherwise localised disease. Reference will be given to why we may state that exhaled NO is really NO and why we can say that it is mainly of airway origin. Recent data show that in normal individuals more than 70% of exhaled NO is blocked by submaximal NO synthase inhibitor, underlining the usefulness of exhaled NO measurements.

Diagnostic use of a nasal NO test

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The nasal region represents the largest source of NO in the human airways and this NO can be measured non-invasively. Nasal NO is altered in certain disorders of the upper respiraory tract and therefore there is hope that this test can be useful in diagnosis and therapy monitoring of airway disease.

In primary ciliary dyskinesia nasal NO is extremely low and there is today enough convincing data to suggest that this test is introduced in clinical practice as an aid in the diagnosis of this disorder. Other possible indications for a nasal NO test include cystic fibrosis, allergic rhinitis and sinusitis. However, more research is needed to explore any clinical value of a nasal NO test in these disorders.

The diagnostic value of nasal NO will be discussed along with recent advances in the methodology of nasal NO measurements.

Exhaled Breath Analysis for Human Exposure Research

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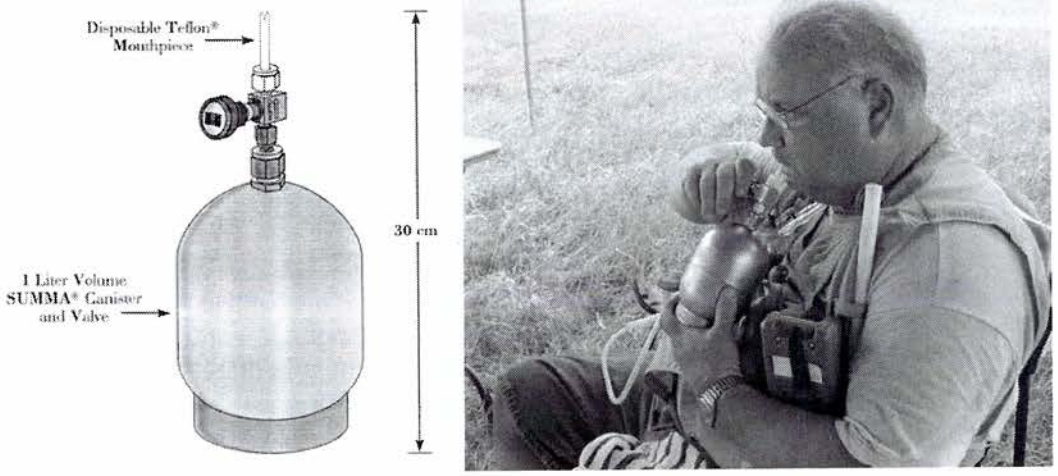
Exhaled breath collection and analysis has historically been used in environmental research studies to characterize exposures to volatile organic compounds. The use of this approach is based on the fact that many compounds present in blood are reflected in the breath, and that unlike blood, breath collection is noninvasive and generally well tolerated by study subjects. This research has focused on demonstrating exposures to volatile compounds, establishing conclusive links between specific activities and corresponding body burdens of xenobiotics, characterizing uptake and elimination kinetics, and illuminating relevant pathways of exposure. In the past few years exhaled breath analysis has evolved to the point where it is now being used to help characterize biological responses associated with exposures to environmental pollutants.

This presentation will explore historical and newly emerging methods for the collection and analysis of exhaled breath for use in environmental exposure assessment studies. We will discuss their applicability and limitations with respect to environmental research. Particular emphasis will be placed on new methods and their utility for examining exhaled biomarkers of environmental pollution.

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

A. B.



A) Single Breath Canister (SBC) for collection of exhaled breath samples, B) the collection of an exhaled breath sample during a field experiment

The unique value of breath biomarkers for estimating pharmacokinetic rate constants and body burden from random/intermittent dose

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Biomarkers are defined as chemicals measured in biological media; these include native compounds (dose chemicals), metabolic products of dose chemicals, and chemically unrelated compounds of response. Biomarker measurements are used in three ways: 1) evaluating the time course and distribution of a chemical in the body, 2) estimating previous exposure or dose, and 3) assessing disease state. Blood and urine measurements are the primary methods employed. Of late, it has been recognized that collecting exhaled breath is an attractive alternative to blood and urine sampling because it is less invasive, and is not restricted by sample volume or time frame. The ensuing discussions are restricted to the first two biomarker applications, time course and dose estimation.

For the purposes of deducing time course and distribution of a chemical in the body, we require a series of time dependent data points, a conceptual model, and knowledge of the exposure route and dose profile. Certainly, urine sampling is a poor choice for time frames shorter than a few hours as sample volume and collection frequency are limited. Blood can be sampled quite effectively even with minutes scale resolution, but is invasive (sometimes uncomfortable), restricted in total volume, and requires trained medical personnel. Breath volume and frequency of sampling are essentially unlimited; the subject never runs out sample and one can collect even adjacent breaths with existing technology. Furthermore, there is a fundamental difference between blood and breath measurement in that blood levels report only the status quo at the time of collection, whereas exhaled breath is reflective of current blood levels as well as an elimination mechanism allowing calculation of real time removal rates. Therefore, from a mathematical standpoint, the time courses of blood and breath are interpreted differently and give complementary information.

The estimation of previous exposure or dose via a spot measurement is fraught with uncertainty. The underlying concept is "biological damping" which refers to the smoothing of response or time lag of appearance of the biomarker. Both the time profile of the dose and the lag time and frequency of sampling affect the variability of the assessment. For example, a stable inhalation exposure to a medical anesthetic like isofluorane or an outdoor environmental

pollutant like carbon tetrachloride can achieve a very stable level in the circulating blood or exhaled breath over time, and thus, any subsequent measurement will reflect the dose or level accurately. Administration of an oral dose of an analgesic such as acetaminophen or ingestion of a disinfection byproduct in water like chloroform will have a smooth but transient response resulting from various biological and metabolic mechanisms; the time window in which the measurement is made thus becomes more important. Finally, there are situations in which the uptake and distribution of the compound is essentially instantaneous as in the case of a bronchial dilator like terbutaline for an asthma attack, or an environmental dermal exposure to a gasoline spill wherein benzene (for instance) appears immediately in the blood and breath. There are also more complex scenarios wherein multiple, random, or intermittent levels of dose are experienced that build up a varying baseline in the body. In general, the native compound is more likely to follow the exposure profile; the biomarker is generally delayed or "damped" because it is slowed by metabolic activity. As such, the exhaled breath biomarker has major advantages over measuring the native compound directly because the exact sample timing does not affect the outcome as much; the time weighted dose of the native compound is more likely represented by its biomarker.

The advantages of breath biomarkers can be illustrated using the example of methyl tertiary butyl ether (MTBE) exposure and environmental classical pharmacokinetics (PK). MTBE exposure is ubiquitous in the U.S. and has been linked to toxicity and cancer in animal studies. It was originally introduced to replace lead in gasoline as an octane enhancer and comprises 1 to 5% of automotive fuel. More recently, it has become the most common "oxygenate" added to fuel to reduce vehicular carbon monoxide emissions in cold climates comprising. MTBE is a volatile, flammable and colorless liquid that readily evaporates and dissolves easily in water; it has a very distinctive odor and (unpleasant) taste at very low levels. Fuel spills, atmospheric deposition, leaking underground storage tanks, and fuel transmission pipe leaks have introduced MTBE into drinking water supplies. Evaporative emissions from auto refueling adds vapor phase MTBE to the ambient air. MTBE has one major metabolite, tertiary butyl alcohol (TBA), which is easily measured in human breath, blood and urine. In a recent EPA study of controlled human exposures, native MTBE and the metabolite TBA were measured during uptake and elimination in blood and breath. Time dependent data were collected for inhalation, ingestion and dermal exposure routes. Using these empirical data, we developed a simple PK model to investigate the disposition of these chemicals and to estimate previous mean exposure.

Specifically, the model takes the form presented in Figure 1. Based on "building block" data from single exposure experiments (Prah, et al, 2004) we recently estimated specific rate constants for transfer among compartments. Based on this model and the calculated parameters, we could predict the levels of metabolite in response to any complex exposure scenario. We consider only one peripheral compartment for simplicity; it serves as an empirical representation (composite) of all of the moderately and poorly perfused tissues. One of the first observations from this model is that once MTBE is absorbed into the central compartment (blood), the rate constants for exchange with the peripheral compartment, elimination via breath, and conversion to metabolite, are all independent of the initial exposure route. One exception is the phenomenon of "first pass metabolism" experienced by ingested MTBE; this possibility is indicated by the dashed path and the rate constant K_{oT} in the diagram. Furthermore, we make the assumption that MTBE is either eliminated via breath or metabolized to TBA; we do not consider any other loss mechanisms.

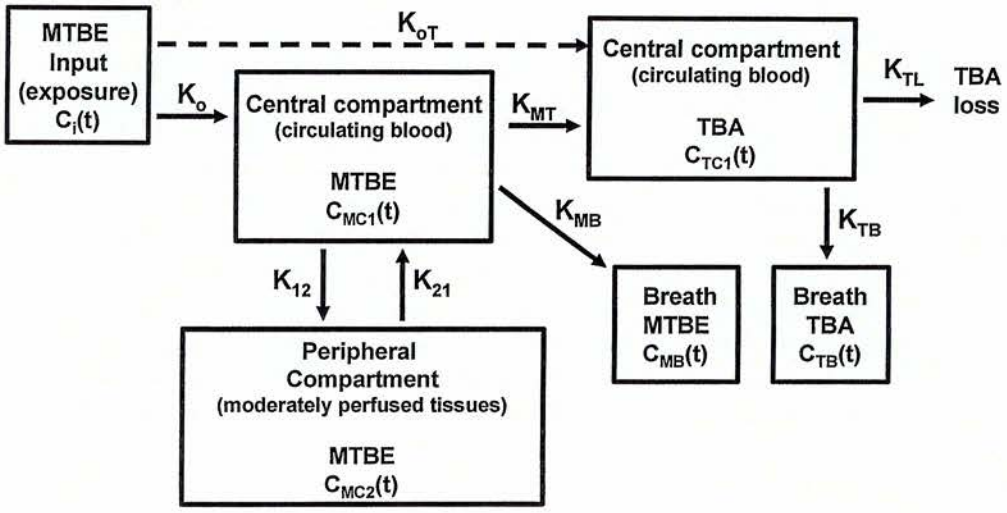

Figure 1. Simple classical pharmacokinetic diagram of disposition of absorbed MTBE among compartments, metabolite, and excretion; the "K's" are first order rate constants except for K_o which is zeroth order for inhalation and dermal exposures. K_{oT} represents direct conversion of ingested MTBE to TBA.

The model and the calculated time constants demonstrate the unique value of using breath data. From the figure, we see that without breath measurements, we would have no access to mass balance for the exposure compound or the metabolite concentrations. Furthermore, we find based on the measured breath levels, that we can infer the blood levels directly without invasive blood measurements. Once the various time constants are established, we can generalize to any input function $C_i(t)$ and determine the time progression of MTBE and TBA in the blood. Finally, from analysis of the respective time constants for the production and elimination of metabolite as compared to exposure compound, we find that the breath measurements of TBA have a damped response to large changes in exposure, and so represent recent exposures regardless of the exact sampling time.

Reference: Prah, J., D. Ashley, B. Blount, M. Case, T. Leavens, J. Pleil, and F. Cardinali, 2004. "Dermal, Oral and Inhalation Pharmacokinetics of Methyl Tertiary Butyl Ether (MTBE) in Human Volunteers", *Toxicological Sciences,* 77: 195-205.

This is an extended abstract of a proposed oral presentation. This work was funded by the National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency and has been approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The background dilemma - Influence of inhaled substances on the results of breath analysis

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Background: Inspired substance concentrations represent a fundamental and crucial problem in breath analysis. All compounds being generated within the body are also found in the inspired air in varying concentrations since there are other natural sources than the patient. Hence, this study was intended to investigate systematically the impact of inspired substance concentrations on results of breath analysis.

Methods: Volatile breath markers were determined in inspired and expired air and in arterial and mixed venous blood of 46 critically ill, mechanically ventilated patients. Volatile organic compounds were preconcentrated via solid phase micro extraction and analyzed by GC/MS.

Results: Mean inspired concentrations were 25% of expired concentrations for pentane, 7.5% for acetone, 0.7% for isoprene and 0.4% for isoflurane. Only when inspired concentrations were low substances disappearance rates from the blood and exhalation rates in breath correlated well (Fig. 1). Exhaled pentane concentrations solely depended on inspired concentrations, exhaled acetone and isoprene concentrations depended on venous concentrations, on inspired concentrations and on cardiac output. Isoflurane concentrations in breath only depended on venous concentrations. Patients with sepsis had significantly higher n-pentane and lower acetone concentrations (Fig. 2) in mixed venous blood than patients without sepsis. N-pentane and acetone concentrations in exhaled air showed no differences between the patient groups. The correlation between concentration profiles in blood and breath did not improve when expired concentrations were corrected for inspired concentrations. Isoprene concentrations in blood and breath did not depend on patients' inflammatory status.

Conclusion: Substance profiles in breath may considerably deviate from profiles in blood depending on the relative amount of inspired concentrations. Due to the non-linearity of these effects in mechanically ventilated patients a simple correction for inspired substance concentration is not possible. Hence, substances having inspired concentrations > 5% of

expired concentrations show now correlation to venous concentrations and should not be used as predictive breath markers for any process in the body of these patients.

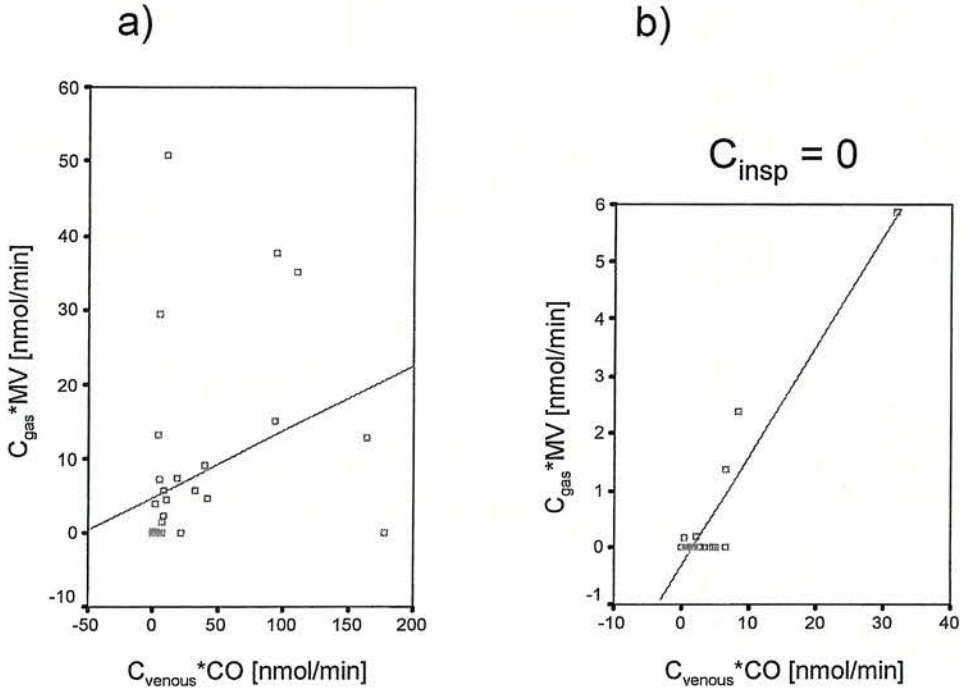

Fig. 1: Pentane production in breath as a function of substance delivery in the blood. Solid line indicates linear regression fit.
a) all measurements, $R^2 = 0.11$, (N = 42) b) measurements with Cinsp = 0, $R^2 = 0.90$ (N = 22)
MV = minute ventilation [l/min], CO cardiac output [l/min]

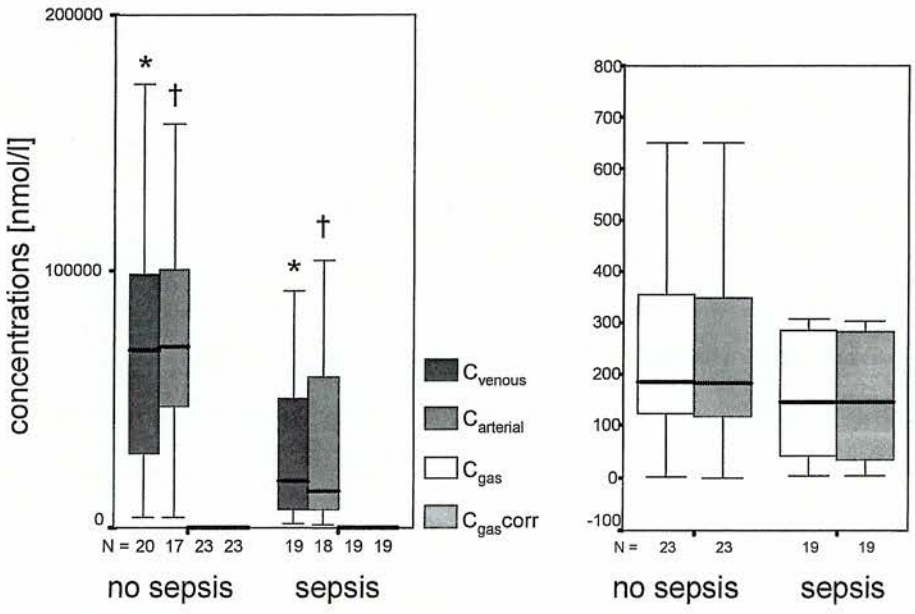

Fig 2: Boxplot of acetone concentrations in mixed venous (C_{venous}), arterial ($C_{arterial}$) blood and in exhaled air C_{gas}. C_{gas}korr = C_{gas} - C_{insp}. *, † indicate statistically significant differences of medians.

Lung Cancer Breath Biomarkers

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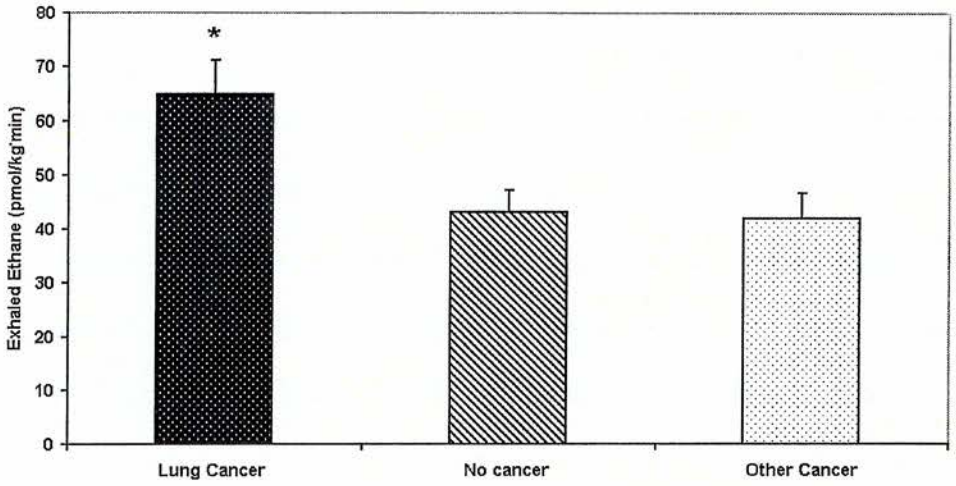
Introduction: Breath biomarkers have been suggested to be indicative of lung cancer. A study was conducted to investigate whether oxidative stress status is increased in lung cancer patients compared with non-lung cancer patients, and to identify novel biomarkers that are unique to malignant lung disease. Ethane, a stable end-product of lipid peroxidation and a sensitive biomarker of oxidative stress status, was analyzed. Nitric oxide, a marker of pulmonary inflammation, was also measured. Patients with lung cancer, chronic obstructive pulmonary disease and non-lung cancer patients were all sampled in the same clinic.

Methods: Organic breath molecules were collected onto thermal desorption tubes for one minute at a flow rate of 80 ml/min, and quantitated by gas chromatography (GC-FID). Molecules were identified by electron impact mass spectrometry (GC-EIMS). For breath collection, a standardized technique was used in which the subject's tidal volume, respiratory rate, end-tidal carbon dioxide, and steady-state carbon dioxide were monitored in real-time. Ambient concentrations of some molecules can exceed endogenously produced concentrations; therefore, room air was collected for each breath sample, and was used to correct the breath concentrations for exogenous molecules. Adjustments for room air were performed using carbon dioxide to estimate anatomical dead space. Subsequently, breath molecule concentrations were normalized to carbon dioxide output or body mass. Nitric oxide was analyzed in real-time using a standardized protocol in which a single exhaled breath was collected at a flow rate of 50 ml/second.

Results: Ethane was significantly increased in the breath of lung cancer patients (n=39) compared to patients without lung cancer (n=29), or with other forms of cancer (n=18).



Acetonitrile was identified in 51% (21/41) of lung cancer cases, but was present in only 8.5% (4/47) of non-lung cancer subjects. However, the presence of acetonitrile can be mainly attributed to smoking since 86% (24/28) of the acetonitrile-positive subjects were current smokers. After adjusting for concurrent smoking, ethane remained significantly associated with lung cancer, but nitric oxide did not. A unique marker was found to be significantly associated with chronic obstructive pulmonary disease (OR=5.7, 95% CI 2.0-16, $p<0.002$). This compound has a mass spectrum similar to dimethyl ether.

Conclusions: Breath analysis of lung cancer patients may reflect an increased oxidative stress status. However, associations between disease pathogenesis and other biomarkers, such as nitric oxide and acetonitrile, may be artifacts related to smoking. Dimethyl ether may hold promise as a biomarker of COPD risk, but further study is required to make a conclusive identification of this compound.

Can inhaled CO be a therapeutic modality in human diseases

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The paradigm that nitric oxide (NO) gas acts as a physiological regulator of blood vessel tone represented a milestone in modern biological research. The discovery that nitric oxide (NO), possesses powerful vasoactive properties identical to those of endothelial-derived relaxing factor spawned a vast body of research investigating the physiological actions of small gas molecules. NO, which arises endogenously through the action of nitric oxide synthase (NOS) enzymes, is a highly reactive gas, that plays significant roles in the regulation of vascular and immune function. Carbon monoxide (CO), a similar yet much more chemically stable gas, occurs in nature as a product of the oxidation or combustion of organic materials. CO also arises in cells and tissues as a byproduct of heme oxygenase (HO) activity, which degrades heme to biliverdin-IXα. Like NO, CO acts as a vasorelaxant, and may regulate other vascular functions such as platelet aggregation and smooth muscle proliferation. CO has also been implicated as a neurotransmitter in the central nervous system. HO–1, the inducible form of HO, confers cytoprotection against oxidative stress *in vitro* and *in vivo*. Recent studies show that CO, when applied at low concentration, exerts potent cytoprotective effects mimicking those of HO-1 induction, effects which include downregulation of inflammation and suppression of apoptosis. Many of the effects of CO depend on the activation of

guanylate cyclase, which generates guanosine 3':5' cyclic monophosphate, and the modulation of mitogen activated protein kinase (MAPK) signaling pathways. We will review the pleiotropic cytoprotective effects of CO in various models of organ injury.

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POTENTIAL APPLICATIONS OF EXHALED BREATH ANALYSIS IN VETERINARY MEDICINE

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Background: The non-invasive nature of the breath test makes this a particularly attractive investigative method for application in veterinary medicine and research. Research at Glasgow University Veterinary School is focused on development of breath tests for assessment of (i) gastrointestinal function, (ii) respiratory inflammation (iii) oxidative stress in animals.

Methods: Breath samples were collected in these studies from horses and dogs using a face mask attached to a non re-breathing valve and a breath collection bag. Methods for collection of exhaled breath have been optimised to induce minimal stress, even in fractious animals. Depending on the specific gas under analysis, samples are either analysed directly (carbon monoxide), stored in Tedlar bags (ethane) or glass tubes ($^{13}CO_2$). Carbon monoxide was analysed using an electrochemical sensor, ethane was analysed by gas chromatography or laser spectroscopy, and $^{13}CO_2$ was analysed by isotope-ratio mass spectrometry. Exhaled breath condensate (ECO) was collected using a face mask connected to a cooled condensing system, and ECO was analysed for H_2O_2 concentration using a spectrophotometric method.

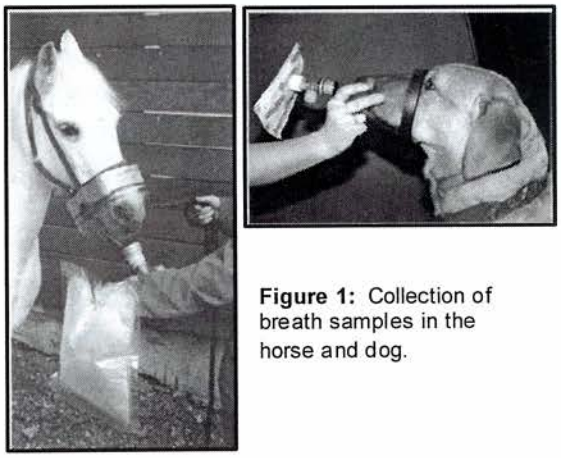

Figure 1: Collection of breath samples in the horse and dog.

Results and Discussion: The ^{13}C-octanoic acid breath test was shown to be a simple and non-invasive method for assessment of gastric emptying in horses and in dogs, and this method was sufficiently sensitive to detect changes in gastric emptying induced by alterations in meal energy composition. The ethane and carbon monoxide breath tests were shown to reflect the degree of inflammation in horses with chronic respiratory disease (recurrent airway obstruction). ECO H_2O_2 was found to be highly variable in healthy animals, and not significantly increased in horses with respiratory inflammation. The ethane breath test was shown to be a useful method for monitoring exercise-induced oxidative stress in race horses and racing Greyhounds.

Conclusions: Breath analysis is a potentially useful investigative method for application in veterinary medicine. Research is ongoing to validate breath tests that can be applied in veterinary clinical practice.

Exhaled Breath Condensate (EBC) alternative or additional diagnostic procedure ?

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Chronic inflammatory airway diseases like COPD, asthma, and cystic fibrosis have an eminent importance in health care. Furthermore the in-time detection of acute respiratory failure needs sensitive and not straining methods.
The qualified care needs more non-invasive and everywhere available diagnostic tools. Traditional lung function tests (PFT) do not give enough information about the disease in early stages. In opposition the anti inflammatory treatments become more and more the main therapeutic basis (controllers) besides the anti obstructive agents (reliever).

Exhaled breath condensate is an new method for non invasive collection of a specimen from the deeper airways. The actual development includes the comparison between traditional airway specimens like induced sputum and bronchial lavage.
EBC makes high demands on collection of the condensate, storage and analysis.
The standardization of the collection of breath condensate needs special effort on breathing pattern, measurement of breathing, standardized cooling of the exhaled air and storage of the sample.
Only for H_2O_2 an on-line possibility for measurement is available. The newly introduced biosensor measuring system for H_2O_2 – ECoCheck™ - is more sensitive than spectrophotometry or fluorometric methods. The biosensor enables the immediate measurement of fresh samples. H_2O_2 gives non specific but sensitive information about airway inflammation. Values below 500 nmol/l seems to be normal, values above 1000 nmol/l are a hint for severe inflammation. A sensitive differentiation between healthy, borderline inflammation and severe exacerbation seems to be significant.
The measurement of pH demands special pH-electrodes because of low conductivity of the sample and the relationship pH-determining agents remains indefinable.
The measurement of other markers is possible by EIA – e.g. LTB_4, $LTCDE_4$, 8-iso-Prosthane. The validation of these markers was possible in comparison to LC-MS.
As a special task for standardization remains the short half-life of markers even in refrigerator.

Breath condensate is even useful in ambulant medicine. Markers in EBC gives in summary more information about airway inflammation than NO, informations about differentiated processes are especially expected.

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Potential for and limitations of exhaled breath analysis in large animal models

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Measurements of volatile substances in exhaled breath have a long history, as exhaled ammonia was measured approximately a hundred years ago. In modern human medicine, exhaled nitric oxide (NO), but also carbon monoxide and other gases, including breath alkanes (e.g. ethane, pentane) are measured in exhaled breath and viewed as markers of airway inflammation. Measurements of NO in the exhaled breath have been reported in elephants, horses, cattle, pigs, sheep, dogs and cats as well as in anaesthetised and ventilated laboratory animals (mice, rats, guinea pigs, rabbits). Following the current knowledge about exhaled NO analysis, however, a number of limitations arise in applying exhaled breath analysis to spontaneously breathing animals (for example: nasal breathing is obligatory in some species, but nasal and paranasal NO should be eliminated from NO measurements in exhaled breath; larger animals also have a higher deadspace to tidal volume ratio- somewhere between 0.5 and 0.75).

As an alternative to measurement of volatile markers in breath, the measurement of inflammatory markers in the **exhaled breath condensate** (EBC) is a rapidly expanding field. The analysis of EBC is based on the hypothesis that water and aerosols (which are present in the exhaled breath) contain a range of compounds which reflect the concentrations within the extracellular epithelial lining fluid in the peripheral broncho-alveolar system. To date, EBC has been collected in horses, calves, pigs, dogs and cats using a variety of different collection methods. As in humans, the volume of EBC collected per unit time depends mainly on individual minute ventilation. Therefore, in small species, a longer period of collection is required to obtain the same volume of EBC as in a larger species.

Different **non-volatile** substances or molecules have been examined in EBC with respect to airway inflammation and respiratory diseases in animal studies. Leukotriene B_4 (LTB_4) has been measured in the EBC of calves, dogs and horses. In the presence of either bacterial or viral infection, EBC LTB_4 was increased. Hydrogen peroxide (H_2O_2) has been measured in the EBC of horses, cats and dogs and has been found to be elevated in inflammatory airway diseases. Under conditions of acute bacterial infection and/or pneumonia, ammonia increased in the EBC (but not in the peripheral blood) indicating a local production of ammonia in the lung. The pH-value in EBC may also reflect airway acidification in inflammatory conditions.

The use of EBC analysis in animals has direct application in animal studies and veterinary medicine, but may also contribute to the understanding of the pathophysiology of human pulmonary disease and dysfunction. Animal models offer the opportunity to undertake challenges that may not be appropriate in human subjects. Direct correlation of tissue histopathology at post mortem with *in vivo* measurements of markers or mediators of lung disease or dysfunction in cohorts of animals under controlled conditions is also possible.

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Capnography in the evaluation of pulmonary emphysema

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Introduction and aims of the study: Nowadays there is an increasing interest in lung function testing methods which objectify and differentiate the respiratory tract by examination of spontaneous breathing subjects. Effort independent assessment of lung function is especially interesting in paediatric and geriatric applications but also in occupational and clinical medicine. New technological solutions and extensive pre-processing allow the development of simple and fast response CO2-analysers, providing advanced intra-breath alveolar gas analysis suitable for emphysema diagnostics and compatible with discriminant analytical methods and distribution analysis of pulmonary ventilation respectively.
In the course of a study in occupational medicine, 91 welders where examined regarding minimal positive results of emphysema caused by Al-dust exposition. Beside capnography, conventional methods such as oscillometry, bodyplethysmography, spirometry, aerosol morphometry and HRCT were employed.

Material and methods: A decisive part at the repeated stimulation of emphysema diagnostics using expiratory CO_2-concentration-volume courses, e.g. capnograms, had the suggestions of Smidt and Worth [1] to use slope b in the Vm/Vin-relation (Vm = a + b?Vin) as diagnostic relevant and specific indicator of emphysema. Vm is the volume-increment between 25 and 50% of the CO_2-endtidal concentration (CO_2et) and therefore located in phase II of the capnogram.
However, Kars [2] found a significant flow-dependence of diagnostic thresholds and at the same time a considerable variability of slope b and intercept a in the Vm/Vin-ratio, when she critically analysed this concept. Her suggestion to replace Vm/Vin by VD/Vin initiated a complete evaluation of all parameters derived from the capno-volumetric method.
As it is nearly impossible to ask emphysematic patients to exhale at constant flow, capno-volumetry was measured "patient-friendly", first at spontaneous breathing, followed with variation of inspiratory deepness of breath, according to the standards of Smidt and Worth.

Results: Beside the determination of various parameter/Vin-regression equations, the assessment of the capno-volumetric examination was completed by time trend graphs which combine the primary signals of volume and CO_2-concentration with secondary parameters derived from the single capnograms like Vtin, CO_2et, Vm2550 or dC3/dV. The evaluation of different functional dead spaces included definitions of Bohr, Fowler, Wolff, Langlay and the threshold dead space.
The entire group of welders was conventionally classified by their residual volume (RV), the effective airway diameter (EAD30) measured with aerosol morphometry and 3 statistical methods in the evaluation of HRCT's. Discriminant analysis was used to compare all methods applied, in their ability to detect latent emphysema. Capno-volumetric dead space determination generated the highest values for both centroid distance/confidence radius of 95% (= 1.6) and eigenvalues (= 1.9) compared with all other testing methods. Traditional classification based on lung volume measurement proved to be superior compared to the classification based on aerosol morphometry or HRCT.

Discussion: Intra breath analysis of expiratory CO_2-concentration courses versus volume instead of time can provide intra-individual coefficients of variation (CV%) which are insignificantly higher compared to effort-dependent spirometry.
Even variation of inspiratory volume was managed by most of the subjects allowing highly specific clinical reports of distribution in ventilation, which is especially helpful in emphysema diagnostics. Beside the recommended Vm mixing volume, especially functional dead spaces are recommended for regression analysis.

Assessment of emphysema on the basis of capnography is complementary to HRCT.

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Extended NO analysis in a random sample population

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Aims

The extended NO analysis has been validated in a previous article (Respir Med 2002; 96:24). The aim of this study was to apply this analysis to a population.

Material and method

Exhaled NO was measured in a sub-sample of a random sample of an adult population in Uppsala, Sweden, as a part of the European Community Respiratory Health Survey (ECRHS II). The focus was on the following groups: healthy non-smoking controls (n=105), allergic rhinitis non-smoking subjects (n=36), allergic asthma +/- allergic rhinitis non-smoking subjects (n=38), healthy smokers (n=20).
We determined the following parameters using the iteration analysis: Caw_{NO} (mean airway tissue concentration of NO), $Calv_{NO}$ (mean alveolar tissue concentration of NO), Daw_{NO} (airway transfer factor for NO) and $FE_{NO0.05}$ (fractional exhaled concentration of NO at a flow rate of 50 mL/s).

Results

Allergic rhinitis and allergic asthma subjects had higher $FE_{NO0.05}$ values than healthy controls. The higher levels of exhaled NO were probably due to the increased Daw_{NO}. The smokers showed significantly decreased $FE_{NO0.05}$ and Caw_{NO}.

	Healthy controls	*Allergic rhinitis*	*Allergic asthma*	*Healthy smokers*
n	105	36	38	20
$FE_{NO0.05}$ (ppb)	18.5 (16.9-20.2)	28.2 (21.9-36.4) **	28.3 (22.7-35.2) **	11.5 (9.26-14.2) **
Caw_{NO} (ppb)	113 (103-125)	133 (111-158)	136 (112-165)	72.3 (57.8-90.4) *
$Calv_{NO}$ (ppb)	1.25 (1.05-1.49)	1.52 (1.22-1.90)	1.51 (1.18-1.94)	0.96 (0.63-1.48)
Daw_{NO} (mL/s)	8.15 (7.36-9.03)	11.3 (9.52-13.4) *	11.0 (9.60-12.5) *	7.64 (5.84-10.0)

The values are expressed as geometric mean (95% CI).
* $p<0.01$, ** $p<0.001$

Conclusion

The iteration analysis was successfully applied to all the studied subjects. The results are in line with earlier publications using this method. Thus the groups with allergic diseases had increased $FE_{NO0.05}$ and Daw_{NO}, while the group of smokers had a decreased $FE_{NO0.05}$ and Caw_{NO}.

DETECTION OF *H. PYLORI* INFECTION BY BREATH AMMONIA FOLLOWING UREA INGESTION

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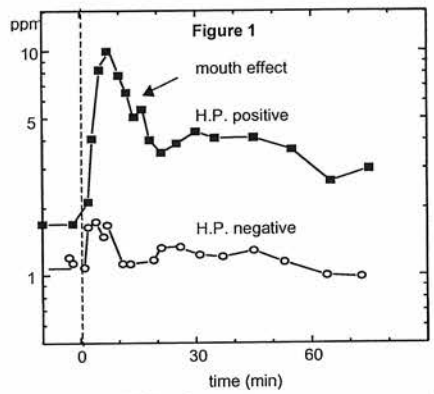
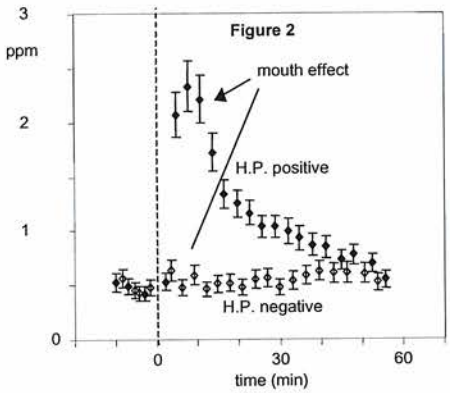
Helicobacter pylori - first isolated from the human stomach mucus layer in 1983 - is identified as the major cause of peptic ulcers and plays a role in the development of gastric cancer. While mostly asymptomatic, it is a very common infection found in up to 50% of the world's population.

Most current non–invasive tests for *H. pylori* depend on the conversion of labelled (^{13}C or ^{14}C) urea, $CO(NH_2)_2$ to labelled carbon dioxide ($^{13}CO_2$ or $^{14}CO_2$) and ammonia (NH_3) by the bacterium's urease enzyme system, with the labelled CO_2 detected in exhaled breath. The use of such labels is associated with significant disadvantages such as cost and the need for complex and time-consuming strategy for the detection of CO_2.

Despite suggestions going back a number of years, the alternative possibility, of using ammonia as the test parameter, has received little attention. However, in 1996, D. Smith and P. Španěl developed selected ion flow tube mass spectrometry, SIFT-MS, with which breath metabolites, including ammonia, can be measured in real time obviating water vapour removal [1,2] with exhalations directly sampled into the instrument with immediate results. They then reported measurement of breath ammonia in volunteers following the ingestion of normal (unlabeled) urea [1]. This pilot investigation indicated an increase in breath ammonia for an *H.pylori* positive volunteer but no real change for an *H-pylori* negative volunteer (Figure 1). The possibility that breath ammonia can be used to detect *H.pylori* is now being explored by The Medical House PLC, using a sensitive chemiresistive sensor [3] able to detect ppm levels of ammonia derived from ingestion of normal urea.

The SIFT-MS study mentioned above has recently been extended in association with TMH with 2 different volunteers (*H.pylori* positive, **A**, negative, **B**). Measurements of breath ammonia were made every 3 min. for an initial 10 min. and then for 40 min. following the ingestion of normal urea (2g in ~ 50ml of water), with both subjects rapidly rinsing their mouth afterwards (Figure 2). The initial ammonia levels for both subjects





were the same, within the measurement uncertainties. Following urea ingestion (time 0), there was a pronounced "mouth effect" in the case of **A**, but only a slight effect for **B**. The sharp rise in the breath ammonia presumably represents the immediate hydrolysis of urea by the urease-associated bacteria of mouth flora. However, it is possible that the large "mouth effect" for **A** is associated with the presence of *H-pylori*. The mean breath ammonia level for **B** over the 60 min. duration does not change significantly, although a slow increase with time may be discernible. In the case of **A**, the decay of breath ammonia level following the "mouth peak" is initially rapid and then relatively slow, indicative of the combination of ammonia production, by *H-pylori*, and its loss by normal metabolic processes. There is a discernible plateau at 20-30 min. after ingestion, with ammonia concentration 2.5 times greater than the pre-dose level; after 60 min. it returns close to the pre-dose level.

The peak breath ammonia concentration following urea ingestion is likely to depend on the degree of *H-pylori* infection, the gastric emptying rate and the rate of loss of ammonia from the blood stream by metabolic processes. These latest results confirm the possible use of breath ammonia to indicate the presence of *H. pylori* infection and provide the basis for a potentially rapid, point–of–care screening / diagnostic breath test for pre and post-eradication. TMH is currently developing a simple, convenient breath test prototype-device based on their ammonia sensor. Clinical trials with proposed protocols are about to start.

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A comparative study of breath ethanol and HDO using SIFT-MS and FA-MS: ethanol metabolism and total body water.

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Selected ion flow tube mass spectrometry, SIFT-MS, is now established as a reliable and accurate method for the analysis of trace gas metabolites in breath and liquid headspace. We have exploited this analytical method for various physiological studies, including real time measurements of the common breath metabolites and the dispersal kinetics and metabolism of ethanol *in vivo*. We have also developed flowing afterglow mass spectrometry, FA-MS, to determine the deuterium content of water vapour in real time. The principal objective of FA-MS is the determination of TBW by breath analysis following ingestion of a known amount of D_2O and the transport of water across the peritoneal membrane of patients being treated by continuous ambulatory peritoneal dialysis.

In the present study, on-line measurements were made of both breath ethanol using SIFT-MS and of the deuterium content of breath water vapour using FA-MS following measured doses of ethanol and D_2O. In this way, the dispersal kinetics and decay of these compounds *in vivo* can be compared and contrasted for particular individuals and their TBW determined. Thus, following the ingestion of a small amount of ethanol by two volunteers in proportion to their body weight (A, weight 60 kg, 5 ml ethanol; B, weight 89 kg, 7.5 ml ethanol), their breath ethanol, acetaldehyde and acetone were measured in single breath exhalations in real time every two to three minutes over a period of about two hours. Typical exhalations as traced by these three compounds are shown in Figure 1.

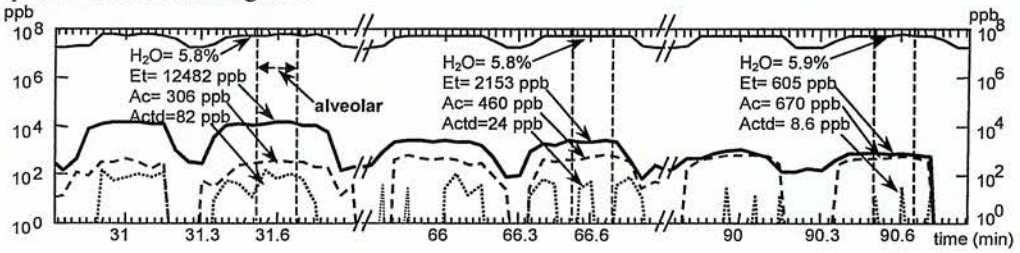

Figure 1. Breath profiles of ethanol, acetone and acetaldehyde at various times after ingestion of ethanol for subject A. Et: ethanol. Ac: acetone. Actd: acetaldehyde. Breath concentrations are shown in ppb.

A similar approach was taken to the FA-MS measurements in that measured quantities of D_2O (A, 16.5 ml; B, 25.5 ml) diluted in about 200 ml of tap water were ingested and the deuterium content of the breath water vapour in single breath exhalations was determined, again over a period of some two hours. Sample data are shown in Figure 2. The experiment was repeated 7 days later for both subjects (see Figure 4).

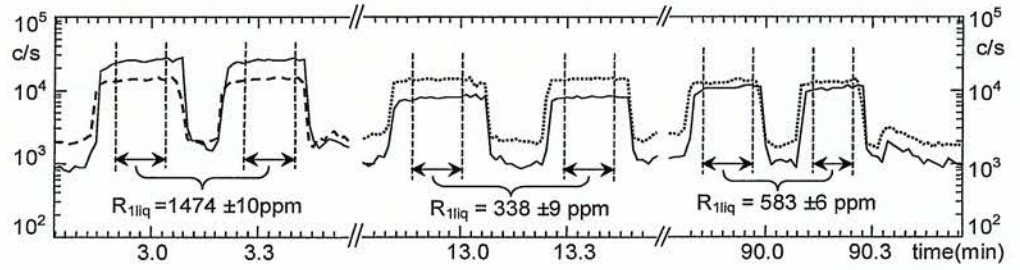

Figure 2. Breath profiles of ions (in counts per second, c/s) at m/z values of 74 and 75 from which the D/H abundance ratios in body water, R_{liq} in ppm, were obtained after ingestion of D_2O by subject A.

The time variations of the breath ethanol (and acetaldehyde and acetone) for both subjects A and B are shown in Figure 3 and the corresponding time curves for the breath deuterium are shown in Figure 4. It can be seen that these plot have common distinctive features in the first 20 minutes following ingestion. The initial ethanol and deuterium content of the exhaled breath are relatively high due to mouth contamination of the ethanol and D_2O (which rapidly converts to HDO in the presence of excess H_2O). This contamination rapidly

falls and minima in the breath ethanol are reached for both A and B whence they increase towards maximum values as the ethanol passes from the stomach, into the small intestine and thence into the blood stream/breath. Note that the increase in ethanol after the minimum values is consistently faster for subject B than for subject A. This may indicate a faster gastric emptying rate for subject B.

The maxima in the ethanol breath/blood levels are due to both dispersal of ethanol throughout the body water and its metabolism. Both these processes occur together as the maxima approach and follow the breath ethanol maxima, but at later times metabolism dominates the loss of ethanol from the blood/breath and a near exponential decay results (see Figure 3). It is interesting that the time constants for decay are very similar for subjects A and B. Note that the ethanol values reach their physiological values after about 2 hours (horizontal arrows). Metabolism of ethanol results in acetaldehyde and the time variations of this compound and acetone in the exhaled breath are plotted in Figure 3. The increase in acetone is due to the gradual onset of hunger.

The minima in the breath deuterium content are obvious for subject A but not for subject B in both sets of data. The data in 4b were obtained 5 days after those in 4a. Note that the pre-dose levels of deuterium were higher because of the residual HDO in the body. The clear difference between A and B is presumably because of the excessive mouth contamination and the relatively rapid gastric emptying rate for B. The decreases in breath/blood deuterium levels are due only to dispersion of HDO into the TBW and thus a constant value in the deuterium content is approached asymptotically as the HDO equilibrates with the TBW. The increase of the equilibrium value above that measured before ingestion coupled with the known dose of D_2O provides accurate values of the TBW, which are given in Figure 4. Note the excellent consistency of the TBW values for both A and B derived from the two data sets. The short time interval needed for sequential breath analyses using both FA-MS and SIFT-MS allows meaningful modeling of flow rates of ingested compounds between body compartments. Thus, these analytical methods have useful applications in physiology and medicine.

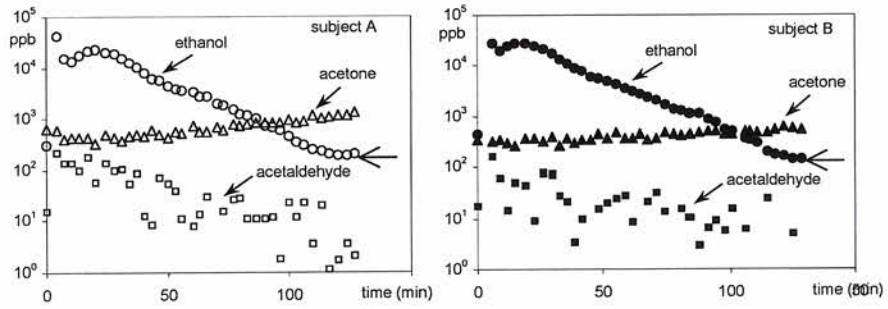

Figure 3. Variations of the levels (ppb) of breath ethanol, acetaldehyde and acetone after ingestion of alcohol.

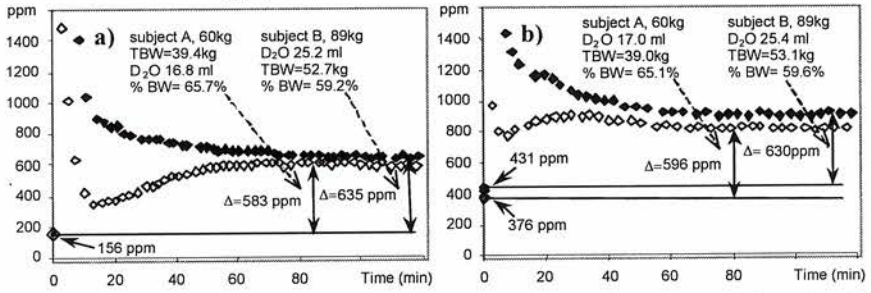

Figure 4. Variations of the D/H ratio in blood/TBW and the derived TBW after ingestion of D_2O. The two sets of data were obtained 7 days apart.

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Title	Occupational Exposure Assessment through Analysis of Human Breath and Ambient Air using Mass Spectrometry
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Keywords	breath analysis, mass spectrometry, non-invasive, industrial medicine, exposure, solvent
Aim of Study	Presentation of a multi-component gas analyzer founded on mass spectrometry for the online and offline analysis of organic and inorganic compounds in exhaled breath. Advantages of this tool are being discussed with regard to simplicity of use and new possibilities in the field of industrial medicine. Examples will be given.
Methods	One online and two offline analyses are to show how an ion-molecule reaction (IMR) – mass spectrometer (MS) works. For an online analysis the subject breathes into the IMR-MS and the selected molecules are recorded continuously. For an offline analysis the subject breathes into a glass vial and the exhaled breath is then analyzed with an autosampler IMR-MS.
Results	a) Online: after exposure to a gas mixture of propene, 1.3-butadiene, benzene and toluene, the subject's exhaled breath is analyzed as to the decomposition of these gases in the low ppb range; this allows conclusions regarding the clearance of these compounds. b) Offline: the analysis of breath samples of workers exposed to the solvents acetone, diethyl ether and dimethylformamide used in a pharmaceutical and glass processing plant showed that solvent concentrations increased with time. Depending on the time of exposure, these concentration levels reached values of up to 80% of those measured in the ambient air. This once more demonstrates how important work breaks and/or resting periods are to minimize occupational exposure.
Conclusions	The online (real time) and offline (passive) analysis of exhaled breath and ambient air provides a simple method to monitor occupational exposure. Good agreements are being reached with blood values.

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Rapid diagnosis of gastrointestinal conditions from faecal volatiles

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There is anecdotal evidence that the odour of diarrhoea can often be associated with a specific infection. Diarrhoea due to infections is a major cause of morbidity and mortality worldwide with circa 7000 people dying every day (1). There are a small number of disease vectors responsible for most of these conditions, for instance enterotoxigenic *E. coli, shigellae* and rotavirus account for most cases of acute diarrhoea in Bangladesh and *C. difficile* and *Campylobacter* are responsible for a large number of cases in the UK (2), circa 55000 cases for the latter. There is often several days between the collection of a stool sample and microbiological assessment. This delays appropriate treatment and can result in death of the patient and cross infection of others.
We have recently undertaken work on the analyses of volatile organic compounds (VOCs) from stool samples (3), (and a literature review on volatiles originating from urine (4)) to assess whether rapid diagnoses can be undertaken by assessment of the volatile profiles. We will present our latest findings. In addition to infectious bowel diseases we have undertaken some preliminary research on VOCs from stool samples donated by patients with Crohns disease, irritable bowel syndrome and ulcerative colitis. An example of our initial findings is shown in Fig. 1 using GC/MS and solid phase micro extraction. Many of these compounds might be expected to be found in breath samples, at lower concentrations.

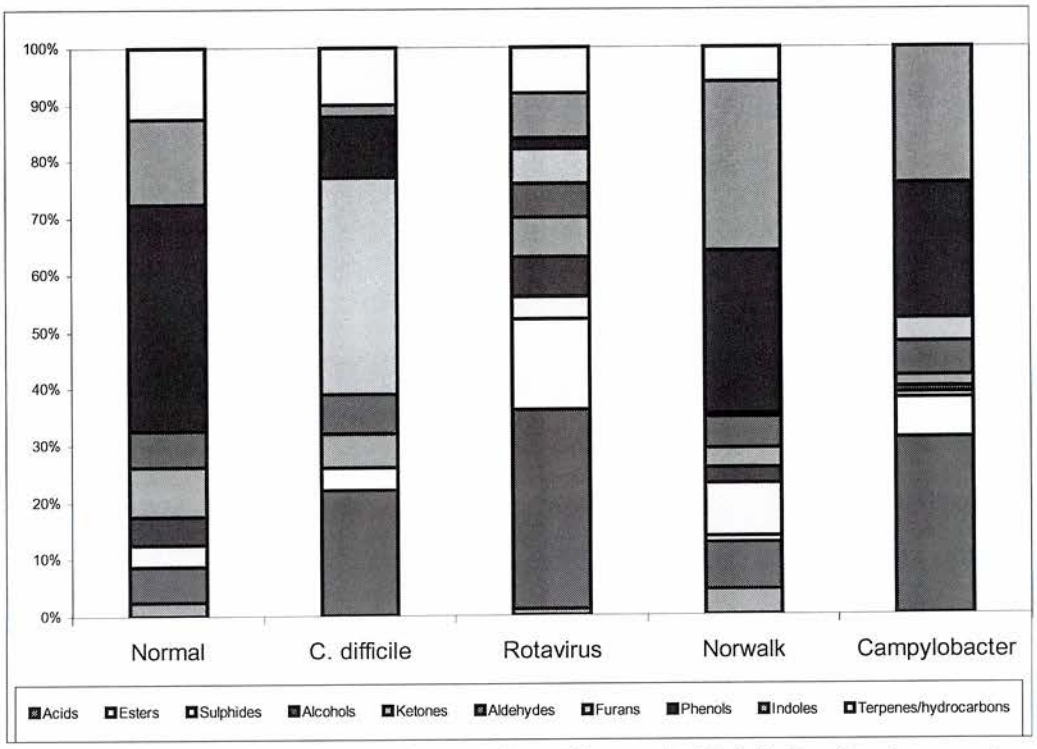

Fig. 1 Comparison of VOC classes from patients diagnosed with infective diarrhoeas and healthy controls

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Increase of acetone in urine headspace; a potential indicator of ovulation.

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Selected ion flow tube mass spectrometry (SIFT-MS) has been developed for on-line, real time analysis of trace gases in air, breath and liquid headspace [1-3], principally for physiological measurements, clinical diagnosis and therapeutic monitoring [4-9]. Recently, we used SIFT-MS to study the daily variations in the acetone and ammonia content of the headspace above urine from a healthy female subject during three separate menstrual cycles. Concurrent with the expected time of ovulation, a sharp increase in the urine headspace acetone concentration was observed in each cycle (range 3-12 fold) [10]. In the present study we measured daily SIFT-MS urine headspace acetone concentrations from seven normally-ovulating healthy women (group 1) and three healthy postmenopausal volunteers (group 2). Urine luteinising hormone and day 21 serum progesterone concentrations were used to confirm ovulation in group 1. Urine headspace acetone levels rose sharply in 5 out of the 7 volunteers in group 1 urines 2-3 days after the urine LH surge (range 3-10 fold increase). No significant increases in acetone concentration were detected in group 2 urines. This study identifies a potentially important physiological phenomenon could be the basis of a rapid, non-invasive ovulation indicator.

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A Model of the Human Cardiovascular-Respiratory Control System

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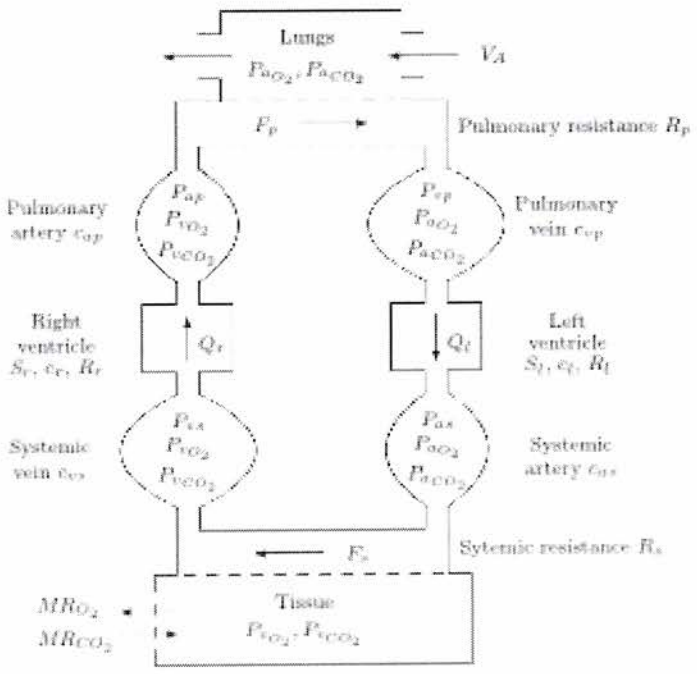
A model describing the interactions of the cardiovascular and the respiratory system is presented.

The respiratory part of the model is based on the model by Khoo et al. [J.Appl.Physiology: Respirat.Environ.Exercise Physiol.53(3), pp. 644-659, 1982]. It comprises two compartments, lungs and lumped body tissue, which are connected by the circulating blood. The events of the respiratory circle are ignored. Mass balance equations for carbon dioxide and oxygen are derived for both compartments. The cardiovascular part of the model is an extension of the mechanical compartment model by Grodins [Q.Rev.Biol. 34 (2), pp. 93-116, 1959]. It consists of four compartments which are the arterial and venous parts of the systemic and pulmonary vascular system. The compartments are connected by systemic and pulmonary resistances and by two pumps, the left and the right ventricle. The events of the cardiac circle are ignored. All pulsatile quantities have to be interpreted as mean values over the length of a pulse. For each of the four compartments, a mass balance equation for the contained blood volume is derived. Heart rate and alveolar ventilation are assumed to be the quantities through which the central nervous system controls the mean arterial blood pressure and the blood gas concentrations.

The model can provide a basis for studying the complex physiological control mechanisms of the cardiovascular system and possible paths of interaction between the cardiovascular and the respiratory control system.



Breath gas analysis and vector-borne disease diagnosis

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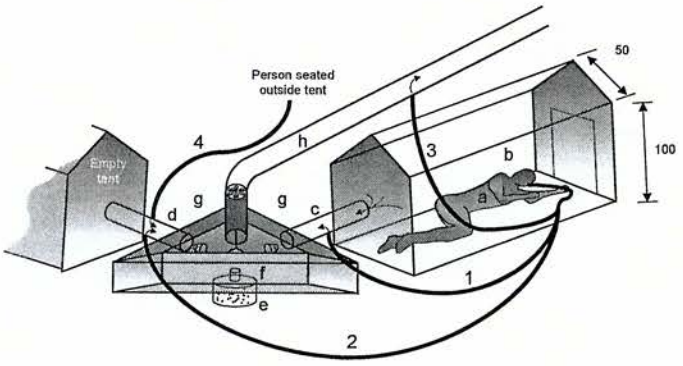
Insect vectors transmit several of the world's most debilitating diseases. Malaria alone causes some 300 millions infections per year, and of the estimated 3 million deaths, more than 90% occur in sub-Saharan Africa. The World Health Organisation advocates early diagnosis and prompt treatment with effective anti-malarial drugs as one of the main pillars to lower disease morbidity and mortality. However, inaccurate diagnosis using classical approaches (thick/thin blood smears and microscopy) is common. Worse, many rural areas in Africa lack even the most basic diagnostic facilities.

It has been postulated that disease pathogens such as *Plasmodium* may alter the host's physiology and indirectly increase attractiveness towards mosquitoes when transmissible stages of the parasites circulate in peripheral blood. Results of host attractiveness studies using elaborate olfactometer analysis in Kenya will be presented (see Figure). Given the presence of millions of parasites in the blood stream, it is likely that olfactory signal changes exploited by insect vectors, may be used as a means to diagnose disease. Our work has shown an allomonal effect of breath on mosquito attractiveness, hinting in this direction. Given the minute changes in VOCs needed to develop diagnostic tools on the basis of breath analysis, research in this area is urgently required.



Non-invasive and rapid diagnosis of vector-borne diseases would significantly enhance disease surveillance efforts, but a variety of possible applications towards active control of disease transmission will be presented. Similar to malaria, it is anticipated that breath gas analysis may be used for diagnosis of African sleeping sickness, dengue fever and lymphatic filariasis.

Detection of $^{13}CO_2$ for Diagnostic Non-Invasive Breath Tests with Stable Isotopes

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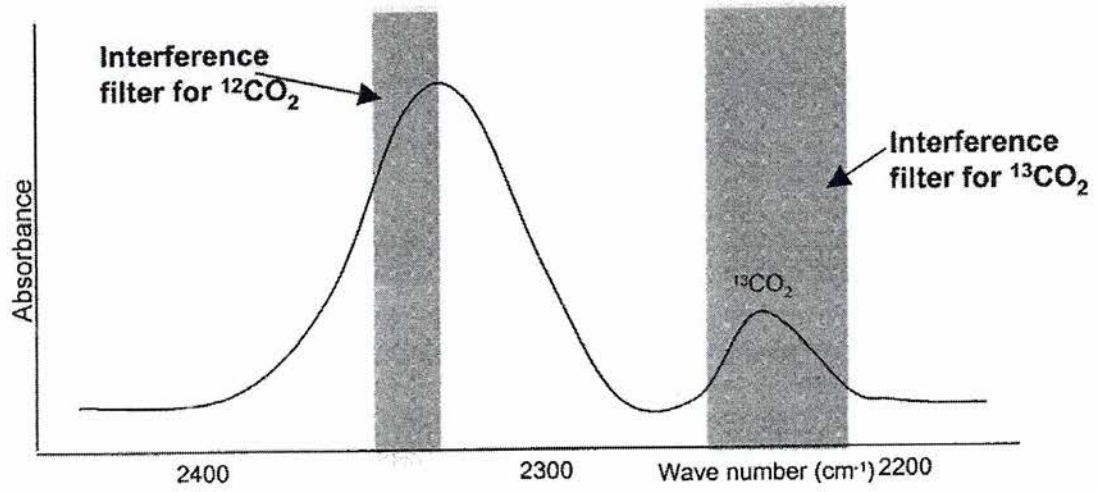
Non-invasive breath tests using stable isotopic compounds have been successfully utilized for various diagnostic tests to detect bacterial infection, liver function, and gastrointestinal disorders. We present two novel diagnostic tests using $^{13}CO_2$ as a metabolite in breath with clinical application. $^{13}CO_2$ and $^{12}CO_2$ in exhaled breath samples is measured by IR spectrometry using the UBiT-IR300 (Meretek Diagnostics, Lafayette, CO). The amount of $^{13}CO_2$ present in breath samples is expressed as a δ over baseline ratio that represents a change in the $^{13}CO_2/^{12}CO_2$ ratio of breath samples collected before and after ^{13}C-substrate ingestion. Recently, Meretek has been successful in developing a cheaper, smaller, and faster IR spectrophotometer named "POCone".

Quantitation of $^{13}CO_2$: The expired $^{13}CO_2$ that enters the spectrophotometer will be distinguished from $^{12}CO_2$ by measuring at a different absorbance, facilitated by the use of interference filters.



The $^{13}C/^{12}C$ isotope ratios will be reported in what is known as delta notation [Δ per mil or $\Delta\ ^{13}C$ (‰)] where the ratio is reported as a parts per thousand (‰) or "per mil" deviation from a known standard. The stable isotope compositions of low mass (light) elements such as oxygen, hydrogen, carbon, nitrogen and sulphur are normally reported as "delta" (δ values in parts per thousand (denoted as ‰) enrichments or depletions relative to a standard of known composition. The symbol ‰ is typically described in several different ways: per mill, per mil, or per mille. The term "per mill" is the ISO term but is not widely used.

δ values are calculated by: (in ‰) = $(R_{sample}/R_{standard} - 1)/1000$
where "R" is the ratio of the heavy to light isotope in the sample or standard.

Delta over baseline (DOB) is expressed as Δ per mil (‰).

The validity of this approach for quantitation of ^{13}C by the UBiT-IR300 infrared spectrophotometer has been demonstrated with the recently approved application to FDA for the *Helicobacter pylori* breath test (BreathTek UBT). The values calculated by the UBiT-IR-300 infrared spectrophotometer were shown to be strongly correlated with those by GC-MS.

UBiT-IR300 spectrophotometer

Example 1. Uracil-2-^{13}C breath test for rapid detection of DPD deficiency.

Dihydropyrimidine dehydrogenase (DPD)-deficient cancer patients have been shown to develop severe toxicity after administration of 5-fluorouracil. Routine determination of DPD activity is limited by time-consuming and labor-intensive methods. Fifty-eight individuals (50 "normal," 7 partially, and 1 profoundly DPD-deficient) ingested an aqueous solution of Uracil-2-^{13}C (6 mg/kg). $^{13}CO_2$ levels were determined in exhaled breath using the UBiT-IR300 spectrophotometer.

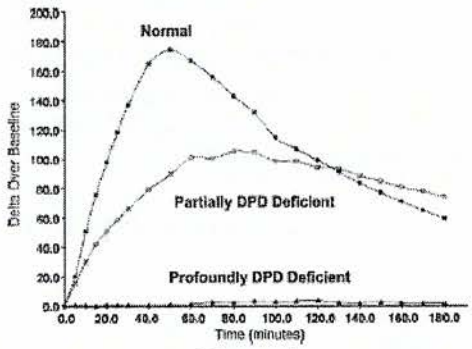


Figure 1

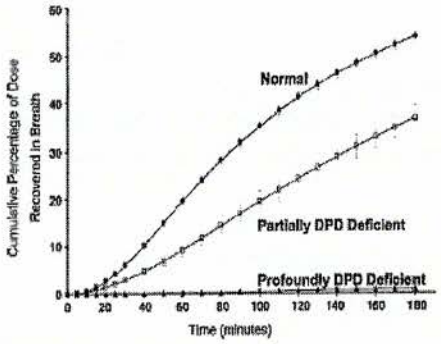


Figure 2

We demonstrated statistically significant differences in the Uracil-2-^{13}C breath test indices (C_{max}, T_{max}, DOB_{50}, and PDR) among healthy and DPD-deficient individuals (Figure 1 and 2). These data suggest that a single time-point determination (50 min) could rapidly identify DPD-deficient individuals with a less costly and time-consuming method that is applicable for most hospitals or physicians' offices.

(Diasio RB, et al. Clin Cancer Res. 2004, 10, 2652-8.)

Example 2. NaH$^{13}CO_3$ (^{13}C-SB) breath test to evaluate $paCO_2$.

^{13}C-SB (1.6 mg/kg) in 100 mL of water was administered to 48 patients referred for ABGs. Breath samples were collected for $^{13}CO_2$ content at various timepoints. 34 patients had normal $paCO_2$ (Mean±SD) *37.5±4.5* and 14 patients were hypercapnic *53±5 mmHg*. PDR's at 30 minutes is an independent predictor of $paCO_2$ ($p<0.001$). PDR_{30} was able to significantly discriminate normocapnic from severely hypercapnic patients ($paCO_2>54$) $p<0.001$ (Figure 3).

(Benzo R, et al. ERS 14th Annual Congress, Sept 2004)

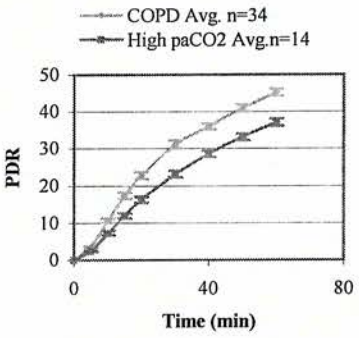


Figure 3

Example 3. Phenylalanine-1-^{13}C breath test for monitoring liver function of post transplant surgery.

We used ^{13}C-labeled phenylalanine (^{13}C-P) administered orally or iv in 7 living liver donors and measured exhaled ^{13}C-P labeled CO_2 to determine the extent of metabolic impairment and liver regeneration (figure 4). We conclude that orally administered amino acids are not well absorbed and/or metabolized in some subjects for weeks after partial hepatectomy whereas iv delivered substrates are much better oxidized by the regenerating liver. These findings may be due to impaired gut motility or portal venous flow that reduces delivery of oral agents after liver surgery. These preliminary findings have wide implications for nutrition and drug delivery in the early recovery phase for living liver donors.

(Freeman RB, et al. ATC Boston May 2004)

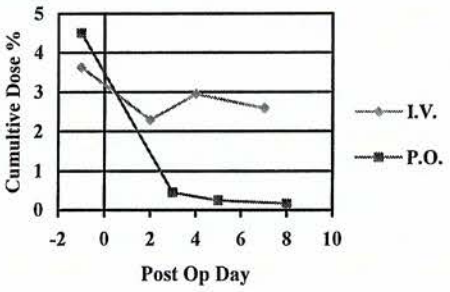


Figure 4

Analysis of breath using SIFT-MS: a comparison of breath profiles of healthy volunteers and seriously ill ICU patients

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Introduction

Breath analysis has great potential to provide a rapid, non-invasive medical diagnostic tool. Selected ion flow tube mass spectrometry (SIFT-MS) is one method for monitoring breath volatile molecules and has previously been developed for analysis of breath and the headspace of body fluids of both healthy volunteers and those with signs of clinical disease. This study examines the use of SIFT-MS breath profiles of healthy volunteers with a normal diet and under different dietary regimes. Breath profiles of several ventilated patients in intensive care units with a variety of medical conditions are also presented, and compared to those of the healthy volunteers.

Methods

There are two ways of introducing samples into the SIFT-MS instrument: either direct breath into the sample inlet, or through taking breath samples at a remote site in sampling bags, and then connecting sampling bags to the sample inlet. In this preliminary study, healthy volunteers provided direct breath samples; ventilated patients provided bag samples.

Seriously ill ventilated patients

Bag samples of breath were taken from 7 seriously ill ventilated patients with a variety of medical conditions and the results are shown in shown in Table 1. The samples were analysed within 4 hours of acquisition. Control samples were taken with healthy volunteers breathing through the ventilator. The same subjects also breathed directly into a sample bag and also provided breath directly into the SIFT-MS instrument at the time of analysis to provide controls. Notable findings from this preliminary study show that very high levels of ethanol and propanol are present in some patients' breath. The reason for this is not immediately obvious. High levels of formaldehyde and acetaldehyde are also present in the sample from the ventilated healthy volunteer, and other results (not shown here) indicate that their presence may be due to plastic components within the ventilator system. Further work is being undertaken to verify this.

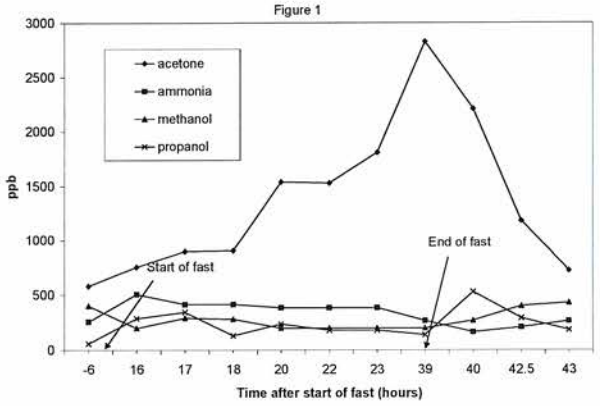
	Patient's bed number							Healthy volunteer			
Breath volatile	**15**	**9**	**18**	**8**	**14**	**7**	**17**	**HV DB**	**HV in bag**	**HV vent**	**bag**
formaldehyde	3	618	0	376	376	0	816	0	0	506	0
acetaldehyde	60	203	72	128	74	0	276	0	0	764	12
acetone	167	48	5	240	190	163	137	294	204	152	6
propanol	150	92	788	691	136	2176	270	120	91	79	9
isoprene	0	97	0	0	72	0	174	48	0	65	0
methanol	48	361	0	173	82	98	170	250	538	186	111
ethanol	3694	9136	518	689	69	1915	71	191	0	82	0
ammonia	1	17	15	108	55	24	24	487	978	1614	0

Table 1. Breath profiles of ill patients and healthy volunteers. HV = healthy volunteer; DB = direct breath into SIFT-MS; Vent = sample taken from volunteer on ventilator; Bag = bag filled with dry air.

Healthy volunteers

Any evaluation of the volatiles present in the breath of patients with clinical conditions must be compared to the range of volatiles present in apparently healthy individuals under various conditions, e.g. during fasting. Figure 1 shows some key breath metabolites observed during 39 hours without food. As expected, acetone rises to a high level. However, it can be seen that propanol peaks soon after resuming eating, and methanol also appears to increase after resumption of food. See: D.Smith, P. Spanel, S. Davies. *J. Appl. Physiol.*, 87 (1999) 1584.

Figure 1



Applications of SIFT-MS in physiology, clinical diagnostics and therapeutic monitoring.

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[a]Institute of Science and Technology in Medicine, School of Medicine, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent, ST4 7QB, U.K.
[b]V.Čermák Laboratory, J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejškova 3, 182 23, Prague 8, Czech Republic.

Selected ion flow tube mass spectrometry, SIFT-MS, was originally developed to achieve on-line, real time analyses of exhaled breath and urinary headspace for clinical diagnosis and therapeutic monitoring, but it has wider applications in such diverse areas as physiology, animal husbandry, food science and environmental science. The major objective of this talk is to demonstrate the versatility of SIFT-MS for breath and urinary headspace analysis via several pilot case studies that have been carried out in the Keele University Medical School. Thus, some results will be presented relating to studies of human physiology in the healthy state and following exposure to toxic gases, and investigations of some specific disease conditions. A scheme of some areas of application we have partially investigated is given in Figure 1.

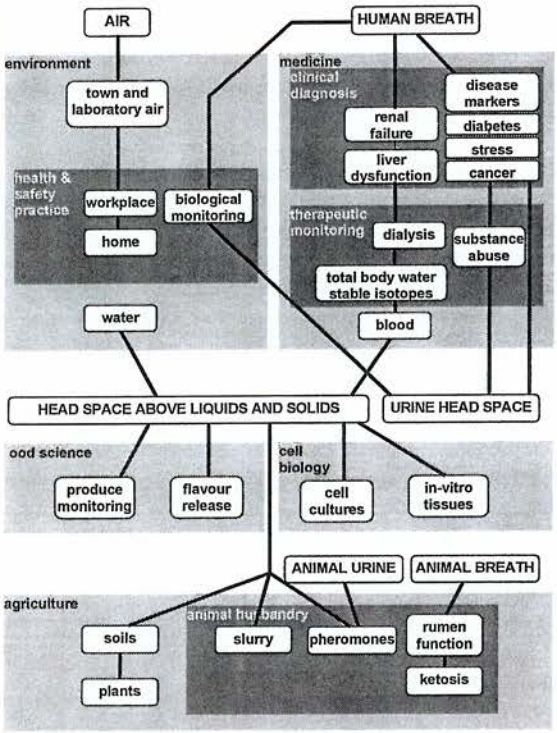

Figure 1 Areas of application of SIFT-MS at Keele University Medical School.

In order to establish the normal levels of some common breath metabolites a longitudinal study of ammonia, acetone, isoprene, ethanol and acetaldehyde in the breath of five individuals over a thirty-day period has been carried out and the results will be presented. Such is shown in Figure 2, which shows the "normal distributions" of ammonia, acetone and isoprene for 4 individuals.

The influence of protein and carbohydrate liquid meals on the levels of common breath metabolites will be illustrated, which shows that level changes of these metabolites are relatively small when compared to the elevated levels seen in specific diseased states. Isoprene is given some attention, since the breath concentration of this hydrocarbon maybe an indicator of physiological and/or oxidative stress. To demonstrate the value of SIFT-MS as a non-invasive analytical tool that allows single breath exhalations to be analysed at a rapid rate without undue distress over a few hours, the results of a study of dispersion and metabolism of ingested ethanol and the associated production of acetaldehyde in the body will be presented, as is illustrated in Figure 3.

To illustrate SIFT-MS as a valuable technique for the analysis of volatile compounds in urinary headspace, the results of a study of acetone and ammonia above the urine of several normally ovulated volunteers will be described that indicate how urinary acetone may be a valuable indicator of ovulation. Biological monitoring of the level of exposure to toxic substances has

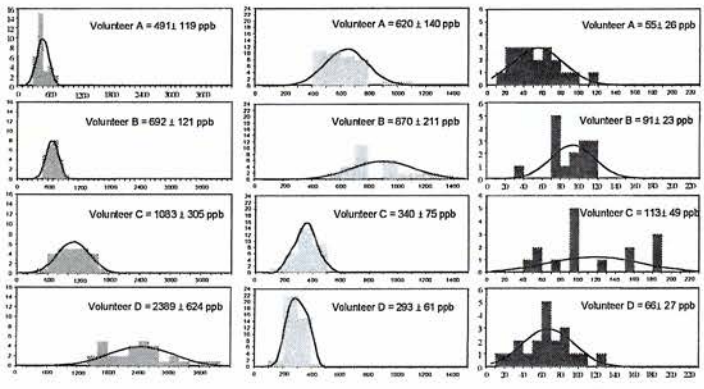

Figure 2 Distributions of breath concentrations of ammonia (left column), acetone (centre), and isoprene (right) for 4 volunteers.

become an essential procedure in health and safety practice. This is normally carried out using blood and urine analysis, but breath analysis is increasingly attractive now that SIFT-MS is available as a reliable non-invasive, real time analytical tool. To illustrate its value in this area, the results will be presented of breath analysis following the controlled exposure of an individual to an atmosphere containing perchloroethylene and exposure to common anaesthetic gases. Further, the results of a parallel study of acetonitrile in breath and urinary headspace of cigarette smokers will be presented, which indicates that this compound becomes systemic. The establishment of the normal levels of breath metabolites in healthy individuals by SIFT-MS paves the way for studies of diseased states. Hence, some diseased states can now be easily recognised and the influence of therapy can be monitored via non-invasive SIFT-MS breath analyses. We present the results of some studies that we have carried out to date. Principle amongst these is end-stage renal failure when breath ammonia is seen to be greatly elevated, but which reduces to normal levels during haemodialysis. The diabetics in the renal patient cohort are clearly identified by high levels of breath acetone, as are the cigarette smokers by the presence of acetonitrile. These indicators are seen in the SIFT-MS spectrum that is given in Figure 1 in the abstract entitled *"Selected ion flow tube mass spectrometry, SIFT-MS: reliable, real time quantification of metabolites in air, exhaled breath and liquid headspace"* of this meeting. Breath ammonia is also shown to be elevated when a person infected with the bacterium Helicobacter pylori ingests a small amount of urea, $^{12}CO(NH_2)_2$ and this offers an alternative to the use of the much more expensive $^{13}CO(NH_2)_2$ and the detection of elevated $^{13}CO_2$ in breath for the detection of H-pylori. Our SIFT-MS studies of the headspace of urine from patients suffering from bladder and prostate cancer showed the presence of formaldehyde that was absent from urine from healthy controls, which suggests a method for the detection of tumours in the body. It is also shown that greatly increased levels of nitric oxide appear in the headspace of bacterially-infected urine that has been acidified with mineral acid, this being a valuable indicator of urinary tract infection. SIFT-MS has numerous potential applications in medicine that are yet to be explored. Further examples from our preliminary work include investigations of substance abuse via breath analysis and the detection of HCN emitted by Pseudomonas bacteria cultures of swab and sputum samples from children suffering from cystic fibrosis (see the spectrum in Figure 4), which might offer a monitoring procedure for this debilitating illness by breath analysis.

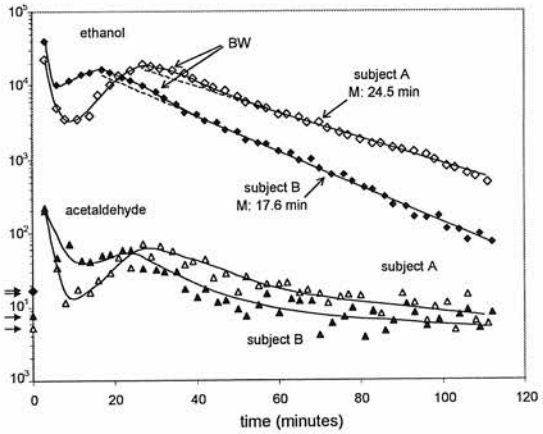

Figure 3 Time dependence of breath ethanol and acetaldehyde concentrations observed after ingestion of ethanol.

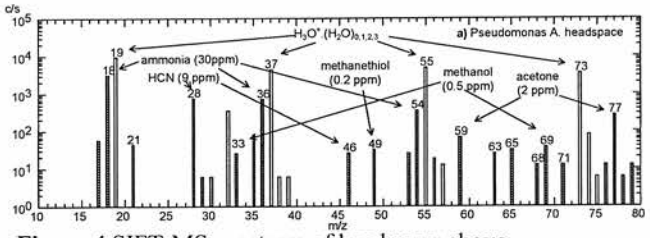

Figure 4 SIFT-MS spectrum of headspace above *Pseudomonas* bacterial culture.

Further reading

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Breath gas as biochemical probe in sleeping individuals

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and Hartmann Hinterhuber[(2)]

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INTRODUCTION There is evidence of a biochemical feedback between the sleep-controlling brain and metabolic activities outside the brain. Perturbed feedback may be a reason for sleep disorders. Online mass spectroscopic analysis of volatile organic compounds (VOCs) in exhaled air is expected to provide insight into the metabolic processes and hence to complement the information about brain activity obtained by polysomnography (PSG).

METHODS Our experimental setup consisting of a proton-transfer-reaction mass spectrometer (PTR-MS, Ionicon FDT-s) combined with PSG (Nihon-Kohden EEG 4317F) was designed for simultaneous online monitoring of the sleepers' exhaled breath and electrophysiological brain signals. Exhaled air was continuously sampled through a catheter in the nasal cavity and conducted to the PTR-MS through perfluoroalkoxy copolymer tubing heated to 43°C. The reported masses are those of the protonated species (molecular mass + 1 u) according to the ionization process used in PTR-MS. The concentration time series are partly smoothed using moving average techniques. Sleep stages were determined according to the rules of Rechtschaffen and Kales. Breathing and pulse frequency (both smoothed) were derived from the recorded thorax excursion and ECG data. All participating test persons, 10 healthy men aged 20 to 28, spent 3 nights in the sleep laboratory. The first night was spent there for the purpose of getting familiar with the experimental setup (adaptation night). Subsequently the test persons were deprived from sleep for circa 40 h, i.e., they had to skip a night of sleep, before returning to the laboratory for sleeping (night after sleep deprivation = recovery night). Thereafter, they underwent their daily routine. In the subsequent night, they returned again to the laboratory and slept there for the third and last time (normal night).

RESULTS

Isoprene (mass 69 u) concentration is influenced by pulse frequency (fig 1) and breathing frequency (fig 2). It tends to increase during night (not shown). There is no obvious difference between normal nights and recovery nights.

Methanol (33 u) concentration decreases during normal nights, whereas it stays rather constant during recovery nights.

The ratio of acetaldehyde (45 u) to ethanol (47 u) concentrations increases during night (fig 3).

DISCUSSION

The evolving concentration of VOCs such as acetone, methanol, ethanol, acetaldehyde or isoprene reflects biochemical processes during the sleep. Sometimes it is more convenient to

consider derived quantities: for example, the time evolution of the ratio of acetaldehyde to ethanol concentrations reflects the alcohol dehydrogenase activity during the night. The concentration course of isoprene is special, as its concentration varies with pulse frequency and depends on breathing frequency. The reported results may be spoiled by wrong concentration values. In fact, concentration measuring may be influenced by condensed water droplets selectively dessolving hydrophilic compounds; or the tubing between probands and PTR-MS may selectively absorb and desorb certain substances. Breath gas analysis in sleeping individuals is non-invasive and enables us to monitor metabolic processes on-line, thus complementing the electrophysiological information obtained by polysomnography. Therefore, we believe that it has great potential as a source of additional information to standard polysomnography.

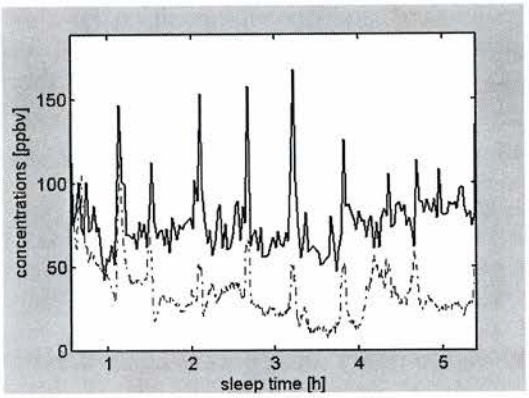

Fig. 1: Volume fraction of mass 69 u corresponding to isoprene (solid) and heart rate (dashed, arbitrary scale) as function of time during one particular night.

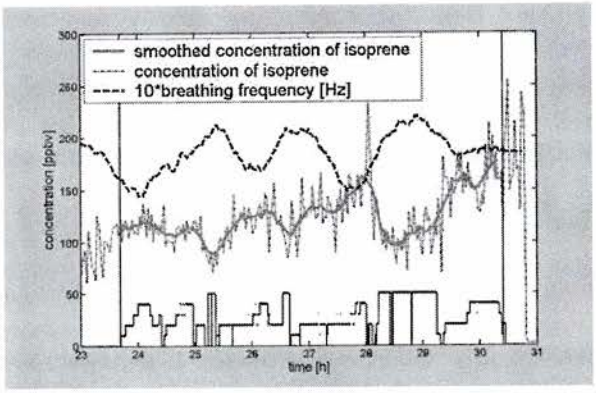

Fig. 2: Volume fraction of isoprene (with and without smoothing), breathing frequency (scaled by a factor 10) and hypnogram (sleep stages, scaled by a factor 10; 0 awake; 1, 2 light sleep; 3, 4 deep sleep; 5 REM) during one particular night.

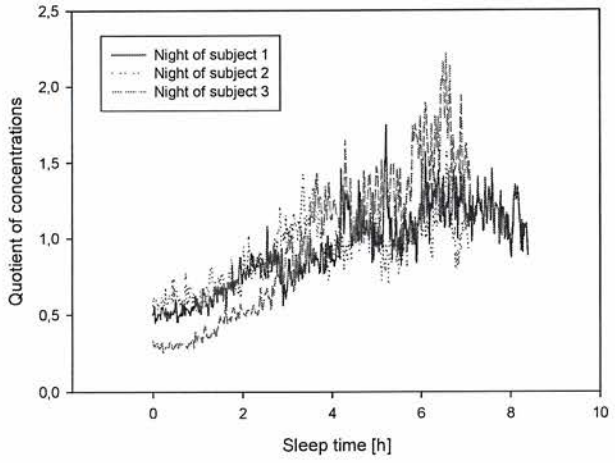

Fig. 3: Ratio of acetaldehyde (45 u) to ethanol (47 u) volume fractions during three particular nights. The ratio increases with time as expected when ethanol metabolizes to acetaldehyde.

Effects of ventilation on the collection of exhaled breath in humans

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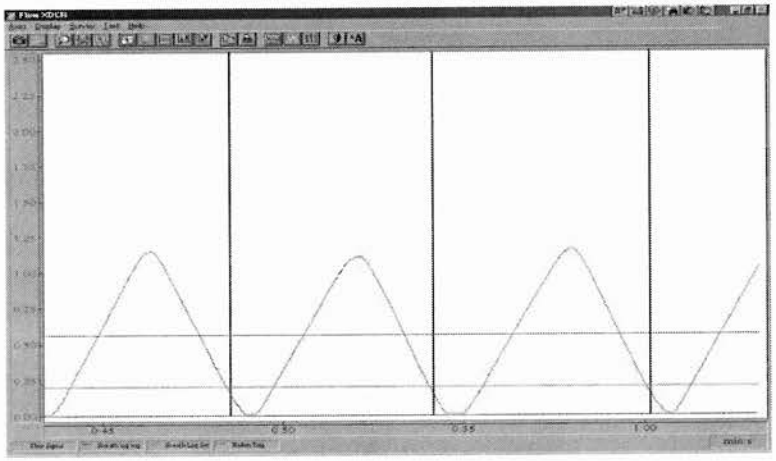
The instantaneous composition of breath varies extensively over the breathing cycle as a result of normal pulmonary physiology. The initial portion of breath in the breathing cycle originates from the conducting airway (anatomic dead-space) and the composition of this gaseous mixture is determined almost exclusively by the composition of the inhaled gas. The remainder of the breath in the breathing cycle consists of mixed expired gas and alveolar gas. In a human subject at rest, almost 80% of the gas that is in contact with the capillary-alveolar membrane is exchanged with each breath. The gas in contact with this membrane will contain approximately 5% carbon dioxide. If a subject is breathing normally, then the concentration of carbon dioxide at the end of the breathing cycle (end-tidal), the concentration of carbon dioxide in arterial blood and their ratio will remain constant from breath to breath. Changing the tidal volume and/or breathing frequency will increase or decrease these concentrations. The rate of alveolar ventilation is controlled by the arterial blood concentration of carbon dioxide. Hypo- or hyperventilation will, by definition, change the composition of exhaled breath and in turn the arterial blood concentration. Variations in tidal volume or breathing frequencies are particularly important when sampling breath from human subjects breathing spontaneously since any method of breath sampling will make the subject conscious of their breathing patterns and the potential to change breathing patterns is increased presumably by shifting control away from the automatic respiration centers to the cerebral cortex. Therefore, it may be necessary to regulate voluntary breathing in order to optimize breath composition for analysis. Additionally, it is preferable to sample multiple breaths in order to ensure that the analysis of the collected breath is

representative particularly when analysis is performed subsequent to collection. To satisfy these requirements a computerized breath collection system has been developed that allows the subject to visualize each breath in real-time so that they maintain consistent ventilation patterns. Continuous measurements of mouth pressure, tidal volume, respiration rate, end-tidal carbon dioxide (ET_{CO_2}) and mixed expired carbon dioxide (ME_{CO_2}) measurements are recorded for each breath.



To date, this system has been used to adjust, correct and normalize endogenous concentrations of breath molecules using carbon dioxide measurements. This system was also used to investigate the inter- and intra-subject variability in the production of breath biomarkers over an eight-hour period and during a one-week study period. Additionally, studies were performed to investigate the effects of exercise (i.e., changes in minute ventilation and cardiac output) on the composition of breath. The results of these studies will be presented.

In Vivo assessments of injury and disease based on breath

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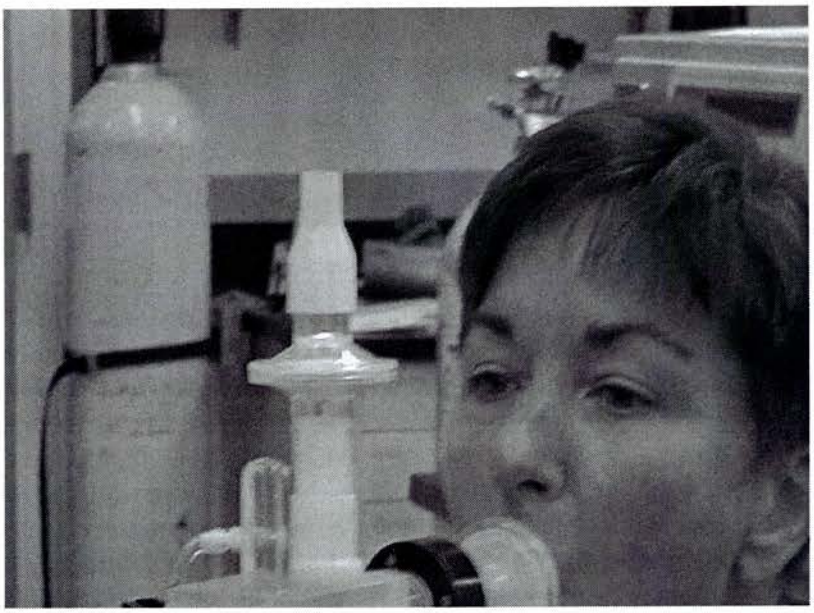
The concept that blood, urine, and other body fluids and tissues can be sampled and studied to yield clinical information for diagnosis of disease states or to monitor tissue injury or therapy is the foundation of modern clinical diagnosis and medical practice. The use of breath as a collectable sample has not received similar clinical use mainly due to the low concentrations of potential marker molecules in breath.

The ability to exchange carbon dioxide with oxygen is essential for many life forms. In animals, this gas exchange occurs at the alveolar-capillary membrane in the respiratory tract. Oxygen and carbon dioxide are passively transported from blood to breath or *vice versa* and the diffusion of these gases is governed by their concentration gradients across the alveolar-capillary membrane. Any additional molecule present in the blood or in the inspiratory air will also pass into the breath or blood respectively. The only requirement for this transport is that the molecule must exhibit a significant vapor pressure. The molecular profile of breath is the product of the composition of the inspiratory air and volatile molecules that are present in the blood. Cells or tissues in the mouth, nose, sinuses, airway and the gastrointestinal tract may also contribute molecules to exhaled breath. The bulk matrix of breath is a mixture of nitrogen, oxygen, carbon dioxide, water vapor and the inert gases. The remainder of breath (<0.000001%) is a mixture of as many as 500 different compounds. These molecules have both endogenous and exogenous sources, however, the concentration is often higher when the origin is exogenous. The concept that breath contains molecules originating from normal or abnormal physiology has its origins in the writings of Hippocrates, the father of medicine. For example, detection of the presence of water vapor in breath has been used as a noninvasive monitor of mortality for thousands of years. Additionally, distinctive breath odors have been used for centuries as indications of diseases such as uncontrolled diabetes, liver disease, renal failure or dental

disease. However, the use of odors of breath for clinical diagnosis can be confounded by the characteristic odors that result from the ingestion of such materials as garlic, onions, fish, spices, mints, and ethanol.

The identification and quantification of molecules present at trace concentrations in breath has a limited history even though breath can be sampled from any human subject from the neonate to the elderly with relative ease, minimum invasion and multiple times.



However, recent advances in analytical instrumentation has suggested that the use of exhaled breath to study disease processes should now be re-examined. Currently, a number of marker molecules have been identified in breath that can be used to identify disease, disease progression, or to monitor therapeutic intervention. It is expected that this list will soon increase dramatically since the analysis of breath is ideally suited for population-based studies in the developed and underdeveloped world.

This talk will review those molecules that have been found in human breath whose biochemical pathways are known or whose biochemical pathways can be postulated and have been shown to be useful clinically.

EXHALED NITRIC OXIDE AND PULMONARY COMPLICATIONS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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BACKGROUND

At present, allogeneic hematopoetic stem cell transplantation (AHSCT) is the only potentially curative treatment for multiple myeloma. Its action is most likely due to an *allogeneic graft-versus-myeloma* effect. Unfortunately AHSCT carries a high risk of complications and toxicities related to the intensive preparative regimen which is traditionally used for pre-transplant myeloablation and to the graft versus host disease (GVHD), which may be life threatening (1).
Pulmonary complications after AHSCT are common (2) and different in nature. Bronchiolitis obliterans is a nonspecific inflammatory injury of the small airways, that has been recognized in early 1980s, particularly among patients with acute and chronic graft versus host disease (GVHD) and in those with respiratory infections. It has been observed that pulmonary complications may be predicted by abnormalities in lung function tests (3).We wondered whether the production of nitric oxide (eNO), that is considered a reproducible marker of airway inflammation (4), increases during AHSCT-induced lung injury.

The aim of this study was to evaluate if the measurement of eNO, together with lung function tests, may help in predicting the morbility and mortality after AHSCT.

METHODS

We examined 26 subjects with multiple myeloma, before and after 3 and 6 months following non ablative AHSCT.
At each examination the subjects underwent the following measurements:

- eNO measured with a chemiluminescence analyser (SIEVERS 280 NOA, Boulder, CO).
- Lung function tests with recording of: total lung capacity (TLC), vital capacity (VC), forced expiratory volume in one second (FEV_1), forced expiratory flow at mid vital capacity (FEF_{50}), FEF_{25-75},
- single breath carbon monoxide diffusion capacity
- arterial blood gas analysis
- methacholine bronchial challenge, using FEV_1 as the index of the responsiveness of the whole airway ($PD_{10}FEV_1$ mcg methacholine) and FEF_{50} as the arbitrary index of bronchiolar responsiveness ($PD_{25}FEF_{50}$).

RESULTS

Out of the 26 patients selected, 8 (31%) died within one month from transplantation (5 for disease progression, 2 for infection and 1 for severe acute GVHD).
All the remaining 18 patients were alive at 6 months; their mean values of eNO, lung function tests, methacholine thresholds and arterial blood gases showed no significant change from either at 3 or at 6 months from transplantation.
In the subsequent period, over 6 but before 12 months after transplantation, 4 patients died, all with pulmonary complications: 2 with severe extensive GVHD and 3 with Aspergillus infection.

As shown in the following three figures, the comparison between the 14 patients survived (GROUP 1) and the 4 died (GROUP 2) showed that the latters had

- progressive increase in eNO (Figure 1)
- progressive decrease in FEF25-75 (Figure 2)
- progressive decrease of bronchiolar threshold to methacholine (Figure 3)

Figure 1

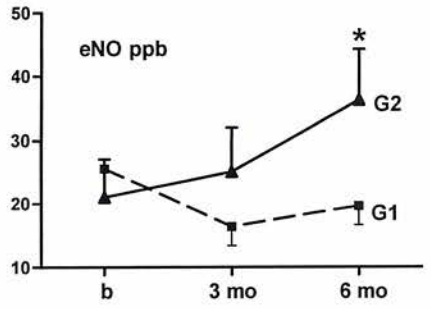


Figure 2

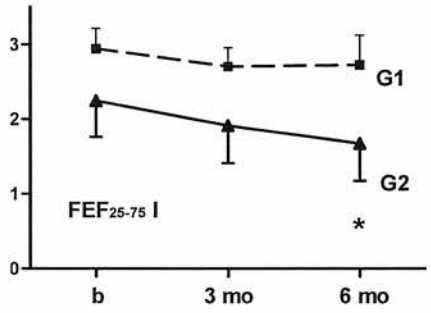
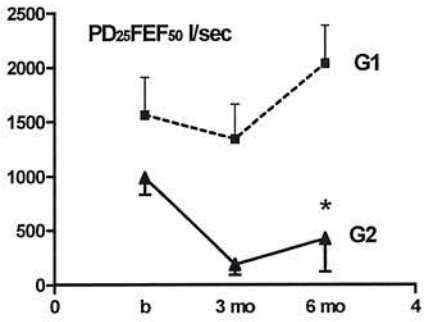


Figure 3



All GROUP 2 patients showed the appearance of ground-glass opacities at lung CT-scan.

CONCLUSIONS

The preliminary results of this study indicate that non myeloablative AHSCT produced short and medium term life threatening pulmonary complications in a consistent number of patients.
Severe pulmonary complications were predicted by increases in exhaled NO and decreases in tests reflecting peripheral airway patency patency. Both functional and CT-scan abnormalities suggest the occurrence of bronchiolitis, that at least in two patients might be due toAspergillus infection.
Exhaled NO resulted to be sensitive enough to reveal ongoing airway damage. These results are in agreement with the findings in lung transplantation, where serial eNO measurements were found to predict the early development of bronchiolitis obliterans syndrome.

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Breath volatiles - making sense of the data

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The modern era of breath testing commenced in 1971 when Linus Pauling used a cold trap to freeze human breath, and gas chromatography to demonstrate that the concentrated breath contained a large number of previously undetected volatile organic compounds (VOCs). In recent years, new instruments have been developed that make it possible to routinely collect breath samples in the field for assay of VOCs by gas chromatography and mass spectroscopy. Normal human breath contains around 200 different VOCs, most of them in nanomolar (10^{-9} M) or picomolar (10^{-12} M) concentrations. The composition of these VOCs varies widely from person to person, and >3,000 different VOCs have been identified in the breath of normal humans. However, a core subset of different VOCs has been observed in all humans that includes isoprene (a precursor of cholesterol) and a number of alkanes and methylated alkanes that are markers of oxidative stress. The breath methylated alkane contour (BMAC) is a three-dimensional display of the abundance of these alkanes and methylated alkanes. Clinical studies have shown that the BMAC changes in disease, and appears to provide a characteristic "fingerprint" in conditions as diverse as lung cancer, breast cancer and unstable angina pectoris. FDA recently approved the first clinical application of the BMAC in humans: Heartsbreath, a breath test for heart transplant rejection.

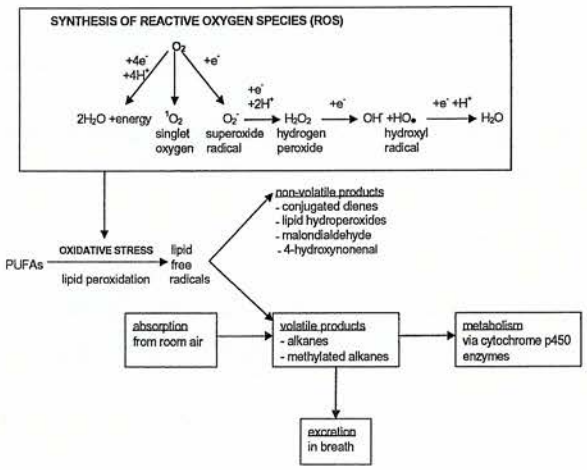

Figure 1: Origin of oxidative stress and the generation of metabolic markers. ROS (superoxide radical, hydrogen peroxide and hydroxyl radical) are synthesized in the mitochondria; electron leak into the cytoplasm generates oxidative stress, a constant barrage of damage to DNA and proteins and polyunsaturated fatty acids (PUFAs). This diagram illustrates the effect of oxidative stress on PUFAs, generating alkanes and methylated alkanes which are either metabolized or excreted in the breath.

Significance of the BMAC as a new "fingerprint" of disease: In clinical studies, the BMAC provided a sensitive and specific marker of different diseases, including heart transplant rejection[1, 2], lung cancer[3, 4], breast cancer[5], ischemic heart disease[6], preeclampsia of pregnancy[7] and diabetes mellitus[8]. An important central finding of these studies is that "increased oxidative stress" is not a simple and nonspecific increase in a single variable, but a complex event which generates different "breath fingerprints" for different diseases. For

example, increased oxidative stress was observed both in normal aging[9] and in Grade 3 heart transplant rejection, yet the pattern of the BMAC was completely different in the two conditions. Future applications include the early detection of diseases associated with increased oxidative stress, such as atherosclerosis, Alzheimer's disease and diabetic retinopathy. In carefully designed clinical studies, breath testing could also measure the impact of new treatments for these conditions.

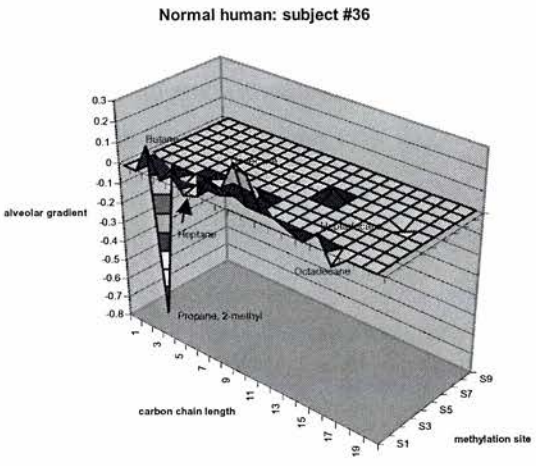

Figure 2. Breath methylated alkane contour (BMAC) in a healthy 30 year old female volunteer. The alkanes and the methylated alkanes in a breath VOC sample collected with the BCA were analyzed by gas chromatography and mass spectroscopy. The x-axis is the length of the carbon chain in the alkane or methylated alkane, the z-axis is the site of monomethylation, and the y-axis is the alveolar gradient (abundance in breath minus abundance in room air).

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Breath Gas Analysis in Patients with Malabsorption Syndromes

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Background: Fructose and lactose malabsorbers are characterized by impaired duodenal fructose transport or the deficiency of mucosal lactase activity, respectively. As a consequence, the non-absorbed saccharides reach the colon, where they are broken down by bacteria to short fatty acids, CO_2 and H_2. Bloating, cramps, osmotic diarrhea and other symptoms of irritable bowel syndrome are the consequence and can be seen in about 50% of carbohydrate malabsorbers. Shortly after the consuption of saccarides H_2 can be detected in the exhaled breath. Therefore breath gas analysis is a quick and adequate method for the diagnosis of carbohydrate malabsorption.

Recently it was found that fructose and lactose malabsorption is associated with early signs of depressive disorders. Therefore it was investigated whether fructose and lactose malabsorption is associated with an abnormal tryptophan metabolism.

Methods: 111 otherwise healthy volunteers (81 females and 30 males) with gastrointestinal complaints were analysed by measuring breath H_2 concentration after an oral dose of 50 g lactose and 25 g fructose after an overnight fast one week apart. They were classified as normals, isolated fructose malabsorbers, isolated lactose malabsorbers and combined fructose/lactose malabsorbers. All patients filled out Beck`s depression inventory questionnaire. In a second study (16 males, 34 females) blood samples were taken additionally for serum tryptophan and kynurenine measurments.

Results: Fructose malabsorption (breath Δ H_2-production > 20 ppm) was detected in 62,5% up to 70% of the tested individuals. 3,6% were only lactose malabsorbers and 11,7% presented both fructose and lactose malabsorption. Isolated fructose malabsorption and combined fructose/lactose malabsorption were associated with significant higher Beck`s depression scores ($p < 0,01$) [Figure 1]. Furthermore, subjects with fructose malabsorption showed significantly lower plasma tryptophan concentrations than those with normal fructose malabsorption [Figure 2].

Conclusion: The data show that fructose malabsorption may play a role in the developement of depressive disorders in females, whereas combined fuctose/lactose malabsorption seems to even more increase the risk of mental depression. Lower tryptophan levels of the affected subjects could be a reason for this finding. High intestinal fructose concentration may reduce the availibility of tryptophan for the biosythesis of serotonin (5-hydroxytryptamine). Carbohydrate malabsorption should be considered in patients with signs of depression and disturbances of tryptophan metabolism.

Figure 1: Beck`s Depression Scores in Fructose and Lactose malabsorption in females

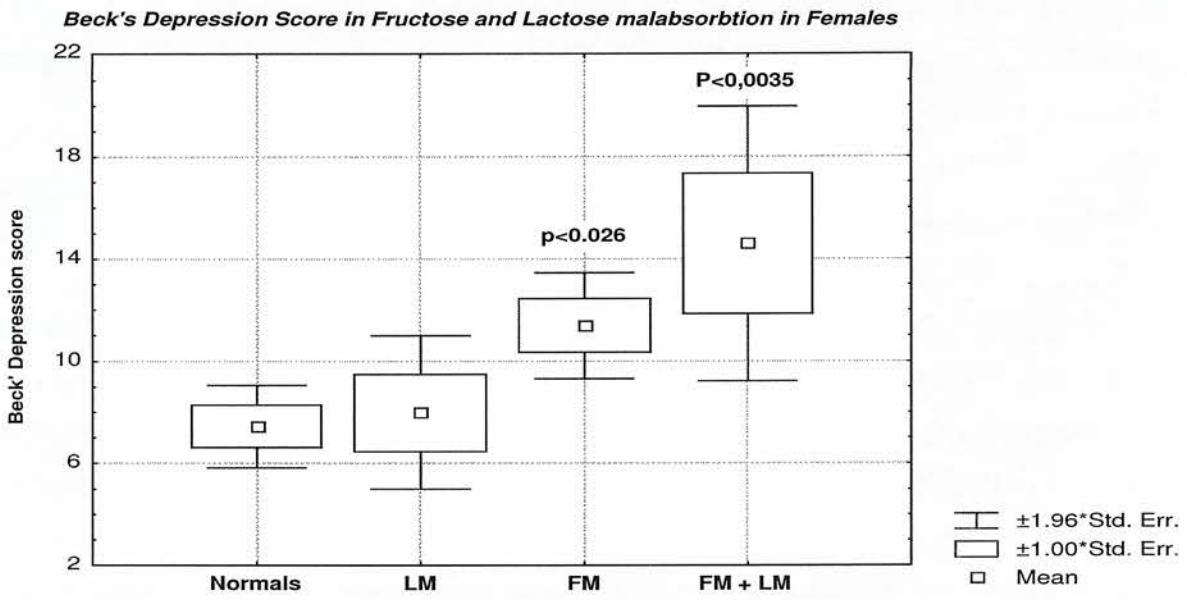

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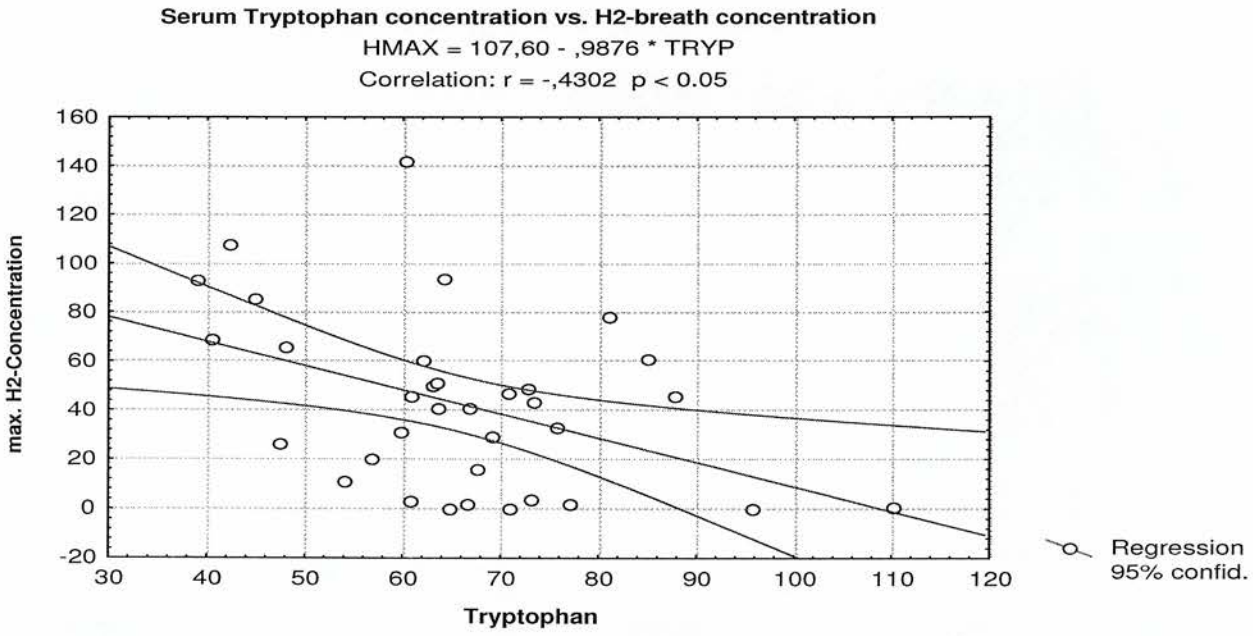
Figure 2: Serum Tryptophan concentration versus H_2-breath concentration



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VOC Breath Markers in Critically Ill Patients - Potential and Limitations

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Breath tests are attractive because they are non invasive and can be repeated frequently in the dynamically changing state of critically ill patients. Volatile organic compounds are produced anywhere in the body and are transported via the blood stream and exhaled through the lung. They can reflect physiological or pathological biochemical processes, such as lipid peroxidation, liver disease, renal failure, allograft rejection, dextrose or cholesterol metabolism. Critically ill patients can be expected to exhale maximum concentrations of volatile markers since clinical conditions change rapidly and pathological conditions are profound in these patients. The collection of gaseous samples represents a crucial issue in breath analysis. In mechanically ventilated patients results may be affected additionally by the ventilator settings and the respiratory pattern. For this reason controlled alveolar sampling (Figure 1) is strongly recommended. Volatile substances usually are preconcentrated (sorbent traps or SPME), separated by gas chromatography, detected and quantified via mass spectrometry or FID.

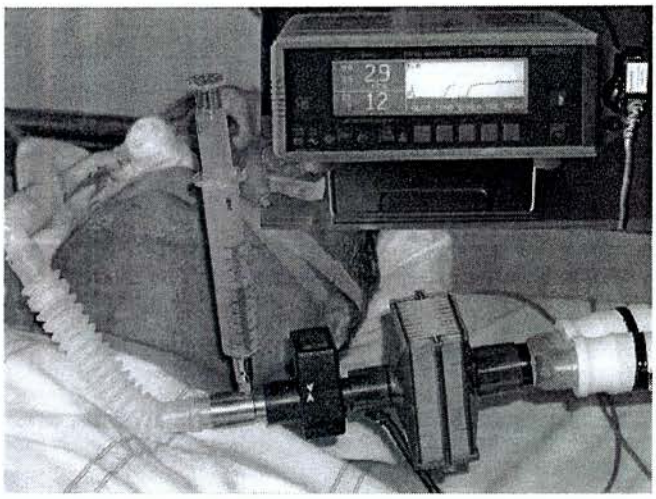

Figure 1: An example of alveolar breath sampling at the bedside. Exhaled air is withdrawn manually by means of a gas tight syringe during the alveolar phase of expiration.

Inflammation and oxidative stress are among the most important reasons of disease and organ damage in critically ill patients. Mechanisms of these diseased states are poorly understood and treatment often remains merely symptomatic. Breath tests involving lipid peroxidation markers, such as ethane and pentane, could enable a better understanding and tailoring of therapy of oxidative stress and

inflammation. Breath volatiles are linked to acute lung diseases such as pneumonia and ARDS and to chronic affections such as asthma or COPD and exhibit, therefore, a considerable potential for diagnostics and surveillance of the critically ill. Since many bacteria produce volatile substances when they are growing even recognition of infectious agents from exhaled air seems possible. In addition, breath volatiles can provide valuable information on metabolic states, organ dysfunction and allograft rejection in the critically ill.

Crucial problems hampering the use of exhaled VOCs in clinical practice are the lack of standardization of analytical methods and the hugh variation of results between different studies. Other parameters that can affect results are inspired substance concentrations. Addition of inhaled concentrations to exhaled concentrations may blur the concentration differences present in the blood. If volatile substances found in the breath are to be used as markers of disease or health their physiological meaning, their pathway of generation and their clinical relevance in terms of sensitivity and specificity should be known. In addition, possible effects of contaminations from the laboratory or from the specific environment have to be taken into account, since qualitative and quantitative composition of background air may vary considerably from one place to the other.

Fast and reliable diagnostic methods play an important role in critical care. New techniques have to be without risk for the patient even if repeated frequently and must provide information beyond conventional analysis of blood and urine. Breath analysis can meet all these requirements. Due to the large number of volatile markers found in exhaled air a great variety of physiological and pathological processes can be monitored via breath analysis. Regarding the ongoing technical progress in this field one may expect that most of the technical problems can be solved in the near future. Promising developments that can be named in this context are automatic CO_2 controlled gas collection, membrane interfaces that could be incorporated into the respiratory system, fast sample preparation via SPME, quick and effective separation through fast or two dimensional GC. If the diagnostic potential of breath analysis is to be used in clinical practice we have to promote standardization and have to learn more about origins, physiological meaning and exhalation kinetics of the volatile substances. The challenge of the next decade will be to reconcile theory and physiology with the complexity of data coming out of a growing number of studies. Ongoing development of real time analysis will further expand the potential of breath analysis as a non-invasive and fast instrument of diagnosis and surveillance in critically ill patients.

Nitric Oxide in Exhaled Breath: a Window on Lung Physiology and Pulmonary Disease

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The discovery that endothelial derived relaxing factor (EDRF) was nitric oxide (NO) brought this highly reactive free radical gas out of relative obscurity as an environmental pollutant and put it on the scientific center stage. This led to an explosion in our knowledge about NO and its role in human physiology and disease. NO is endogenously synthesized by nitric oxide synthases (NOSs) which convert L-arginine to L-citrulline and NO in the presence of oxygen and several cofactors. Three NOSs (type I, II and III) have been identified and are widely expressed in various tissues including the lungs. Once produced, NO is freely diffusible and enters target cells activating soluble guanylate cyclase to produce guanosine 3', 5'-cyclic monophosphate (cGMP) which mediates the majority of NO effects. NO also diffuses into the airway and can be detected in exhaled breath of all humans. NO is formed in high concentrations in the upper respiratory tract (nasopharynx and paranasal sinuses). Our studies have also conclusively demonstrated that the lower respiratory tract is a significant source of NO in exhaled breath. Furthermore, we demonstrated that the pulmonary circulation serves as a biologic sink for NO, and is not likely to contribute to NO in exhaled breath.

The functions and effects of NO in the lung/airways reflect its key roles as a vasodilator, bronchodilator, neurotransmitter, and inflammatory mediator. The unique lung anatomy with the close proximity of the airways to the blood vessels allows NO that is produced in high levels in the upper and lower airways by NOSII to affect the pulmonary vascular tone in concert with the low NO levels that are produced by NOSIII in the vascular endothelium. We have demonstrated that endogenous NO levels in the lung change rapidly in direct proportion to inspired oxygen which strongly supports a critical role for NO as mediator of ventilation-perfusion coupling in the lung.

NO also plays a major role in the pathophysiology of lung disease. Patients with pulmonary hypertension (a group of diseases characterized by high pulmonary artery pressures and pulmonary vascular resistance) have low levels of NO in their exhaled breath. Although this is a far more complex issue than the simple lack of a vasodilator, replacement of NO seems to work well in treating the problem and patients on effective therapies have normal NO levels. Patients with asthma have high levels of exhaled NO in their exhaled breath and high levels of NOS II enzyme expression in the epithelial cells of their airways suggesting a role for NO in asthma pathogenesis.

In summary, the ability to measure NO levels in exhaled breath has provided us with a unique opportunity to gain insights into lung physiology and to better understand the pathophysiology of lung disease.

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Diagnostic aspects of exhaled nitric oxide and carbon monoxide in cardiothoracic anaesthesia

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Nitric oxide (NO) plays an important role in both the physiological control of the pulmonary vascular bed and in the pathophysiology of several lung diseases [1,2]. Pulmonary vascular endothelial cells and airway epithelial cells continuously generate NO from the terminal guanidino group of the amino acid L-arginine, the physiological precursor of NO by the action of NO synthases [3]. In the lungs NO has been identified as one of the important regulators of intercellular communication modulating many critical aspects of pulmonary vascular and airway functions. This is either a direct effect of NO as a signaling molecule or is related to the generation of the second messenger cGMP through activation of soluble guanylate cyclase (sGC) in the vascular and airway smooth muscle cells. There is growing evidence to suggest that constitutive NO release is a major protective mechanism against vasoconstriction and airway hyperreactivity. NO also appears to be critical in the determination of cellular phenotype, kinetics of cell cycle, apoptosis, survival and proliferative responses in both the vasculature and airways [4].

Given the pivotal importance of all of these changes in pulmonary inflammation associated with cardiothoracic surgery and particularly acute lung injury (ALI), it is likely that there are considerable changes in the production and bioavailability of NO and in the cellular responses to available NO in the lung. Recent experimental and clinical investigations revealed many insights into basic mechanisms of ALI [5]. In addition, technological developments allowing direct measurements of NO in the expired air have provided an exciting opportunity to evaluate changes in NO production and consumption in the clinical setting [6]. Exhaled NO has become a diagnostic and monitoring tool in chronic lung pathologies in co-operating and spontaneously breathing patients [7-9]. Similarly, there is recent progress in our understanding of the characteristics of NO kinetics in acute lung injury in mechanically ventilated and critically ill patients [10,11].

Similarly to exhaled NO, exhaled carbon monoxide (CO) appears to be a promising non-invasive tool to assess lung inflammation in a variety of conditions [12]. However, the origin and determinants of exhaled CO remains controversial. Particularly, the magnitude of local airway production of CO and contribution from delivery from the pulmonary and systemic circulation has not been established.

The aim of this lecture is several fold. First we will discuss technical aspects of exhaled NO and CO measurements in intubated and ventilated patients. We will then overview data regarding cellular and molecular origin of gaseous NO and CO and their implications to data interpretation in the context of ALI. This will be followed by a review regarding the different mechanisms of altered NO and CO production and bioactivity in the setting of ALI. Finally we will discuss our own experience at the Harefield Hospital regarding exhaled NO and CO in lung inflammation associated with cardiothoracic surgery as seen following cardiopulmonary bypass and lung transplantation.

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Exhaled Nitric Oxide in Hepatopulmonary Syndrome

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The term Hepatopulmonary Syndrome (HPS) is used to indicate abnormal oxygenation (at least alveolar arterial oxygen gradient, $AaDO_2$, higher than 20 mmHg) due to intrapulmonary vascular dilatations (IPVD) in a patient with hepatic disease, most commonly liver cirrhosis.

It is well known that hypoxemia may be associated with liver disease in the absence of cardiac or pulmonary disease. Right-to-left intrapulmonary shunting , alveolar capillary diffusion limitation , or alveolar ventilation-perfusion (VA/Q) mismatch have been the physiopathologic mechanisms to which hypoxemia of liver cirrhosis has been variously attributed. The frequency of oxygenation abnormalities has not been established in the cirrhotic population as a whole : while severe hypoxemia ($PaO_2 < 60$ mmHg) is found in 5-7 % of patients referred for liver transplantation, a widened alveolar-arterial oxygen gradient has been reported in up to 70 % of patients undergoing pre-transplant assessment of pulmonary function . Intrapulmonary vascular dilatations (IPVD) may lead to impaired oxygen exchange as a result of an increase in O_2 diffusion distance across the dilated pulmonary capillaries (diameter ranging from 15 to 500μm, compared to the diameter ranging from 8 to 15μm of normal capillaries) and a decreased transit time of blood through the pulmonary circulation due to hyperdynamic circulation (the so-called diffusion-perfusion defect) (Krowka MJ et al. Chest 1994). Moreover, as a result of vasodilation, there is excess perfusion (Q) in relation to ventilation (V) that is most marked in the low V/Q units of the basal regions of the lung .

NO theory

NO is a powerful local vasodilator which contributes to the normally low pulmonary vascular tone. Vallance and Moncada (Lancet 1991) postulated that an increased production of nitric oxide (NO) may account for the hyperdynamic circulation of liver cirrhosis. Actually serum levels of stable NO metabolites (NO_2^- / NO_3^-) have been found to be elevated in cirrhosis , particularly in biliary cirrhosis . The increased levels of circulating NO metabolites have been related to the elevated concentration of endotoxin .The potential causes for systemic endotoxemia in cirrhosis are many, including decreased clearance function of the liver, increased gut permeability and small intestinal bacterial overgrowth, which is associated with bacterial translocation (Bauer TM et al. Am J Gastroenterol 2002). Exposure to bacteria and their endotoxins, directly or involving cytokines, such as TNFα, has been associated with increased synthesis of NO . Recent investigations have shown that eNOS is the major enzymatic source for NO overproduction in splanchnic circulation of portal hypertensive rats (Wiest R et al. J Clin Invest 1999). It is interesting that endotoxin and TNFα, which are well known stimulators of iNOS , can directly increase the activity of eNOS . Supporting the important role of endotoxin and bacteria in promoting vasodilation through NO overproduction is the anedoctical report of improved oxygenation in HPS following antibiotic treatment . Moreover, in a rat model of HPS, Rabiller et al. (Am J Respir Crit Care Med 2002) reported that prophylactic treatment with norfloxacin decreased the incidence of gram-negative bacterial translocation , the number of macrophages sequestered in pulmonary microvessels, the

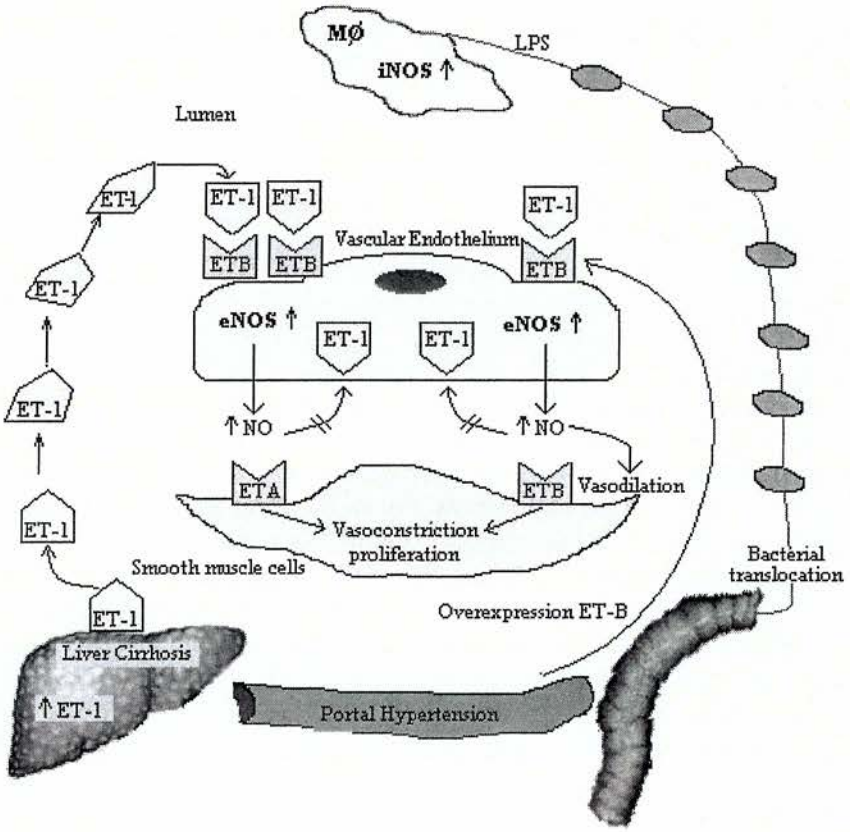
expression and activity of lung iNOS, and the severity of HPS. In a similar rat model of liver cirrhosis, obtained by ligation of common bile duct, Fallon et al. found overactivity of eNOS in endothelial cells of lung vasculature, possibly due to stimulation of endothelyn-1 B type receptors by increased circulating levels of ET-1 . The model implies that HPS develops when both portal hypertension and liver damage coexist, the first by promoting overexpression of ET-B receptors in the lung vaculature, the second by increasing liver and circulating ET-1 levels (Luo B et al. J Hepatol 2003). While ET-A receptors, which are present in vascular smooth muscle cells, mediates vascoconstriction and proliferation, the ET-B receptor, which is found in endothelium and smooth muscle cells, mediates endothelium dependent vasodilation through the release of NO. Actually, increased plasma ET-1 levels have been reported in patients with liver cirrhosis, both with and without HPS (Figure).

Exhaled NO

Increased NO output in exhaled air has been reported in patients with advanced cirrhosis, in whom exhaled NO was positively correlated to cardiac index (Matsumoto A, Ann Intern Med 1995). In a study of 45 cirrhotic patients, exhaled NO output and serum NO_2^-/ NO_3^- have been shown to be significantly higher than in normal controls and in all the patients a significant correlation between exhaled NO and alveolar-arterial oxygen gradient was found (Rolla , Hepatology 1997).In the same study the nine patients who met the criteria for the diagnosis of HPS had also the highest values of exhaled NO . By using the technique of multiple flows analysis of NO output , Delclaux et al. have recently demonstrated that the increased levels of exhaled NO reported in cirrhosis is of alveolar origin (Am J Respir Crit Care Med 2002) and it was correlated with AaDO2. These observations reinforce the hypothesis that NO locally produced in the lung may play an important role in determining oxygenation abnormalities in patients with cirrhosis. A few clinical studies have investigated the relationship between changes in NO produced in the lung and changes in oxygenation abnormalities in liver cirrhosis. In one case of severe HPS, Rolla (N Engl J Med 1994) reported that i.v. methylene blue (a dye that inhibits the effect of NO on soluble guanylate cyclase and thereby prevents the cascade of events leading to vasodilation) acutely improved oxygenation, through a marked decrease in pulmonary shunting. The observation was confirmed by Schenk (Ann Intern Med 2000) , who showed that i.v. methylene blue improved hypoxemia and hyperdynamic circulation in 7 patients with liver cirrhosis and severe HPS . A significant correlation between the decrease in exhaled NO after liver transplantation and the improvement in oxygenation has been reported in 18 patients with cirrhosis who did not have obvious cardio-respiratory diseases (Rolla , Ann Intern Med 1998). Five of these patients met the criteria for the diagnosis of HPS before transplantation and the syndrome was cured by transplantation. The correlation between the decrease in exhaled NO after liver transplantation and the improvement in oxygenation reinforces the hypothesis that NO is an important mediator of impaired oxygenation in patients with cirrhosis. Very recently, in a case of HPS associated with HCV related cirrhosis, nebulized N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthesis, acutely improved oxygenation, because of a decrease of intrapulmonary vascular dilatations, as evaluated by contrast-enhanced echocardiogram (Brussino , Lancet 2003).

In conclusion, many clinical observations support the theory that NO plays a major role in oxygenation abnormalities of patients with liver cirrhosis, complicated by hepatopulmonary syndrome. Alveolar NO concentration might be used to assess the development and the severity of HPS. However, the enthusiasm for inhibiting NO as therapeutical strategy in HPS has been mitigated by the observation of Carter , who showed that the vasodilatory action of NO alone did not completely account for the abnormal vasoreactivity of cirrhotic rat lungs . There is evidence

that NO can induce heme oxygenase-1 (HO-1) expression and HO-1 derived CO may contribute significantly to pulmonary vasodilation . A partial reversal of HPS was obtained by inhibiting HO-1 in rats with HPS induced by common bile duct ligation (Zhang J , Gastroenterol 2003). These observations suggest that the development of HPS is a multifactorial process, involving not only NO, but, at least, also HO-1 and carbon monoxide (Rolla , J Hepatol 2003).



Legend of Figure. Increased production of NO in the alveolar region in hepatopulmonary syndrome. (partially modified by Rolla G. Dig Liv Dis 2004; 36: 303-308)

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A 'Breath Test' for Plants: Photoacoustic Trace Gas Detection and its Application in Plant Science

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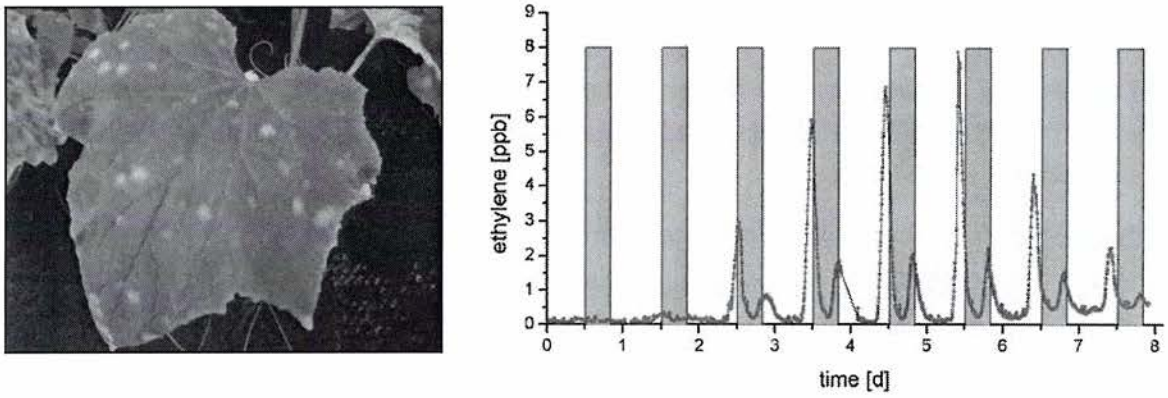
Breath analysis is known as a tool with high application potential in medical diagnostics. Monitoring the emission of volatile organic compounds (VOCs) is expected to yield essential information on the condition of the organism in a non-invasive way.

If taken as a more general term, "breath analysis" is, however, not limited to medical applications. Like any other living organism green plants are known to emit a wide range of VOCs, from simple molecules as methanol or ethylene to rather complex compounds like terpenoids. As in medicine, these molecules may carry very different messages about the metabolic state of the organism, the action of diseases, pathogens or feeding herbivores, or the presence of stress situations. In order to receive these messages, one needs to have the right tools, providing detection limits at the parts-per-billion (ppb) level or even below and a selectivity adequate for the compound mix emitted by the plants.

A tobacco plant in the physicists lab. A "breath" analysis may reveal important information about its health.

Infrared spectroscopic methods are particularly suitable for the detection of small molecules, ranging from methane over ethylene and ethane to isoprene. The talk summarizes our recent work on trace gas detection with parts-per trillion (ppt) sensitivity using photoacoustic spectroscopy (PAS). Taylored to the requirements of plant biological measurements they allow us to continuously monitor the emission of the above mentioned compounds with a time resolution of minutes for periods of days and weeks. These analytical tools are now being used in numerous collaborations with partners from plant biology, ecology and agriculture. Some examples will illustrate which messages we received from the plants and how they were decoded:

The **ethylene** molecule (C_2H_4) was pivotal for the establishment of trace detection in plant biology. Using photoacoustic spectroscopy with CO_2 lasers, it can be detected down to low ppt concentrations. And despite its simple chemical structure it is an important plant hormone: Ethylene is involved in numerous developmental processes ranging from seed germination over growth and flowering to senescence. In addition to that, this molecule is known as a stress indicator: Increased levels of ethylene emission are frequently observed in connection with different types of exogenous or endogenous stress factors. The continuous observation of the emitted ethylene thus allows us, for example, to monitor the interaction between a plant and a pathogen or a herbivor over several days in a non-invasive way. Other applications include the analysis of signal transduction pathways in plants by applying different kinds of elicitor compounds and



A cucumber plant with developing powdery mildew colonies (left) . The ethylene emission from the leaf, measured over several days (right), is highly synchronized. How does this happen? And why?

monitoring the temporal evolution of the ethylene signal. Here a continuous monitoring has the clear advantage over simple sampling techniques, since it allows to discriminate different factors which may have an influence on the ethylene emission.

Based on the solid base of experience collected with ethylene monitoring, the target list was extended to other molecules: **Ethane** (C_2H_6) is known to be a product of lipid peroxidation in cells, and it's monitoring is expected to give information on the oxidative status in the tissue. With a simultaneous ethylene detection it helps to observe how an external stress condition may transform into a damage.

Isoprene (C_5H_8) is a compound emitted in large quantities both in plant and animal kingdom. While its role in plant biology is still not fully understood, it is of great importance as a biogenic trace gas in tropospheric chemistry, maily due to its high reactivity of OH, one of the "cleansing agents" of the atmosphere. Infrared spectroscopic isoprene detection opened one possible way for the detection of different isoprene isotopomers, as it is required for labelling studies. Such experiments help to better understand the regulation of isoprene synthesis by green plants under different conditions and thus to develop better models for atmospheric chemistry.

Other molecules which have also been studied include **acetaldehyde** and **ethanol**, which are produced under anoxic conditions, and **methanol**, the most simple volatile emitted by plants.

All these applications require different infrared laser systems and the development of an infrastructure which allows to conduct experiments according to the requirement of plant biology. While such plant biological experiments are clearly simpler to conduct than medical studies, they may quite well deliver some very helpful experience concerning the analysis of "breath samples" with high sensitivity and selectivity.

The talk summarizes the intensince work over several years on the application of photoacoustic trace gas detection to plant biology. The studies were financially supported by the German Ministry for Science and Education (BMBF) and the Deutsche Forschungsgemeinschaft (DFG). The authors greatfully acknowledge the fruitful collaboration with colleagues from plant physiology, agriculture and ecology and the contributions from numeorus graduate students which were essential for the success of the work.

Deuterium analysis of water vapour using flowing afterglow mass spectrometry, FA-MS: total body water and the transport properties of the peritoneal membrane.

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We have developed flowing afterglow mass spectrometry, FA-MS as a new method for the measurement of the deuterium to hydrogen abundance ratios, D/H, in water vapour, in particular that in exhaled breath and above aqueous liquids such as urine and dialysate fluid. Major objectives were to make possible real time physiological measurements, specifically total body water, TBW, determinations and water transport across the peritoneal membranes of patients on peritoneal dialysis.

In FA-MS, a swarm of H_3O^+ precursor ions are created in a flowing helium carrier gas by a weak microwave discharge. These precursor ions react with the H_2O, HDO, $H_2{}^{17}O$ and $H_2{}^{18}O$ molecules in the water vapour in a humid air sample introduced into the carrier gas forming the cluster ions $H_3O^+(H_2O)_3$ at a mass-to-charge ratio, m/z, of 73 and their isotopomer variants $H_8DO_4^+$ and $H_9{}^{17}OO_3^+$ at m/z 74 and $H_9{}^{18}OO_3^+$ at m/z 75. These ions are detected and the signal levels recorded by a downstream analytical mass spectrometer. In principle, both the D and ^{18}O content of the water vapour can be obtained from a proper statistical analysis of the signal levels of the ions at m/z values of 73, 74 and 75 making allowance for the contribution of the $H_9{}^{17}OO_3^+$ isotopomer ions to the m/z 74 signal level. However, attempting to measure the larger count rates of the m/z 73 ions simultaneously with the much smaller count rates of the m/z 74 and 75 isotopomers is inherently inaccurate with the particle multipliers (counters) available (e.g. channeltrons). So we adopt the known fractional abundance of ^{18}O in water vapour, and accounting for the contribution of the isotopic ions $H_9{}^{17}OO_3^+$ at m/z 74, a measurement of the m/z ratio 74/75 (see Figure 1) provides the fractional D abundance in the water vapour. Using FA-MS, the D abundance in single exhalations of breath can be determined, on-line and in real time. If this can be achieved to sufficient accuracy then valuable near-patient measurements can be achieved in the clinical environment (see below).

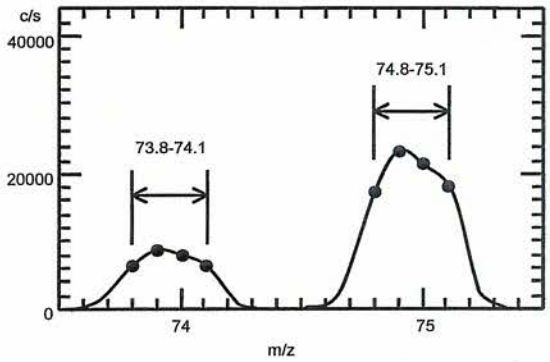

Figure 1 FA-MS spectrum of ions at m/z values of 74 ($H_8DO_4^+$ and $H_9{}^{17}OO_3^+$) and 75 ($H_9{}^{18}OO_3^+$).

The accuracy and precision of FA-MS for absolute D abundance measurements in water vapour has been established using standardised tap water/D_2O mixtures prepared gravimetrically, within the range from 155 ppm (local tap water) to 1100 ppm, This shows that a precision and accuracy of 1% can be achieved, which is quite adequate for most physiological measurements, including the rapid non-invasive measurement of TBW using deuterium breath analysis following D_2O ingestion.

The ability to measure TBW accurately and non-invasively represents an important advance in body composition research. Breath analysis by FA-MS provides the opportunity. After obtaining a baseline measurement of D (combined in HDO) in breath water from only single breath exhalations, an accurately measured dose of D_2O is ingested. The breath water D is determined, again from single exhalations over a period of two hours at frequent intervals (typically two to three minutes depending on how many people are simultaneously involved in the study). The data for all individuals show (see Figure 2) an initial immediate peak due to HDO

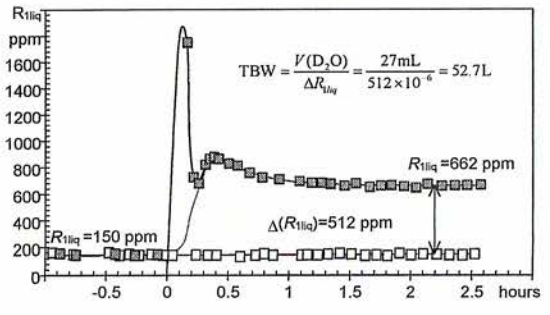

Figure 2 Time dependence of deuterium abundance in the TBW after ingestion of 27 ml of D_2O.

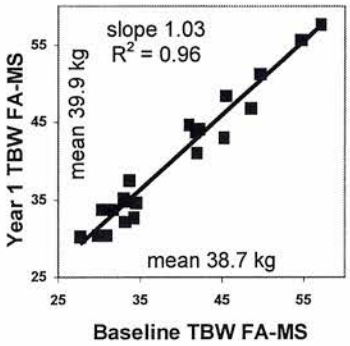

Figure 3 TBW measurements of 24 volunteers (x axis) plotted against the TBW obtained one year later (y axis) for the same subjects.

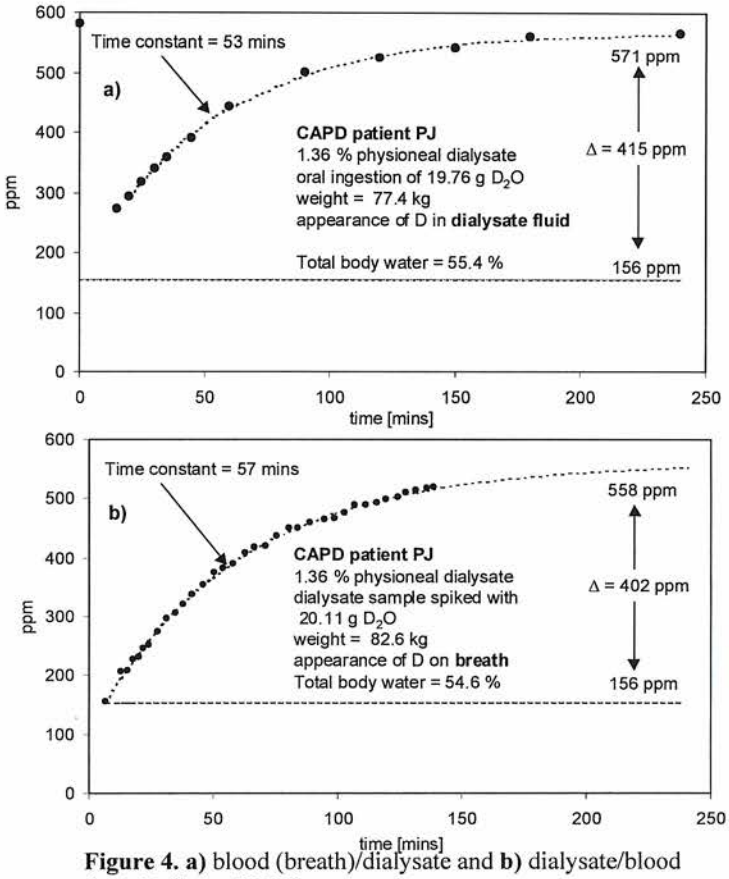

Figure 4. a) blood (breath)/dialysate and **b)** dialysate/blood (breath) flow of HDO.

remaining in the oral cavity, then a second peak as the breath deuterium increases to a maximum due to gastrointestinal absorption into the blood stream (and hence the breath), which then falls to an equilibrium as the deuterium equilibrates with the TBW. The increase of the deuterium from the baseline value to the equilibrium value is used to calculate the TBW. A study of the TBW of 24 volunteers using FA-MS (see Figure 3) and also bioimpedance measurements have shown that the mean TBW of these volunteers varied only by as little as 1 kg over a period of twelve months.

FA-MS has also been used to study the rate of increase of HDO in dialysate fluid (used by patients being treated by peritoneal dialysis) following an oral dose of D_2O (Figure 4a). This is allowing the transport properties of peritoneal membranes to be studied. In parallel studies, the increase of breath water D has been studied following the introduction of a known amount of D_2O to the dialysate fluid. Breath samples can be taken at time intervals as short as 30 second, which provides quality data (Figure 4b) that allows accurate modeling of the transport properties of the peritoneum. Such measurements are assisting the renal physician to provide more appropriate dialysate concentrations.

Further reading

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New approaches to the analysis of small hydrocarbons in exhaled breath: Breath test for the monitoring of ischemia-reperfusion injuries

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Breath tests are attractive because they are non invasive and can be repeated frequently in the dynamically changing state of critically ill patients. Ischemia/reperfusion injuries are a major cause of morbidity and mortality in critically ill patients. Lipid peroxidation represents a fundamental mechanism of these processes. Any method assessing lipid peroxidation markers in a fast and easy way could considerably improve monitoring and treatment in critical care. Small hydrocarbons, such as ethane and pentane, are regarded as the most reliable markers of lipid peroxidation. Multibed adsorption techniques and SPME were used to establish correlations between the chemical composition of breath and clinical conditions of laboratory animals and patients suffering from ischemia-reperfusion injury.

Methods: Ischemia/reperfusion was induced in pigs by clamping of the upper mesenteric artery for at least 60 min. Patients were investigated following extracorporeal circulation for CABG or valve replacements. Exhaled breath was collected at the laboratory into inert tedlar bags. 100 ml of breath were preconcentrated on multibed sorbent traps containing Tenax, charcoal and Carboxen. Samples were analyzed using automatic thermodesorption, cryofocusation, GC/MS and GC/FID. For SPME 10 ml of alveolar breath was sampled from mechanically ventilated patients by means of a CO_2 controlled method. Samples were analyzed using automatic solid phase extraction, desorption and GC/MS.

Results: Exhaled ethane concentrations increased after abdominal ischemia in pigs. In patients undergoing cardiac surgery with extracorporeal circulation (ECC) pentane concentrations increased immediately after skin incision/sternal sawing but not following ECC. Volumes of exhaled air required for analysis could be reduced when optimized sample preconcentration and cryofocussation were used. Methane and ethane could be determined in the upper ppt range by means of FID. Inspired C1, C4 and C6 concentrations were higher than exhaled concentrations.

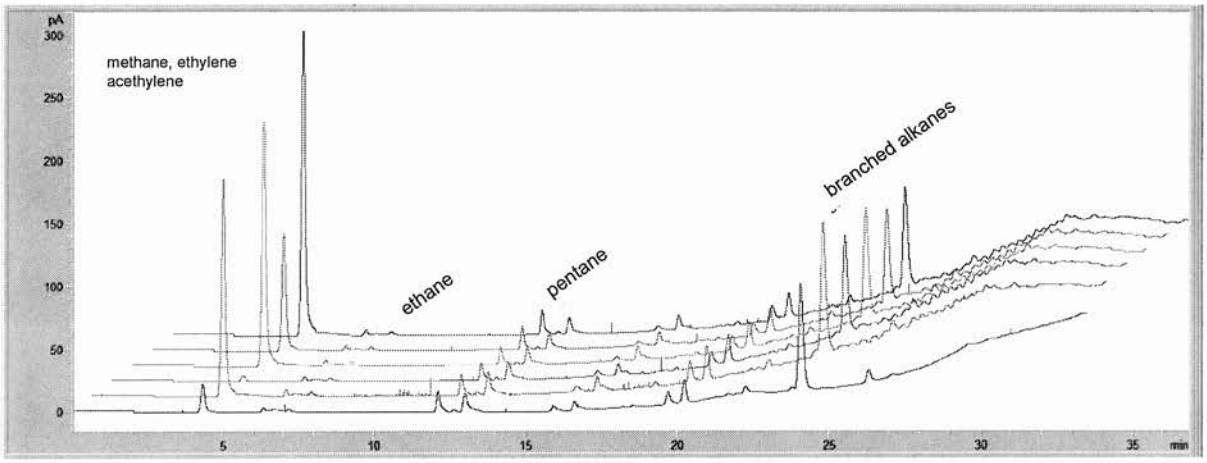

Figure 1: concentration profile of volatile breath markers during ischemia/reperfusion in pigs. (clamping of upper mesenteric artery for 70 minutes). The bottom line was measured during ischemia, the next lines correspond to 1 min, 5 min, 10 min, 15 min, 20 min after unclamping (reperfusion).

Conclusions: Concentrations of exhaled breath markers vary during ischemia/reperfusion. Mechanisms of these diseased states are poorly understood and treatment often remains merely symptomatic although the impact on patients' survival is high. Breath tests involving lipid peroxidation markers, such as ethane and pentane, could enable better understanding and tailoring of therapy of ischemia reperfusion injury. Hence, breath analysis via multibed adsorption or SPME may help to improve survival of critically ill patients.

Abstracts of posters

A standardized CO_2 controlled alveolar breath sampling technique

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Background: A crucial issue in the analysis of exhaled breath is the collection of gaseous samples. Mixed expiratory air that has frequently been used for the analysis of volatile breath markers is contaminated with variable amounts of dead space gas. Hence, the analysis of pure alveolar gas is the method of choice if contamination of samples is to be minimized. A controllable and effective method of alveolar gas sampling is the CO2 controlled technique described by Schubert et al [1]. The purpose of this study was to evaluate a simplified version of this method using visual CO_2 control by means of a commercially available capnometer.

Methods: After approval by the local ethics committee and after having obtained informed consent by the patient or his next of kin 22 mechanically ventilated patients of an interdisciplinary ICU were enrolled into the study. Without changing patients' ventilator settings alveolar and mixed expiratory gas, and arterial blood was sampled. PCO_2 in blood and gas was determined in a blood gas analyzer (Radiometer, Copenhagen, Denmark). In addition, end tidal PCO_2 was monitored in all patients by main stream capnometry allowing real time CO_2 detection. Alveolar gas sampling was done in the following way: a stainless steel Tee and the CO_2 measuring cuvette were introduced into the respiratory system near the endotracheal tube [Figure 2]. 10 ml of exhaled air were drawn into a gastight syringe connected to the stainless Tee. Taking the gaseous samples was visually synchronized with the expired CO_2 in the way that gas was only withdrawn during the alveolar phase of the capnogram [Figure 1].

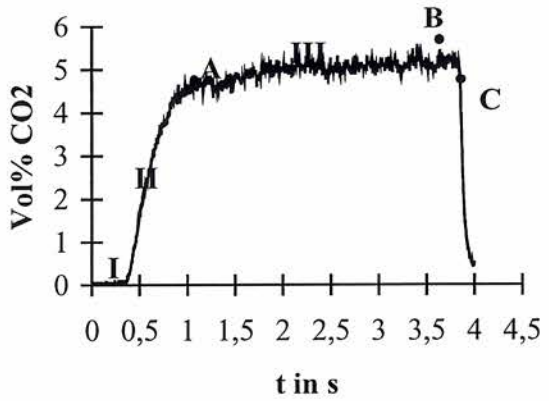

Fig. 1 Tracing of exhaled CO_2 concentrations (vol% CO_2) and points of visual controlled sampling. A: start of alveolar sampling; B: end of alveolar sampling (maximal CO_2 concentration); C: End of expiration
I: CO_2 free inspiratory phase; II: mixing phase; III: alveolar phase

Each measurement was done in duplicate using two different respiratory cycles. To assess the reproducibility of the alveolar gas sampling technique CO_2 and isoprene concentrations were determined in 10 alveolar gas samples of one patient.

Results:

Alveolar CO_2 contents measured during two different respiratory cycles were identical (p for difference 0.86). The variation of the CO_2 and isopren content during 10 measurements in one patient was lower than 4 %. CO_2 contents in visually controlled alveolar samples, in mixed expired samples, in arterial blood and end tidal CO2 content were significantly different from each other ($p<0,05$). (Table1). Arterial PCO_2, PCO_2 in alveolar gas and end tidal PCO_2

showed positive correlation. CO_2 analysis in mixed expiratory gas did not correlate with the other CO_2 measuring methods.

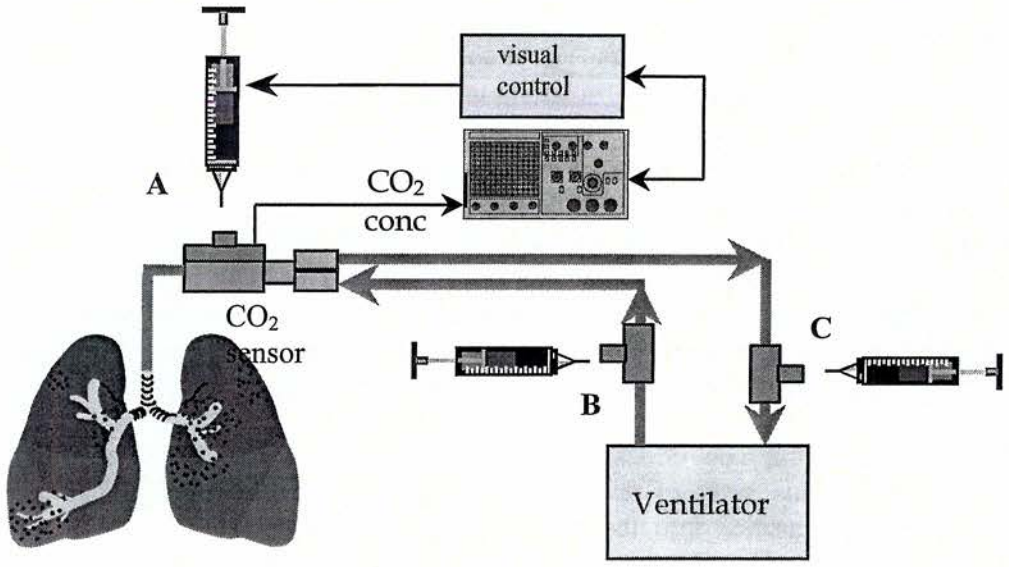

Fig. 2 Schematic drawing of the visual CO_2-controlled sampling device
A: Tee for alveolar gas sampling close to the endotracheal tube; B: Tee in the inspiration limb of the ventilator; C: T piece for mixed expired sampling in the expiration limb

	Blood	Alveolar gas (CO_2 controlled)	Capnography	Mixed exhaled breath
N	22	22	22	22
Mean(kPa)	5,46	4,37	4,66	2,43
Std.Dev	0,62	0,65	0,56	0,81

	Alveolar gas probe 1 (CO2 controlled)	Alveolar gas probe 2 (CO2 controlled)
N	22	22
Mean(kPa)	4,37	4,29
Std.Dev	0,65	0,69

Table1: Alveolar and mixed exp. CO_2 concentrations Table2 CO_2 concentrations (repro)

Discussion: Sampling of alveolar gas by means of visual CO_2 control proved to be reliable and reproducible. Alveolar, mixed expiratory, end tidal and arterial PCO_2 being significantly different from each other proved the consistency of our data. End tidal CO_2 contents have to be higher than alveolar contents measured under visual CO_2-control since the latter represent maximum expired concentrations whereas alveolar PCO_2 is obtained by averaging CO_2 during the alveolar phase. For reasons of respiratory physiology, arterial PCO_2 always is higher than any concentration in exhaled air. Alveolo-arterial differences *($aADCO_2$)* calculated from end tidal (0,8 kPa) or alveolar PCO_2 (1,09 kPa) were comparable to results in healthy volunteers.
Conclusion: The visually CO_2-controlled sampling technique of alveolar gas is a reliable and reproducible method that can easily be performed at the bed side without sophisticated equipment. It represents an important step in simplifying and standardizing breath analysis in mechanically ventilated patients.

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Breath gas analysis in paediatric patients suffering from propionic acidaemia

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INTRODUCTION

Propionic acidaemia (PA) is caused by propionyl-CoA carboxylase (PCC) deficiency [1]. PCC is a mitochondrial enzyme that catalyzes the ATP-dependent carboxylation of propionyl-CoA to D-methylmalonyl-CoA (fig. 1). Propionyl-CoA is produced in catabolism of isoleucine, valine, methionine, threonine, odd-chain fatty acids, thymine, uracil and cholesterol. Besides elevated excretion of organic acids, Menkes reported the urine of PA patients to contain large amounts of the four-carbon ketone butanone (a by-product of isoleucine catabolism) as well as of the five- and six-carbon pentanones and hexanones [2]. Today, a standard PA therapy includes protein-defined nutrition, substitution of essential amino acids, vitamins and treatment of complications, i.e. seizures. So far, no breath gas analysis has been performed in PA patients to determine these unusual metabolites.

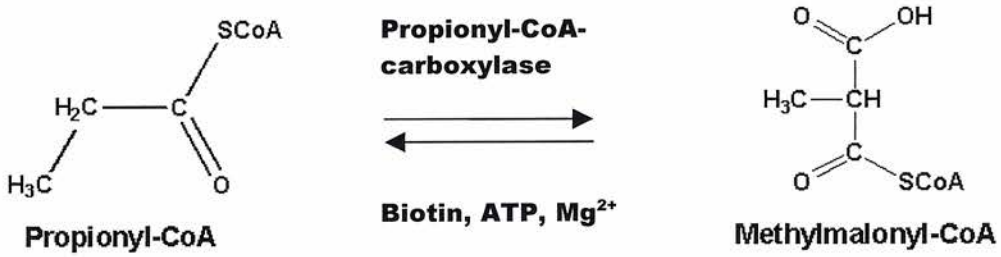

Fig. 1: Reactions catalyzed by the enzyme propionyl-CoA-carboxylase, whose concentration is reduced in patients suffering from propionic acidaemia

METHODS

Breath gas was collected in 3-L Tedlar® (polyvinylfluoride) bags, the reference ambient air inhaled by patients or probands in 1-L glass bottles. All collected samples were analysed at 40°C by means of proton-transfer-reaction mass spectrometry (PTR-MS, Ionicon FDT-s) for component quantification. The reported masses are those of the protonated species (molecular mass + 1 u) as detected in PTR-MS. Additionally, some samples were further investigated using gaschromatography with mass spectrometric detection (GC-MS, Agilent 6890N-5973N) to tentatively identify a few interesting breath air components by a library search (Wiley7n library).

PATIENTS

We collected 2 breath gas samples per patient (1 exception) at an interval of ca. 1 h from 4 patients (2 girls and 2 boys aged 6, 15, 17 and 19) suffering from propionic acidaemia confirmed by molecular-genetic and enzymatic tests and treated by standard therapy. Two of them tested on a further day, giving a sample total of $N = 11$. Altogether 44 healthy children producing 2 breath gas samples each provided $N = 88$ control samples.

RESULTS

The volume fraction differences (expiratory – inspiratory value) of mass 73 u and mass 115 u observed by PTR-MS were found to be significantly increased in the breath gas of PA patients compared to the control group. They are summarised in tab. 1 and depicted in fig. 2.

	mass 115 u patients	(controls)	mass 73 u patients	(controls)
N	11	(88)	11	(88)
mean	8.5	(0.2)	14.0	(-1.7)
median	8.9	(0.2)	12.8	(-1.4)
SD	5.5	(0.2)	4.0	(3.1)
min	-0.02	(-0.3)	6.7	(-12.9)
max	19.7	(0.7)	20.2	(6.2)

Tab 1:
Expiratory – inspiratory volume fractions (in ppb) of mass 115 u (tentatively 3-heptanone) and mass 73 u (butanone or butanal?) in PA patients. *N* is the number of samples in the respective group (number of patients $n=4$, number of control children $n=44$).

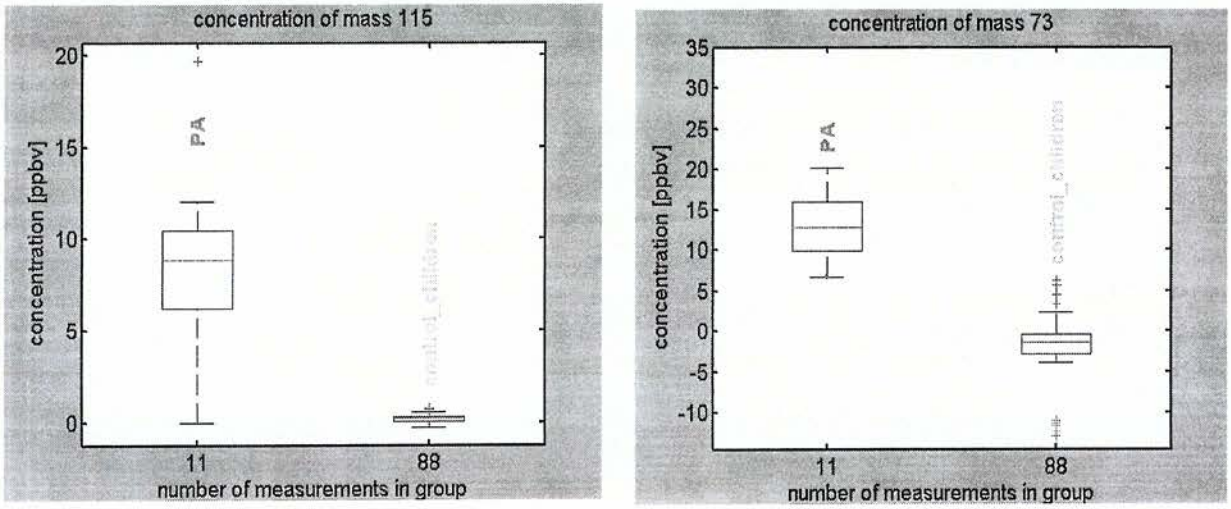

Fig. 2: Expiratory – inspiratory volume fractions of mass 73 u (butanone or butanal?) and mass 115 u (3-heptanone according to Wiley7n MS library search, quality factor 90) in boxplot representation (median value and 25- and 75-percentiles).

DISCUSSION

The compound exhibiting mass 73 u detected by PTR-MS could be butanone or butanal. Furthermore, GC-MS analysis of the substance detected at 115 u by PTR-MS suggests the presence of 3-heptanone (according to Wiley7n library search, quality factor 90). Should this be confirmed later on, enhanced ketone exhalation through the lungs would add to the already known increased urinary excretion as a PA symptom. Therefore, we speculate that breath gas analysis will reveal itself a useful tool for the long-term follow-up of PA patients.

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Sampling of breath gas using Tedlar® bags

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INTRODUCTION

In our studies, the patients' or probands' breath gas is usually collected in Tedlar® bags unless it is continuously sampled for online monitoring. Since we reuse the bags many times, proper cleaning after each use is important in avoiding artefacts. In case of reuse, the manufacturer, which extends its warranty to single use only, recommends thorough cleaning (at least 3-fold flushing) with purified air or nitrogen (N_2) and background verification before sampling again. The following is a short communication about preliminarily work still in progress.

METHODS

4 Tedlar® (polyvinylfluoride) 3-L bags (SKC 232 Series bags) already used several times were thoroughly flushed with N_2 (quality 5.5) and finally inflated by a healthy test person aged 25 to 45. Another unused bag was also thoroughly flushed with N_2 (quality 5.5) before being filled with N_2 (quality 5.5) for reference. Subsequently, all bags heated to 40°C were analysed by proton-transfer-reaction mass spectrometry (PTR-MS, Ionicon FDT-s) for component quantification in the mass range 20–230 u, the reported masses being those of the protonated species (molecular mass + 1 u). Then they were all evacuated and flushed with N_2 (quality 5.5) before being reanalysed the first time, followed by 3 more such evacuating-flushing-analysing cycles. Additionally, after preparation the reference bag and one filled with breath air were further analysed using gaschromatography with mass spectrometric detection (GC-MS, Agilent 6890N-5973N) in order to tentatively identify through MS library (Wiley7n) searching the most prominent compounds of the range 33–230 u.

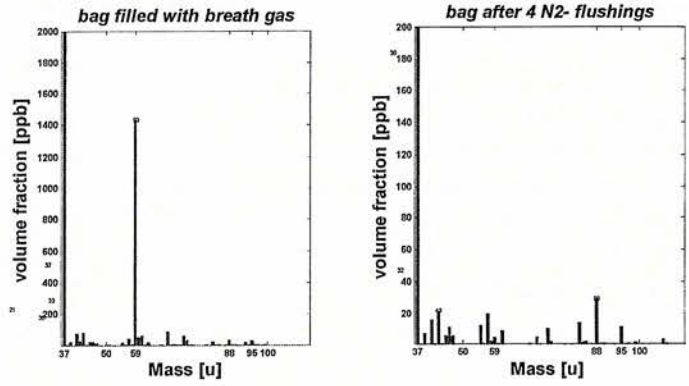
RESULTS

The table shows concentrations of different components for breath gas of 4 test persons collected in Tedlar bags and the same bags after *n* flushings with nitrogen. Components are acetone (mass 59 u), water clusters (mass 37 u and 55 u), and bag-specific compounds phenol (mass 95 u) and dimethylacetamid (mass 88 u).

	components	c(0)	Rates of decrease: c(n)/c(0) after the nth flushing			
			n=1	n=2	n=3	n=4
N2 Bag	37,55 (water cluster)	898	0.90	1.25	0.94	1.16
testbag1		2917	0.40	0.42	0.37	0.35
testbag2		3137	0.42	0.44	0.40	0.37
testbag3		2665	0.54	0.55	0.58	0.51
testbag4		2909	0.56	0.57	0.55	0.49
mean/ std		**2907 +/- 193**	**0.50 +/- 0.09**	**0.50 +/-0.08**	**0.48 +/-0.10**	**0.43 +/-0.08**

N2 Bag	88, 89 phenol 95,96 dimethylacetamid	4300	0.79	1.01	0.67	0.8122
testbag1		230	0.69	0.67	0.52	0.55
testbag2		104	0.70	0.68	0.54	0.60
testbag3		64	0.67	0.65	0.59	0.67
testbag4		92	0.54	0.57	0.49	0.53
mean/ std		**124 +/- 74**	**0.65 +/- 0.08**	**0.64 +/- 0.05**	**0.53 +/- 0.05**	**0.59 +/-0.06**
N2 Bag	59 acetone	10	0.691	0.95	0.79	0.779
testbag1		1832	0.016	0.01	0.00	0.004
testbag2		522	0.030	0.02	0.01	0.014
testbag3		1486	0.018	0.01	0.01	0.004
testbag4		943	0.025	0.01	0.01	0.008
mean/ std		**1196 +/-579**	**0.022 +/- 0.007**	**0.010 +/-0.005**	**0.008 +/-0.004**	**0.007 +/- 0.005**

The fig shows a PTR-MS measurement of breath gas for one test person after sampling with a Tedlar bag, and the same bag after 4 nitrogen flushings. Observe the high concentration at masses 88 and 95 (bag-specific compounds dimethyl-acetamide and phenol).



DISCUSSION

Sampling of breath gas is a delicate and not standardized matter. Different techniques are used: Cartridges filled with activated carbon, Tedlar bags, Teflon bags, metal canisters, glas tubes, solid phase microextraction (SPME), etc. A comparison of results based on different sampling techniques is often impossible. In the present study, the use of Tedlar bags for sample collection and its influence on concentration measurements are investigated. Three flushing cycles are sufficient for most components in breath gas to avoid contamination of samples (due to previous use with another test person). Phenol (mass 95 u) and dimethylacetamid (mass 88 u) are bag-specific compounds, whose concentrations remain high even after several flushing cycles.

Real-time detection of Common Microbial Volatile Organic Compounds (VOCs) from medically Important Fungi by SIFT-MS.

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The headspace above actively growing fungi, Aspergillus flavus, Aspergillus fumigatus,Candida albicans, Mucor racemosus, Fusarium solani and Cryptococcus neoformans grown on or in malt extract agar (MEA), Columbia agar (CA), Sabouraud's dextrose agar (SDA), blood agar (BA) and brain heart infusion broth (BHIB) was examined. The common abundant VOCs ethanol, methanol, acetone, acetaldehyde, methane thiol and crotonaldehyde (2-butenal) were detected and quantified. The fingerprints of the VOCs were strongly dependent on the culture medium. (1=very low counts (<25), 5= very high counts (>1000). Numbers in parenthesis are uncertain.

Fungus and Medium	Ethanol	Acet-aldehyde	Acetone	Methane-thiol	Croton-aldehyde
Aspergillus flavus					
• **BHIB**	5	4	-	-	(1)
• **CA**	3	2	3	-	-
• **MEA**	5	5	-	-	-
Aspergillus fumigatus					
• **BHIB**	1	-	(2)	-	-
• **CA**	2	-	-	-	-
• **MEA**	5	3	(1)	-	-
Candida albicans					
• **BHIB**	5	3	-	-	(3)
• **CA**	-	-	2	2	-
• **MEA**	5	3	-	-	3
Cryptococcus neoformans					
• **BHIB**	5	2	-	-	(2)
• **CA**	-	-	-	-	-
• **MEA**	5	5	2	-	3
Fusarium solani					
• **BHIB**	4	-	-	(1)	-
• **CA**	-	-	-	3	(2)
• **MEA**	5	5	-	-	3
Mucor racemosis					
• **BHIB**	5	3	-	-	3
• **CA**	3	-	3	2	2
• **MEA**	5	5	-	-	3

Applications of selected ion flow tube mass spectrometry (SIFT-MS) in addiction research.

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The technique of trace gas analysis of exhaled air and liquid headspace using SIFT-MS has been developed at Keele University [1] and has resulted in the development of clinical applications of the technique [2 3]. The technique is unique in that it provides real time, online analysis of breath and of liquid headspace and is non-invasive. The use of breath analysis in addiction studies has been limited apart from the detection of alcohol.
The diagnostic potential of breath sampling was described in 1983 [4], but there have been limited practical applications in addiction. Verstraete [5] describes the potential for detection of cannabis on the breath but acknowledges that most studies have been hampered by problems with techniques which require complex analytical methods on prepared stored samples.

The application of SIFT-MS with its ability to detect trace gases to the parts per billion (ppb) in real time may overcome these issues and lead to the development of non-invasive diagnostic techniques for use in addiction treatment and research.
The SIFT-MS technique uses precursor ions H_3O^+, NO^+ and O_2^+ which are generated in a discharge ion source, mass selected by a quadrupole mass filter and then injected as selected ionic species into a fast-flowing helium gas carrier. The sample gas to be analysed is introduced into carrier gas through an inlet port where the trace gases react with the chosen precursor ion species. The precursor ions and the product ions of the reactions are then detected and counted by a downstream quadrupole mass spectrometer.
We will describe the first stages of a programme of research focusing on developing a methodology for breath detection of markers of the use of cannabis. A major objective of the reported study is to investigate whether or not these compounds are amenable to individual identification and quantification using SIFT-MS. The m/z values of the parent ions of THC and CBD molecules (see Figures 1 and 2) are beyond the range of the current SIFT-MS system and this study explores identification of marker ions of lower m/z resulting from either pyrolysis or fragmentation in the analytical ion-molecule reactions.

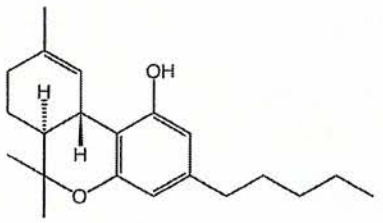

Figure 1 Delta-1-THC delta-9-Tetrahydrocannabinol, (THC) $C_{21}H_{30}O_2$ (Mol.wght 314.46)

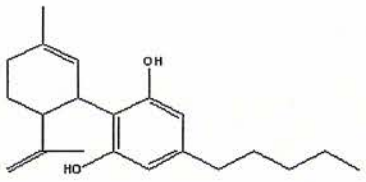

Figure 2. Cannabidiol (CBD) $C_{21}H_{30}O_2$ (Mol wght 314.46)

Two preliminary studies will be described. The first involves the analysis of the volatile compounds in the air above prepared concentrated samples of THC and CBD provided by GW Pharmaceuticals and stored under a Home Office Licence. The second study investigated the vapour produced by heating these samples in a commercially available "drug vaporizer" commonly used for inhalation of cannabis fumes.

Experiment 1 – using samples of the air above THC and CBD at room temperature

We will describe the techniques used to identify monitor ions of lower m/z than those of the protonated or ionized parent compounds and the determination of estimated concentrations of THC (80ppb using O_2^+ precursor ions), ethanol (230ppm using H_3O^+) and terpene (3ppm using H_3O^+) in the air above the THC samples. The corresponding data for the CBD will also be presented, CBD (140ppb using O_2^+), ethanol (110ppm) and terpene (8ppm). Monitor ions for THC and CBD are identified for THC (m/z231 and m/z244) and CBD (m/z231, m/z244 and m/z246) as the major ions in the 70 eV electron ionization spectra.[6]

Experiment 2 – using the vapour from heating samples in a commercially available "drug vaporizer" commonly used for inhalation of cannabis.

Three phases of the pyrolysis of the THC and CBD samples are identified in Figure 3.

Phase 1 occurs when the low boiling points/low molecular compounds vaporise. **Phase 2** occurs when the higher boiling points/high molecular weight compounds vaporise. **Phase 3** is the vaporising / pyrolysing phase.

In **Phase 3**, the highest molecular weight compounds begin to vaporise and also probably partially pyrolyse as the temperature approaches 200C°. The ions observed at m/z of 104 and 204 using NO^+ precursor ions in this phase (Figure 4) are either due to the presence of molecules of molecular weight 104 and 204 (formed by pyrolysis) or are formed from larger molecules by the dissociation that can sometimes occur in the analytical ion-molecule reactions.

Having identified suitable marker ions using headspace and pyrolysis studies, the project will continue by analysing urine headspace of subjects smoking cannabis to attempt to identify additional lower m/z monitor ions. Breath samples will then be obtained from subjects using cannabis which will be analysed by SIFT-MS techniques.

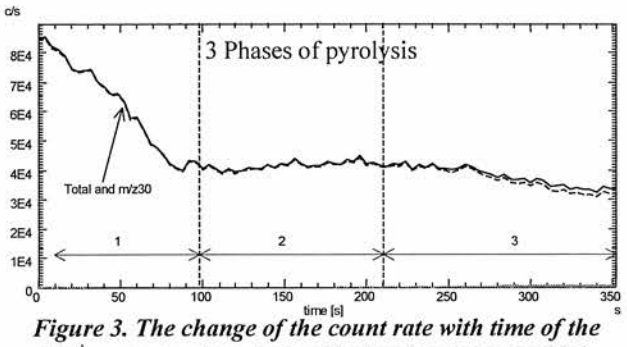

Figure 3. The change of the count rate with time of the NO^+ precursor ions during the heating process which identifies the three phases.

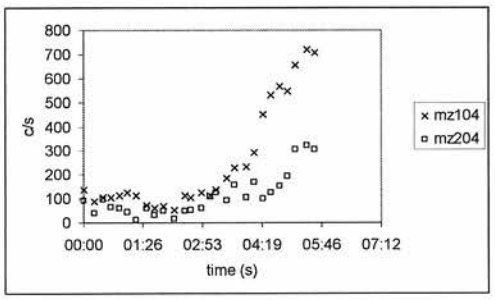

Figure 4. The increasing count rate of the ions at m/z104 and 204 with time during the heating process

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ASSESSMENT OF THE EFFECT OF MAXIMAL EXERCISE ON EXHALED ETHANE AND CARBON MONOXIDE IN HUMAN AND ANIMAL ATHLETES

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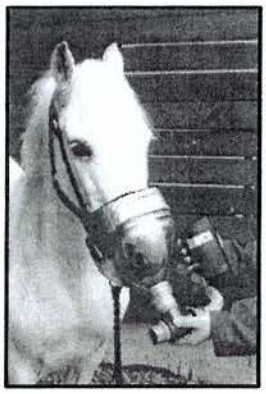
[3] Department of Physics and Astronomy, University of Glasgow

UNIVERSITY *of* GLASGOW

Background: Exercise is associated with production of free radicals and increased oxidative stress in human athletes. Oxidative stress can be assessed *in vivo* using the carbon monoxide (CO) and ethane breath test. Oxidation of cell membrane lipids, and generation of ethane occurs as a consequence of oxidative stress, when anti-oxidant defences are overcome by free-radical mediated attack. Haemoglobin is degraded *in vivo* by the enzymes haem oxygenase-1 and -2 (HO-1 and HO-2) resulting in release of bilirubin, carbon monoxide (CO) and iron. Oxidative stress associated with maximal exercise may induce HO-1 and cause increased exhalation of CO.

Aim: The aim of this study was to investigate the effect of maximal exercise on exhaled ethane and CO in human, canine and equine athletes.

Methods: Human athletes (n = 7) performed a maximal exercise test on a treadmill, and canine (n = 12) and equine (n = 10) athletes exercised at gallop on a sand racetrack. Breath samples were taken at regular intervals before and after exercise in the human athletes, using a mouthpiece connected to a non re-breathing valve. Breath samples were collected immediately before and after exercise in the canine and equine athletes, by allowing the animal to breathe through a face mask connected to a non re-breathing valve and a breath collection bag. Breath samples were stored in gas-impermeable bags for analysis of ethane by laser spectroscopy, and CO was measured directly using an electrochemical CO monitor.



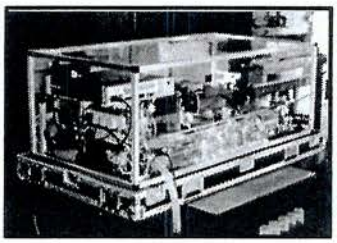


Figure 1: Analysis of exhaled carbon monoxide in a horse (left), and the laser spectroscopy system for analysis of exhaled ethane.

Results and Discussion: Maximal exercise was associated with significant increases in exhaled ethane in the human, equine and canine athletes. There was no difference in exhaled CO detected after maximal exercise in the human athletes, compared to rest; and CO was rarely detectable in the canine and equine athletes. While the CO breath test requires further refinement to provide the necessary sensitivity to detect possible exercise-related changes in HO-1 induction, the ethane breath test can clearly discriminate exercise-induced changes in oxidative stress in the physiological range both in human and animal athletes. This suggests the potential for exploring the adaptive potential of oxidative stress mechanisms in longer-term exercise and also in disease conditions characterised by exercise intolerance.

PROTOCOL FOR PRODUCING AN ION MOBILITY SPECTROMETRY LIBRARY OF VOC IN HUMAN BREATH

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Exciting pioneering studies demonstrated how IMS may be used to monitor human subjects for disease biomarkers. This study is developing a library of IMS data of biomarkers associated with respiratory diseases.

Adsorbent-based sampling approaches suitable for human subjects with impaired respiratory functions are described. These systems enable the researcher to selectively sample different phases of the exhaled breath cycle. The data produced, from thermal desorption GC-MS are combined with studies of the biochemistry and metabolitic features of the disease to identify candidate biomarkers.

Mixtures of biomarkers are injected onto GC-IMS and GC-DMS systems producing data sets that yield response surfaces showing the effect of mass flux on the ion mobility spectrum. Control of temperature, pressure, water concentration and reactant ion chemistry enables a range of potential instrument conditions to be recorded.

This research is leading to the development of clinical IMS and DMS applications.

Poster Presentation:
Breath Gas Analysis in Patients with Malabsorption Syndroms

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Introduction:

Fructose and lactose malabsorbers are characterized by impaired duodenal fructose transport or the deficiency of mucosal lactase activity, respectively. As a consequence, the non-absorbed saccharides reach the colon, where they are broken down by bacteria to short fatty acids, CO_2 and **H_2**. Shortly after the consumption of the malabsorbed saccharides H_2 can be detected in the exhaled breath (**H_2 breath test**).

Recently it was found that fructose and lactose malabsorption is associated with early signs of depressive disorders (1). For this purpose it was investigated whether fructose and lactose malabsorption is linked with an abnormal Tryptophan metabolism (2). Furthermore it was of interest if a sorbitol and fructose reduced diet could influence not only gastronitestinal complaints, but also depressive disorders which are associated with carbohydrate malabsorption (3).

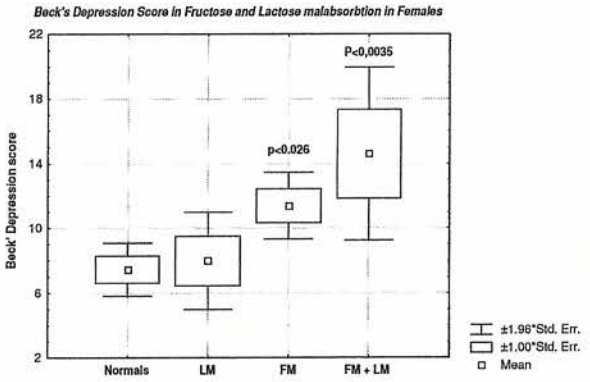

Figure 1: Beck's Depression Scores in Fructose and Lactose malabsorption in females

Methods:

In total three consecutive studies were carried out. Over all 214 volunteers with gastrointestinal complaints were analysed by measuring breath H_2 concentration after an oral dose of 25 g fructose. 111 subjects underwent a H_2 lactose (50 g) test one week after additionally. They were classified in four groups (normals, isolated fructose malabsorbers, isolated lactose malabsorbers and combined fructose/lactose malabsorbers). All patients filled out Beck's depression inventory questionnaire. In the second study blood samples were taken additionally for serum Tryptophan and Kynurenine measurements. In a third study questionnaires with arbitrary scales for measurement of meteorism, stool frequency and quality of life were filled out by fructose malabsorbers before and after a four week sorbitol and fructose reduced diet auxiliary.

Results:

Fructose malabsorption (breath Δ H_2-production > 20 ppm) was detected in 62,5% up to 70% of the tested individuals. 3,6% were only lactose malabsorbers and 11,7% presented both fructose and lactose malabsorption. Isolated fructose malabsorption and combined fructose/lactose malabsorption were associated with significant higher Beck's depression scores ($p < 0,01$) [Figure 1]. Furthermore, subjects with fructose malabsorption showed significantly lower plasma Tryptophan concentrations than those with normal fructose absorption. A sorbitol and fructose reduced diet lowered depression scores by 65,2% and there was a significant decrease of meteorism and stool frequency after four weeks.

Conclusion:

The data show that fructose malabsorption may play a role in the development of depressive disorders in females, whereas combined fructose/lactose malabsorption seems to even more increase the risk of mental depression. Lower Tryptophan levels of the affected subjects could be a reason for this finding. High intestinal fructose concentration may reduce the availability of Tryptophan for the biosynthesis of Serotonin (5-Hydroxytryptamine). A fructose and sorbitol reduced diet in fructose malabsorption seems to increase signs of depression and decrease gastrointestinal complaints such as meteorism and stool frequency. Therefore carbohydrate malabsorption should be considered in patients with signs of depression and disturbances of Tryptophan metabolism.

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Breath Tests in Gastroenterological Practice – carbohydrate malabsorption

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Introduction:

Since the time of Hippocrates physicians have known that the aroma of human breath can provide clues to medical diagnosis. Besides many other diagnostic possibilities in medicine, breath tests (BTs) offer a huge potential of diagnostic possibilities for gastroenterological purposes. Despite the fact that they are a simple, safe and inexpensive alternative to the so called „gold standard" more invasive diagnostic strategies (endoscopy, biopsy), they are underutilized in gastroenterological practice.

The aim of this work is to give an overview of breath testing in gastroenterological practice and to demonstrate their capability of diagnostic possibilities especially in the detection of carbohydrate malabsorption.

The principle of breath testing in gastroenterological practice is to analyse physiological and metabolic processes in an indirect way and to find potential rate limiting factors by tracing metabolites of a specific test substance in the exhaled breath. Predominantly two different sorts of breath tests are performed: 1. Tests with ^{13}C–labelled test substrates (stable, non-radioactive isotope) where CO_2 ^{13}C enrichment in the exhaled breath is determined over a certain period of time. 2. H_2- breath tests (HBT): H_2 doesn't naturally accumulate in the human intestine, only if a non-absorbable, but fermentable substrate reaches the colon and is broken down by colonial bacteria. In HBTs the change of H_2 in the exhaled breath is measured over a certain period of time.

HBT in carbohydrate malabsorption syndromes:

Although all the carbohydrates used for testing yield both H_2 and CO_2 when digested by bacteria, HBTs are used for the diagnosis of carbohydrate malabsorption most of the time. To get satisfactory test results, patient preparation is indispensable. Baseline H_2 is measured before the test substrate is given. Afterwards H_2 levels are measured in defined time intervals for a certain period of time. The length of time over which measurements should be taken is controversial (1). Depending on the specific test and substrate, different H_2 level progressions and peaks show if the test is positive or negative. For the lactulose H_2 BT it is advised to measure H_2 levels every ten to fifteen minutes. A significant rise of H_2 before 60-90 min (which is considered as a normal oro-coecal transit time), indicates bacterial colonisation of the small intestine, no significant increase at all is sign of non-H_2-producers. Half hour steps are scheduled in HBTs with fructose, sorbit and lactose. Fructose-, sorbitmalabsorption and lactosemaldigestion are indicated when the H_2 level of the exhaled breath rises more than 20 ppm over the base level. If the test result is not satisfying it is advised to additionally perform a ^{13}C-breath test before a biopsy specimen needs to be taken (2). Specificity and sensitivity in HBTs lie within 90-100% (1).

Conclusion:

In the past years ^{13}C-breath tests and especially H_2-breath tests have established themselves as simple, accurate and practicable methods for the diagnosis of gastrointestinal disorders. The fact that they are non invasive and inexpensive clarifies their importance even more. Nevertheless they are not the "gold standard" of gastroenterological diagnostics. New studies show that there is a huge potential in breath gas analysis for other diagnostic purposes than the ones shown above (3). However some BTs (liver and pancreatic function etc.) are not yet recommended for routine use (1). Therefore further development and validation have to be carried out in the future.

RATE LIMITING FACTOR	INDICATION FOR	BREATH TEST / TEST SUBSTRATE
Motility	Gastric emptying	^{13}C breath test / ^{13}C-Octanoate (fluids); ^{13}C-Acetate (solids)
	oro-coecal transit time	H_2 breath test / 1g/kg, max 20g lactulose
Bacterial Colonisation of the gastrointestinal tract	Helicobacter pylori	^{13}C-breath test / urea
	SIBOS	H_2 breath test / 1g/kg, max 20g lactulose or 2g/kg, max 50 g glucose
Malabsorption of nutrients	Fructosemalabsorption	H_2 breath test / 1g/kg max.25g fructose
	Lactosemaldigestion	H_2 breath test / 2g/kg, max 50g lactose
	Sorbitmalabsorption	H_2 breath test / 0,5g/kg, max 12,5g sorbit
	Xylitmalabsorption	H_2 breath test / 0,5g/kg, max 12,5g xylit
	Starchmaldigestion	^{13}C-breath test / glucose enriched starch
Efficiency of Resorptioncapability	Celiac disease, malabsorption of nutrients	H_2 breath test/ 1g/kg, max 25g D-xylose
Pancreatic function	Carbohydrate digestion	^{13}C-breath test / cornflakes

Table 1: List of breath tests that can be used in gastroenterological practice.

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Are ethane, pentane and isoprene produced locally in the airways?

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Introduction:
Exhaled ethane, pentane and isoprene have been suggested as markers of oxidative stress in the airways. The main objective was to investigate whether these substances are produced locally in the airways.

Method:
Exhaled air from subjects with physician-diagnosed, stable asthma (n=13) and healthy controls (n=14), all non-smokers, was collected using a single-breath system with controlled exhalation flow-rate, oral pressure and duration. Fraction of exhaled nitric oxide (FENO) was measured on-line at the same time. Three tests were performed: 1. inhalation of purified air (1 breath, 1 and 4 min tidal breathing) before exhalation, 2. breath holding (0, 10 and 20 s) before exhalation, and 3. exhalation at different flow rates (50 and 200 ml s^{-1}). The air from dead space and alveolar space was collected separately. The air samples were transferred to adsorbent tubes and were thermally desorbed and analyzed with gas chromatography (GC)/flame ionization detection (FID).

Result:
Purified air inhalation: There was a rapid and substantial decline in ethane concentrations after inhalation of purified air (1 breath: 35%, 1 min: 47%, 4 min: 58%). Pentane levels decreased more slowly (4 min: 27%). Isoprene levels increased (4 min: 24%). All changes were statistically significant. FENO levels were not significantly changed.
Breath holding: No significant changes in ethane levels could be observed after breath holding. Pentane levels increased after 20 s of breath holding ($p<0.05$). There was an increase in isoprene levels, which was most pronounced after 20 s of breath holding ($p<0.001$). The same was true for FENO levels ($p<0.0005$).
Exhalation flow rate: The ethane concentrations at the lower exhalation flow rate were statistically elevated, compared to concentrations at the higher flow rate ($p=0.0041$). For FENO, the increase at the lower exhalation flow rate was even more pronounced ($p<0.0001$). Pentane and isoprene levels were significantly increased at the higher flow rate ($p=0.0002$ and $p=0.0005$). In subjects with asthma, the ethane concentrations at the lower exhalation flow rate were statistically elevated, compared to concentrations at the higher flow rate ($p=0.0063$), but no difference was observed in healthy subjects.

Conclusion:
The exhaled ethane concentrations in patients with asthma seem to be exhalation flow rate dependent, which might indicate a central airway origin. The major part of pentane and isoprene seem to be of systemic origin.

SELECTIVITY OF SENSOR RESPONSE TO THE BREATH GAS COMPONENTS AS A METHOD OF NONINVASIVE DIAGNOSTICS OF HUMAN DISEASES

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Development of methods of noninvasive express-diagnostics is both vital medical problem and important goal for the up-to-date investigations. Definitely, in the nearest future methods for exhaled breath analysis should take forward among relevant diagnostics technologies with an account of an essential number of markers (see, for example, [1]) of organism state in the breath medium. Efficient approach to the problem is to apply a sensor technique (i.g., [2]).

We developed a series of samples of gas sensors for an analysis of breath constituents. Alkylquinolinium and alkylisoquinolinium derivatives of 7,7,8,8-tetracyanoquinodimethane (TCNQ) complex salts were used as a gas sensitive material [3]. The principle of our samples operation is based on changing of electrical conductivity of a sensor material under gas agent action. The output current signal of a sensor varies under influence of definite components of a breath gas and serves as an indicator of its composition. For metrological and statistical investigation we used an autonomous portable measurement complex, which included a sensor, a power supply, an electric current recorder and a special device to safe an exhaled breath chemical composition. This device ensures an opportunity to avoid influence of any side factor beyond a sample of medium under study.

The main metrological characteristics of sensors have been investigated when composition of exhaled air is being constant or variable. The experiments show high reproducibility of a sensor response signal at constant composition of medium under study. The variation rate of basic metrological characteristics was as less as 5-10 per cent. At the same time exhaled air of tested volunteers with different state of health caused different sensor behavior and changed character and parameters of response essentially. For example, variations of the output signal parameters such as decreasing time and maximum amplitude of response signal were very high and may run as much as 300 per cent in many cases. In particular, it was revealed higher value of decreasing time of response signal for persons with ulcerous disease.

Thus obtained results testify the prospects of gas sensors development based on the TCNQ derivative compounds and their application for breath gas analysis as a way of noninvasive express-diagnostics of human diseases.

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TCNQ DERIVATIVES-BASED SENSORS FOR EXHALED BREATH ANALYSIS

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Synthetic metals are significant among the new prospective materials developed for engineering lately. Their investigation is of essential interest because of a wide spectrum of physical-chemical properties and attractive prospects of possible applications. Last years it was shown that synthetic metals allow broadening of the resourcefulness of electronic devices (see, for example, [1]) and filling up essentially an arsenal of sensor technique [2].

Complex salts of 7,7,8,8-tetracyanoquinodimethane (TCNQ) are among the most thoroughly studied organic conductive substances and now there is a possibility to design their application. We developed and produced samples of gas sensors based on the TCNQ compounds. Design of our sensitive elements (transformers) is created on the principle of the response signal registration by conductivity measurement. In this case output signal of transformer is generated by the changes of conductive properties of synthetic metal interacting with the components of gas mixture under investigation. Sensitive element was prepared as a thin film of a synthetic metal deposited on a dielectric substrate. The opposite sides of thin film were connected with current feeding copper or silver wires. The film was manufactured from complex TCNQ salts with N-alkylquinolinium (N-Alk-Qn) and alkylisoquinolinium (N-Alk-iso-Qn) cations. The general formulas of the used compounds are [N-Alk-Qn](TCNQ)$_2$ and [N-Alk-iso-Qn](TCNQ)$_2$.

Response of the sensitive element has been investigated by registration of electrical current through the given sensitive element contacting with breath gas medium. Experiments were carried out for different types of sensors, which can work in a passive or active regime. Principal characteristics of a sensor such as delay time of signal increasing and decreasing, time of increasing and decreasing of the response signal, time constant, maximum level of signal were determined experimentally. It was shown that a level of the output signal is proportional to the length of a complex cation of the carbon-hydrogen radical. Samples of sensitive elements with a surface area up to 10 mm^2 yielded output signals up to 3 mkA under influence of an exhaled breath in a passive regime and up to 1 mkA in an active regime.

As follows from obtained results TCNQ complex salts can serve as prospective material to create sensors for analysis of breath gas composition.

This work was partly supported by STCU and Ministry of Education and Science of Ukraine.

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Exhaled nitric oxide levels in relation to allergic sensitisation

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Aims

The aim was to investigate the relationship between sensitisation to allergens and exhaled nitric oxide (NO) using the extended NO analysis which describes the contributions of NO from different compartments of airways.

Material and method

The study comprised 283 adult subjects from a population study that were investigated regarding exhaled NO and sensitisation to four different allergens (cat, timothy, mite, mould). We focused on the following parameters determined using the iteration analysis: Caw_{NO} (mean airway tissue concentration of NO), $Calv_{NO}$ (mean alveolar tissue concentration of NO), Daw_{NO} (airway transfer factor for NO) and $FE_{NO0.05}$ (fractional exhaled concentration of NO at a flow rate of 50 mL/s).

Results

Subjects that were sensitised to at least one allergen had higher levels (geometric mean) of $FE_{NO0.05}$ (24.9 vs 17.5 ppb), Daw_{NO} (10.4 vs. 8.1 mL/s) and Caw_{NO} (125 vs. 107 ppb) ($p<0.05$), while no difference was found regarding $Calv_{NO}$ between sensitised and non-sensitised subjects (1.4 vs. 1.2 ppb). Looking at individual allergens, the largest difference in $FE_{NO0.05}$ between sensitised and non-sensitised subjects was found for cat and mould sensitisation (*Cladosporium*) (Table 1). No significant relation was found between sensitisation to mite and NO-levels.

Table 1. $FE_{NO0.05}$ levels (ppb, geometric mean (95% CI)) in relation to type of sensitisation.

Allergen	Non sensitised	Sensitised	p-value
Cat	17.8 (16.6-19.2)	27.9 (23.6-32.9)	<0.0001
Timothy	18.5 (17.2-20.0)	26.2 (22.0-31.2)	0.0001
Mite	19.8 (18.4-21.3)	22.8 (16.5-31.5)	0.26
Mould	19.7 (18.3-21.1)	37.6 (17.1-82.7)	0.004
Any	17.5 (16.1-18.9)	24.9 (21.8-28.4)	<0.0001

Conclusion

The level of exhaled NO was significantly increased in subjects sensitised to inhalation allergens particularly cat and mould. This increase was related to higher NO concentration in airway tissue and a larger airway transfer factor for NO. Sensitisation to allergens was not related to an increased NO concentration in the alveolar tissue.

Comparison of Linear Response in the Detection of Aroma Compounds by APCI-MS and PTR-MS.

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Direct inlet mass spectrometry has become popular for measuring volatile organic compounds (VOCs) in human breath. Atmospheric Pressure Chemical Ionization-Mass Spectrometry (APCI-MS) and Proton Transfer Reaction-Mass Spectrometry (PTR-MS) are two of the techniques that have been widely used for food, medical and environmental applications. This research directly compares APCI-MS and PTR-MS. The objectives of this research are 1) to determine the linear response regions for several VOCs using APCI-MS and PTR-MS, 2) to discuss the optimum conditions of each instrument, and 3) to better understand the advantages and disadvantages of using APCI-MS and PTR-MS for breath analysis.

Gas phase samples of VOCs were studied using a customized exponential dilution chamber. Exponential dilution was chosen so that the measured concentration could be compared to the concentration predicted by the following equation.

$$C(t) = C_0 e^{-(F/V)\cdot t}$$

where C(t) = concentration (ppb) of volatile at time, t
C_0 = initial volatile concentration (ppb) in exponential dilution chamber
F = flow rate of carrier gas (ml/sec)
V = volume of exponential dilution chamber
t = time (sec)

The linear regions and detection limits were calculated for ethyl butyrate, ethyl isovalerate, and cis-3-hexenol. Results show that both APCI-MS and PTR-MS have wide linear regions and almost equivalent detection limits.

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BREATH ANALYSIS USING PHOTOIONIZATION MASS SPECTROMETRY

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REMPI (Resonantly Enhanced Multi Photon Ionization) is an ultra-sensitive analytical technique based on the unique combination of laser spectroscopy and mass spectrometry that can identify and quantify vapor-phase constituents present at part per trillion levels rapidly and without the need for sample collection, pre-concentration, or sample pre-treatment. The real-time nature of the instrument allows these measurements to be made in minutes or even seconds and therefore diagnose breath components in a fraction of the time it takes today. Measurements may also be made as a function of time (for example, on an hourly basis, and/or over many days) to observe fluctuations of specific breath components in order to monitor changes due to, for example, disease development, as a function of therapeutic treatment, change of drugs, drug concentration, and other conditions.

Our main focus has been to examine human breath samples using the REMPI method; partly to confirm its anticipated improvement in sensitivity over GC/MS, and partly to verify that a simple photoionization approach to breath analysis is viable. As is our practice, we began by doing some GC/MS surveys to establish what compounds are present and their relative signal intensity. Both parameters are important for comparison with photoionization.

The first step in doing the GC/MS and photoionization work was to establish a uniform sampling protocol that would collect and release representative vapor samples from expired human breath. We determined that the Solid Phase Micro Extraction (SPME) fibers are the most reliable and straightforward method to use. The breath samples were acquired from SRI volunteers. Two 30-second breaths were collected on a SPME fiber under conditions that minimized collection of room background vapors.

The SPME fiber was desorbed in the inlet of the GC/MS and the total ion chromatogram (TIC) measured. All peak identifications were made by searching the NIST MS library and selecting the most realistic compound whose mass spectra fit the measured signal.

A breath sample from the same volunteers was collected following the same protocol for examination using the photoionization instrument. Because we wanted to see as many volatile aromatics as possible from this breath sample, we configured our instrument to perform multiphoton ionization using a fixed wavelength of 266 nm. This well-established method gives very efficient ionization of aromatic compounds and does not required scanning the laser. This is particularly important when using the SPME sampler as the sample is present in the inlet for only 15 seconds during the thermal desorption which is insufficient to perform a wavelength scan required to do a REMPI survey. A conventional GC injector with our direct capillary inlet was used to desorb and transfer the sample from the fiber to the photoionization region.

We will show results of the very first photoionization analysis of human breath. The measured mass spectra of the breath samples as well as the corresponding background spectrum were acquired over a period of 10 seconds (100 laser shots). A number of mass peaks appear in both the breath sample and the background (benzene, toluene, and xylene), while some aromatics appear only in the background (phenol, cresol). Likewise, a set of mass peaks is readily observed *only* in the breath samples.

Based on the observed results, we estimate our limit of detection for the photoionization system is a factor of 40-100 times lower than we can achieve using the newest GC/MS instrument. Of more importance perhaps, compounds such as benzene, toluene or even dichlorobenzene could readily be detected using our method in real time directly in expired breath, that is, without collection on a SPME fiber.

This preliminary experiment served to: (1) establish sampling via SPME fiber as a useful technique, (2) confirm that fixed wavelength photoionization is a viable means of detecting trace aromatics in expired human breath, (3) verify that photoionization is more sensitive that GC/MS for these compounds by a factor of 1-2 orders of magnitude, and (4) at least for aromatic vapors, direct, real-time breath analysis is possible using our instrument.

This work was supported by SRI internal funding.

Automated and intra-breath shape analyses of expirograms on a breath-by-breath-basis - potential for diagnostic applications

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The shape of so-called expirograms is subject of scientific interest since many years. Thereby, information content of these plots is unquestionable – problems arose from designing robust algorithms to assess these curves automatically and independently from the observer. In this study, we introduce a system that serves for a stable automated shape analysis of expirograms on a breath-by-breath basis.

10 subjects with chronic heart failure (NYHA II-III), 10 subjects after long-term cigarette smoking and mild emphysema, 2 subjects with advanced sclerodermia, and 10 age matched controls without any heart or lung disease were underwent an exercise test on the cycling ergometer (starting at 20 Watt, 10 Watt/min increment) up to subjective exhaustion. Gas fractions for O_2, CO_2, Nitrogen, and Argon were recorded with a respiratory mass spectrometer system (AMIS 2000 Quadrupol, Fa. Innovision, DK) at intervals of 30 ms time duration. Special software routines were produced, firstly, for the time alignment between the signals for gas concentrations and flow. Secondly, each resulting expirogram for CO_2 and O_2 was fitted to a nonlinear model using a Marquardt-Levenberg algorithm. As examples of this model optimization, we present a robust calculation of the physiological lung deadspace (VD) and a parameter K that represents steepness of phase II.

For all groups of subjects, characteristic exercise related trends regarding VD, VD/VT, and other expirogram-derived parameters could be observed. Particularly, the subjects with emphysema showed an initial increased VD/VT with flat phase II (low K) and a further increase of VD/VT during exercise. Subjects with chronic heart failure, but without clinical signs of emphysema, showed an elevated VD/VT against normal subjects, but without an exercise related increase. The two patients with sclerodermia presented remarkably lowered values for VD/VT at rest and during exercise.

Intra-breath shape-analysis of expirograms on a breath-by-breath basis seems to be a promising approach for differential pulmonary diagnostics under several conditions. Further areas of application have to be discussed.

Sensitive monitoring of the UV-induced lipid peroxidation in human breath

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Abstract

Trace gas analysis of the breath composition gives information about various processes occurring inside the human body. One such process is lipid peroxidation in which free radicals induce oxidative degradation of the polyunsaturated fatty acids, causing cell damage and cell death. As a marker of free radicals-mediated damage in the human body, the measurement of the exhaled volatile hydrocarbons ethylene (C_2H_4), ethane (C_2H_6) and pentane (C_5H_{12}) represents a good and non-invasive method to monitor lipid peroxidation.
In this work, two very sensitive trace-gas analyzers based on photoacoustic detection are presented. We used them to study the effect of UV-radiation on the human skin by on-line monitoring the ethylene and ethane emission in exhaled air and directly from the skin. These detectors have an achieved detection limit of 10 parts per trillion and demonstrated their potential to become powerful tools for breath analysis.

Novel hand-held device for exhaled NO-analysis in research and clinical applications

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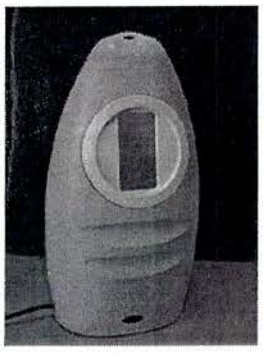
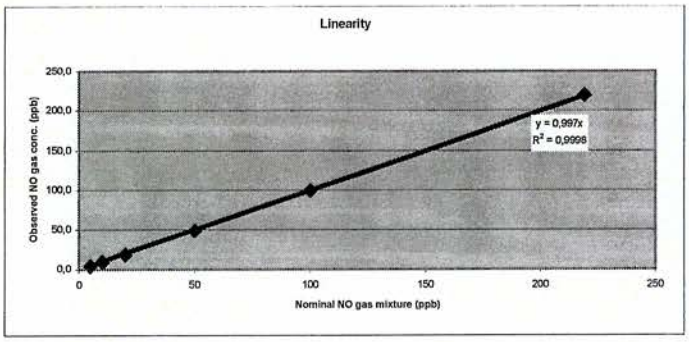
Nitric oxide (NO) is a biologically active molecule which is formed e.g. in the airways. Changes in expired NO (F_{ENO}) occur in airway inflammation and has proved to be important in the monitoring of disease processes such as asthma. We set out to develop a novel NO-analyzer with a performance comparable to the present relatively costly and complicated chemiluminescence technique but at the same time smaller, cheaper and easier to handle. The new instrument is based on an improved electrochemical sensor. To be able to measure NO down to single digit parts-per-billion concentrations, we have developed a novel sampling technology, compensating for the relatively slow response properties of the electrochemical sensor. Also, we have developed techniques for highly accurate conditioning of the gas sample before analysis. The sensor element is rendered specific to NO by selection of filter materials and optimization of the electrochemical properties of the analyzer cell and the signal processing. We obtained an LDL (Lowest Detection Limit) in NO-analysis from reference gas tests of less than 3 ppb and an average precision in human breath measurements of 1.4 ppb. We show an agreement with the existing 'gold standard' F_{ENO} measurement technique, within 0.5 ppb in a group of 19 subjects. We also find a high linearity and accuracy compared to reference gases. The new hand-held analyzer enables affordable monitoring of inflammatory airway diseases in research as well as clinical routine work in hospitals, private clinics and home monitoring, primarily for the asthma patient.





Breath Analysis by on-line TDS/GC/MS

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Analytical methods such as gaschromatography/mass spectrometry (GC / MS) offer a great potential for environmental analysis, in life sciences and in medicine.

In the field of breath analysis GC/MS is a fast, powerful, cheap and non–invasive alternative to support common clinical diagnosis like invasive and x-ray methods. Lung diseases especially lung cancer are often detected in a late stadium, i.e. the possibility to heal the illness and the life expectancy decrease very fast. Therefore, beside others GC/MS technique should be used for example in surgeries and hospitals to get early hints for lung diseases which initiate further diagnostics to enlarge the time window.

Our method based on an adsorption of breath samples on tenax in glass tubes. Analytes are set free by thermodesorption (TDS) in a very moderate way. They are traped in a cold injection system (CIS) at –80°C, separated by high resolution capillary gaschromatography and analysed by mass spectrometry. One of the main interests for a good, suitable method is an effective drying step of the breath sample to avoid too much water in the GC-system. This aim can be reached by condensation effects, by the use of diffusion and adsorption techniques and by the change of physical parameters. Whereas, the drying must avoid the loss and the destruction of hydrophilic analytes such as alcohols, aldehydes and ketones.

Up to now it is not possible to find concrete biomarkers for special diseases in literature. Published biomarkers vary in between hundreds of compounds (alkanes, alcohols, aromatic compounds, terpenes, etc.) [1], statistical information for special groups of compounds (alkanes, aromatic compounds) [2] and more detailed lists for small analytes (ethane, isoprene, pentane, etc.) [3]. Therefore, samples have to be checked very carefully and one has to take into consideration which analytes may be appropriate biomarkers because of their special frequency or proportion among each other.

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Rapid 3D - spectra classification, identification and quantification of gaseous metabolites in human breath using ion mobility spectrometers

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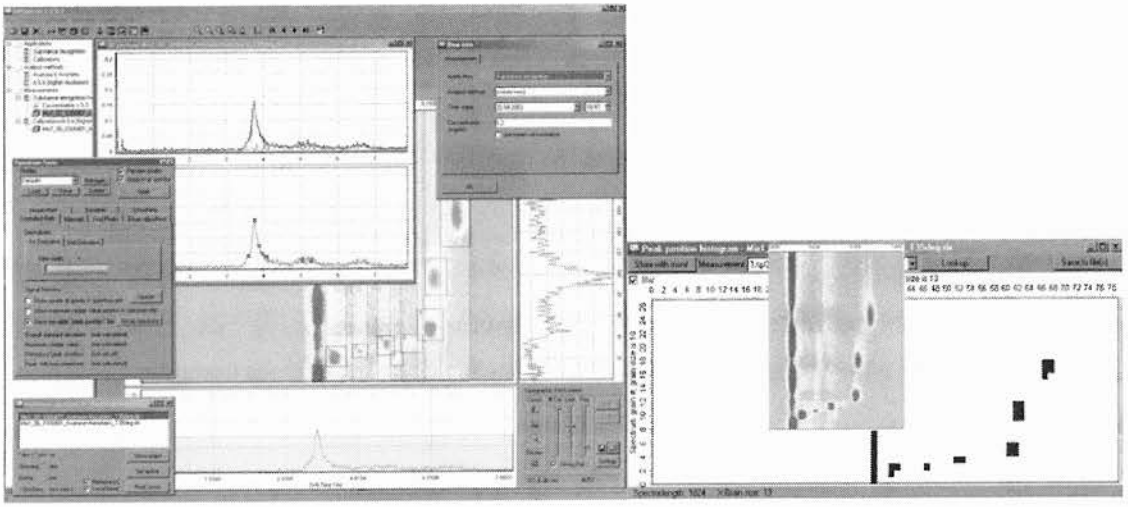
Ion mobility spectrometers (IMS) are well known as fast, sensitive and selective tools for trace gas analysis in air down to the pg/L and ppb_v-range. A further enhancement could be reached by coupling the IMS with different gas-chromatographic columns. Thus, new challenges occur not only with respect to the instrumentation (sensitivity, detection limits, dynamic range), but also for rapid substance classification. Additionally, a fast identification and quantification of analytes requires also new calibration procedures as well as new concepts in the field of operation of the spectrometer. Peak-height-diagrams and more dimensional data presentations will be completed by fast classification procedures like cluster analysis and calibration procedures realised automatically. We discuss the advantages of the new features of the software packages GASpector® with respect to the operation of the ion mobility spectrometer, spectra classification, identification and quantification of gaseous metabolites in human breath in detail.



Interleukin 6 and Total Protein in Exhaled Breath Condensate of CF-Patients - methodical examinations.

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Introduction:

Interleukin 6 (IL-6) is an important marker for inflammatory processes. It be responsible for the systemic answer on local inflammations. IL-6 is increased in chronic neutrophile inflammation. There is an correlation between IL-6 and the increasing or decreasing of acute phase proteins.

Some papers has described the concentration of IL-6 in exhaled breath condensate (EBC). Concentrations between 2,6 pg/ml and 34,4 pg/ml were found.

Methods:

37 CF patients with exacerbation were sampled initially at hospital admission and 30 patients after two weeks antibiotics therapy.

EBC sampling was done with JAEGER-EcoScreen. Samples were frozen with –80 °C and freeze dried. The residue was resolved with tenth of original volume.

A High Sensitivity ELISA (R&D-Systems) was used. The sensitivity, reproducibility and the influence of storage temperature and material of tubes (glass, inactivated glass, polypropylene, Teflon) were checked. Native EBC with artificial IL-6 concentration of 2 pg/ml were analysed under different conditions.

Total protein (TP) was measured with Micro BCA-Method.

Results:

There is no difference for IL-6 ($p = 0.34$) and TP ($p = 0.37$) before and after therapy. The quotient between IL-6 and TP is increased after antibiotic therapy (before: 0.77 ± 0.59; after 1.05 ± 1.02 pg/mg TP) but not significant ($p = 0.11$).

IL-6 in EBC is stable overnight at room temperature. There is no influence of tube material on IL-6 values.

Detection limit in EBC after tenfold enrichment is 2 fg/ml.

Conclusions:

CF patients has an measurable IL-6 concentration in EBC. But the measurement is only possible with high sensitivity ELISAs after enrichment.

It seems useful to calculate the quotient between IL-6 and total protein as result.

IL-6 in EBC is not a useful parameter to observe the anti-inflammatory effect of antibiotic therapy in CF patients.

Exhaled breath condensate nitrite as a marker of overdistension of the lung parenchyma

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Mechanical ventilation may damage the lung. Low tidal volume is protective. Tidal volume (V_t) is scaled to body weight and may be high in functionally small ARDS lungs. BAL NO_2^- concentration increases with tidal volume in an isolated perfused lung model. We hypothesized that the relation of exhaled breath condensate (EBC) NO_2^- and tidal volume is indicative of the extent of mechanical stress in the lung parenchyma. Moreover, in obstructive lung disease, such as COPD, EBC NO_2^- might also be influenced by the degree of lung overdistension.
Exhaled breath condensate was collected from 35 consecutive patients requiring mechanical ventilation for more than 24h. MURRAY lung injury score was obtained at the time of EBC collection. EBC was collected for 30min by inserting a specialized conduit fitted to the EcoScreen (Jaeger, Toennies, Germany) into the expiratory limb of the ventilator. EBC NO_2^- concentration was photometrically determined using the GRIESS reaction. In 50 patients (10 healthy smokers; 24 COPD: stable; 15 COPD: acute exacerbation) we investigated EBC NO_2^- as well as EBC cytokines (IL-6, IL-8, IL-1 beta, TNF alpha, IL-12, IL-10). Lung function was performed in all patients investigated.
EBC-NO_2^- was found to be closely correlated to V_t [ml/kg BW] ($r=0.79$, $p<0.0001$) but not correlated to positive end-expiratory pressure (PEEP) or to peak inspiratory pressure (PIP). If EBC NO_2^- truly reflects alveolar distension, and if increased alveolar distension occurs at equivalent tidal volumes with increasing lung injury, the relation of EBC NO_2^- and V_t (i.e. EBC-NO_2^-/ V_t; [V_t in ml/kg BW]) should be related to the extent of lung injury as expressed by the individual Murray score. EBC-NO_2^-/ V_t i.e. the amount of nitrite released at a given V_t was indeed closely correlated to Murray lung injury score ($r=0.84$, $p<0.0001$). In spontaneously breathing patients we observed a nonlinear, logarithmic correlation of EBC NO_2^- with residual volume (RV: $r=0.74$, $p<0.0001$), total lung capacity (TLC: $r=0.53$, $p<0.0001$), and thoracic gas volume (ITGV: $r=0.73$, $p<0.0001$) but not with resistance, FEV1, vital capacity with EBC cytokine levels. Analysis of the subgroups listed above demonstrated that this correlation was stronger in COPD subgroups.
Because EBC is easily and rapidly measured EBC NO_2^-/ V_t qualifies for an indicator of alveolar distension/ overdistension in mechanical ventilation. This indicator appears to be useful in an effort to consequently follow the experimental and clinical suggestions to employ lung protective strategies in mechanical ventilation. We also conclude that EBC NO_2^- in COPD is also likely to reflect overdistension of the lung parenchyma due to hyperinflation rather than inflammation in the airways.

http://www.physics.gla.ac.uk/Optics/

Real-time sub-ppb ethane measurement using a field-robust laser spectrometer

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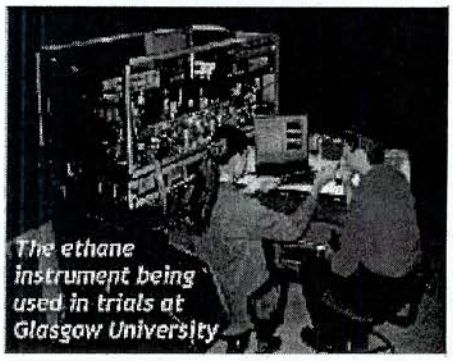
The Instrument

The instrument developed at the University of Glasgow is an infra-red spectroscopy system for the ultra-sensitive measurement of ethane. It is based around a lead-salt laser passing through a multi-pass optical delay line through which a breath sample flows. The system was originally designed for an oil prospecting application.



The ethane instrument being used in trials at Glasgow University

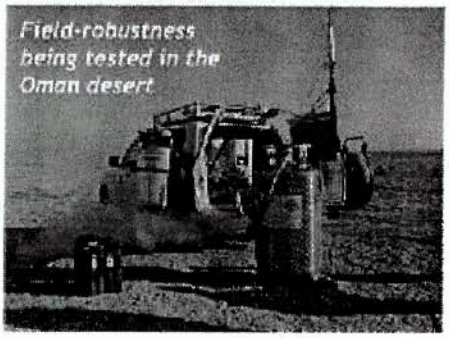
♦ *key features* ♦

ultra-sensitive ethane detection down to 100 parts per trillion (1 part in 10^{10})

measures ethane essentially in real time (~1s response time for 250ppt sensitivity)

turn-key operation via LabVIEW controlled operating system

field-robust for use in the field and on-site use in hospitals



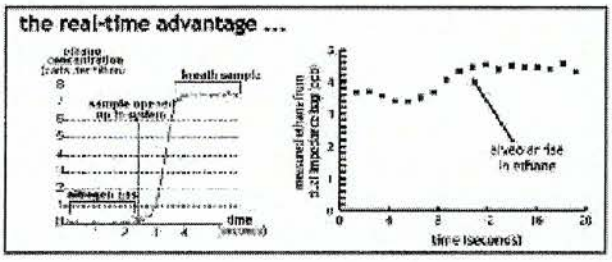
Field-robustness being tested in the Oman desert

Oxidative Stress and Ethane

Ethane is produced in the body as the end-product of the oxidative degradation of cellular omega-3 fatty acids by free-radical attack (lipid peroxidation). The resulting equilibrium of this process (the level of oxidative stress) can therefore be measured using ethane as a non-invasive marker. Unlike many of the higher-chain alkanes ethane is poorly soluble in tissue and fat and is not easily metabolised, although concentrations are low. Having been qualified for industry, our system is proven to be sensitive to ethane at 0.1ppb with a 1s response.

♦ *clinical advantages of the spectrometer* ♦

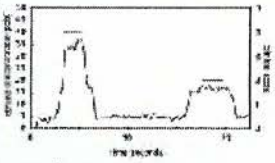
100s of samples per hour can be accurately analysed - essential for larger scale trials



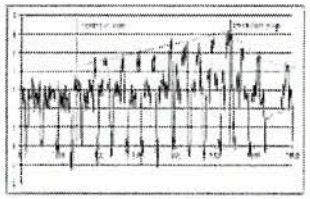
our system is fast (left) allowing real-time breath ethane info to be gained, for example, we can measure the alveolar gradients in COPD patients (right)

Current Biomedical Applications

Our biomedical investigations can be divided into diagnostic and monitoring studies. We have engaged in studies using breath ethane as an indicator of oxidative stress in human and animal healthcare as summarised below.

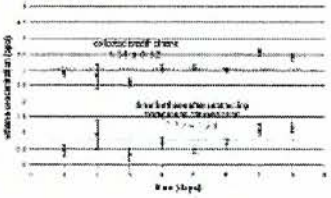


equine respiratory inflammation



exercise-induced oxidative stress

Studies of ethane in healthy adults, essential in assessing the "noise" in clinical trials



Air volatile chelates preconcentration and determination by graphite furnace of atomic absorption spectrometer

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Metal content in organs and tissues can be correlated with metal concentration in environment and with some human diseases.
The today's most widely used method for monitoring of metal poisoning in the human body is examination of blood, hair, nails, etc. The method limitations are related to the necessity of sample preparation and the high noise, unselective absorption, in the final spectrum analysis (e.g. atomic absorption spectrometry, AAS). Moreover, bleeding for analysis (blood assay is the method most frequently used for microelement identification) is a hazard as far as hepatitis and AIDS are concerned.

An alternative to this method may be metalorganic compounds destruction accompanied with aerosol deposition in high gradient electric field in the inner surface of graphite tube followed by AAS determination.
The electrothermal atomic absorption spectroscopy (ETA-AAS) using graphite furnace (GF) is a reasonably inexpensive method for metal determination at the 10^{-14} g level. Graphite tubes are not expensive. Metal impurities can be removed by high temperature heating (at 3000°C) in argon atmosphere. The metal content in graphite tube can be controlled easily by absorption signal under atomization.

The first attempt to use the corona fee precipitation in analytical practice was made by Torsi [1]. Metal impurities from air were precipitated in a high gradient electric field on the graphite tube surface, not into its volume. Very high efficiency was demonstrated [1]. Detection level can be diminished by increase of probe volume.

The objective of our work is to design a new high-performance instrument for monitoring of breath out air MOC. A new instrument should offer improved analytical capabilities and ease of operation under real clinical conditions.

The special device was made for mounting the graphite tube, pumping through it air with chelate and applying high voltage for corona discharge.The precipitation (or accumulation) of the MOC in ETA under corona discharge was proposed to use followed by AAS determination for non –invasive breath test.

Volatile metal chelates were choosen as model substances for precipitation studies in corona fee on graphite tube because of unknown nature (origin) of breath out MOC. Metal chelates have low volatility at room temperature. Beryllium chelates were used because of their low clark in environment
There was shown the possibility to use volatile metal chelate as model substances, and not found corona fee voltage fall in humid atmosphere and no decrease of absorption signal.

These developments hold enormous promise to produce compact device for collection MOC from breath. There was supposed that analysis of exhaled breath could open a valuable new window into human metabolism and illuminate its function in health and disease.

References

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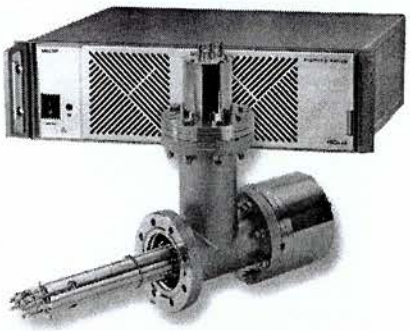

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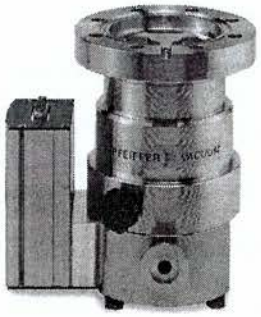

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