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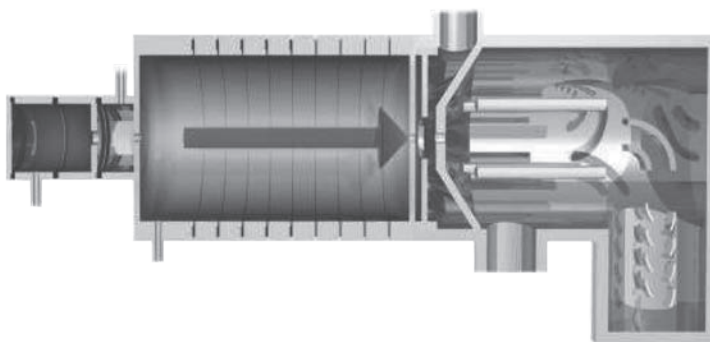
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4th International Conference on Proton Transfer Reaction
Mass Spectrometry and its Applications



Contributions

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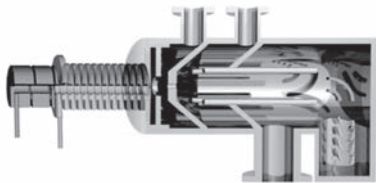
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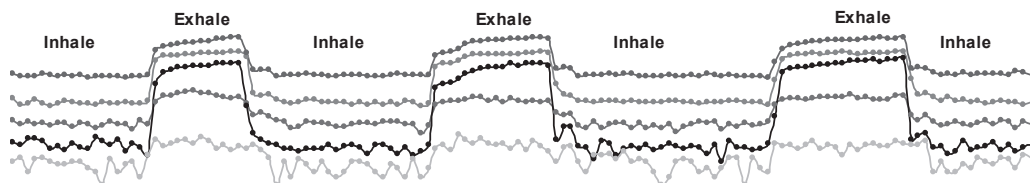
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Foreword

PTR-MS (Proton Transfer Reaction - Mass Spectrometry) is a relatively new technology developed at the Institute of Ion Physics and Applied Physics at the University of Innsbruck in the 1990's. PTR-MS has been found to be an extremely powerful and promising technology for the online detection of volatile organic compounds (VOCs) at trace levels (pptv) in gaseous media. PTR-MS has been successfully employed in many fields of research including environmental research, life sciences, and food & flavor technology.

More than ten years ago the spin-off company Ionicon Analytik GmbH (www.ptrms.com) was founded to provide PTR-MS instruments to a growing user community and to develop the technology further. In 2004 Ionimed Analytik GmbH (www.ionimed.com) was founded to provide trace gas solutions for the fields of biotechnology and medicine. Today more than 120 research institutions and companies throughout the world use this technology.

The intent in initiating and organizing the 1st International PTR-MS Conference in January 2003 in Igls, Austria was to bring together active scientists and technology experts involved in mass spectrometric measurements of VOCs. The 4th PTR-MS conference continues this biennial series to provide a discussion forum for PTR-MS users and scientists from both academia and industry. More than 110 conference participants are expected at the conference site in Obergurgl. This year's conference is organized in plenary sessions and focused parallel sessions. The program will start with a plenary session with key note speakers presenting interdisciplinary overviews in environmental science, food science and medicine. On the following two days the conference topics PTR-MS in Environmental Science, Food Science, and Medicine & Biotechnology will be discussed in parallel sessions. Finally PTR-MS technology and future development requirements will be discussed in a concluding plenary session. This session is part of a satellite workshop supported by the European Commission within the Marie Curie Industry-Academia Partnership and Pathways Programme.

I would like to thank the session chairs Saskia von Ruth & Franco Biasioli (Food Science) and Nancy Hecker-Denschlag (Medicine and Biotechnology) for putting together an exciting programme which exemplifies the growing number of PTR-MS applications in various scientific disciplines.

Special thanks go to Jürgen Dunkl, Martin Breitenlechner and Sandra Naschberger who worked very hard to organise this conference. Finally I would like to thank the UNIVERSITY of INNSBRUCK, IONICON ANALYTIK, IONIMED ANALYTIK and the EUROPEAN COMMISSION for support. IONICON ANALYTIK also sponsors the poster award which will be bestowed to the three most impressive and innovative posters presented at the conference.

Armin Hansel

Innsbruck, January 2009

1. Plenary Session

Emissions and Chemistry of Atmospheric VOCs: New Insights from PTR-MS Measurements Onboard Research Aircraft and from a Tall Tower

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Abstract

The use of PTR-MS has led to significant new insights into the emissions, chemistry and loss processes of volatile organic compounds (VOCs) in the atmosphere. It is the aim of this presentation to review our most recent results from measurements made onboard the NOAA WP-3D research aircraft and from a movable platform on a 300-m tall tower.

Introduction

Volatile organic compounds (VOCs) play an important role in the chemistry of tropospheric ozone and organic aerosol, and research at NOAA is focused on elucidating this chemistry. Three recent projects will be discussed at the meeting. First, airborne measurements of isoprene in 2006 and earlier were used to evaluate the accuracy of several different emission inventories. Second, measurements of vertical VOC profiles from a tall tower in Colorado gave new insights into the vertical stratification of the nighttime atmosphere and processing of VOCs and nitrogen oxides. Finally, airborne measurements were made in the springtime Arctic, and gave detailed insight into the origin of the extensive biomass burning plumes that were observed in the region.

Experimental Methods

The VOC measurements in this work were made by proton-transfer-reaction mass spectrometry (PTR-MS) and additional data were obtained by gas chromatographic methods, both on-line and from canister sampling. We have analyzed the specificity of PTR-MS using a gas chromatographic pre-separation method [1,2], and using inter-comparisons with alternative methods [3]. Most of this work has been discussed in a recent review paper [4].

Results and Discussion

Isoprene is one of the most important VOCs in the atmosphere: its total emissions as well as its reactivity are the highest among all VOCs, and as a result it plays a significant role in the chemistry of tropospheric ozone and, potentially, organic aerosol. In atmospheric chemistry models, isoprene emissions are represented using emission inventories that combine a land-use database with a parameterized emission model. In this work, we have used airborne

measurements of isoprene using PTR-MS and compared the results directly to the mixing ratios expected from the emission inventory. Two different approaches were developed for this purpose; in one approach the emission inventory is incorporated into the Lagrangian transport model FLEXPART. Figure 1 shows the comparison between measurements and model for one research flight over Northeast Texas. The agreement is within a factor of 2, and we generally found this to be the case for other emission inventories and regions of the U.S.

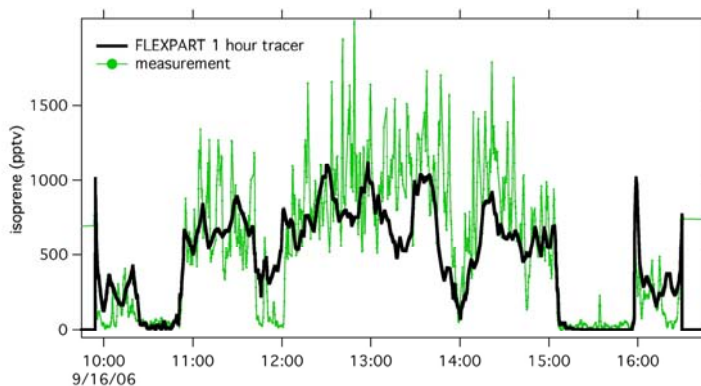


Figure 1: Comparison between isoprene measured by PTR-MS during a flight over Northeast Texas and a model calculation that combines the BEIS-3 emission inventory with the transport model FLEXPART.

The emissions of isoprene go to zero at night, but because the photochemistry slows down significantly in the later afternoon and early evening, a significant amount of isoprene can remain in the atmosphere at night. The lifetime of isoprene at night is controlled by the nitrate (NO_3) radical. Studies of this chemistry are complicated by the strong stratification of the nighttime, lowest atmosphere. To overcome these difficulties, we have used a 300-m tall tower in Colorado that is equipped with an exterior elevator to measure altitude profiles of VOCs, nitrate radicals and other chemical species in the nighttime atmosphere. The tower is situated in an agricultural area to the north of the Denver metropolitan area; local isoprene emissions are low.

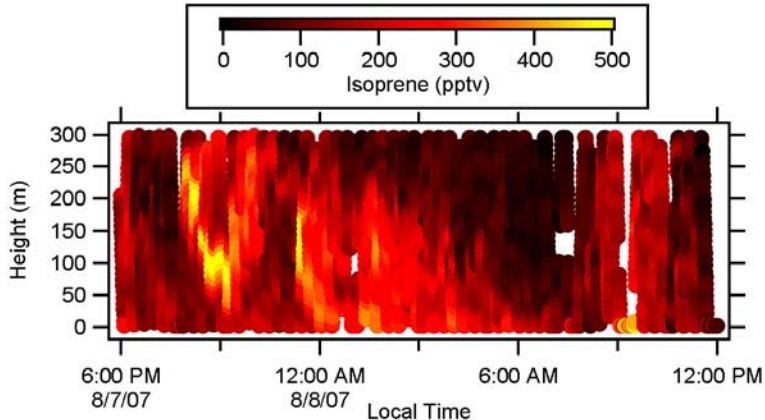


Figure 2: Nighttime vertical distribution of isoprene in the lowest atmosphere measured from a 300-m tall tower in Colorado.

Figure 2 shows the results for isoprene for one night and morning in the summer of 2007. The highest isoprene is observed around 9 PM, but not at the surface. Later in the night, the layer with the highest isoprene has descended to the surface. In the early morning isoprene is only present in a very shallow layer at the surface. After sunrise, isoprene increases and is more uniformly distributed. Clearly, this type of data gives unique insights into the nighttime chemistry of the lowest atmosphere that surface and/or aircraft measurements cannot obtain. Preliminary results indicate that isoprene plays an important role in determining the nighttime removal of NO_y , which may impact the transport of NO_x from urban to rural areas.

Finally, measurements of VOCs and other species were made in April of 2008 onboard the NOAA WP-3D aircraft during flights over and to the north of Alaska. One of the main goals of the project was to identify the origin of pollutants that contribute to “Arctic Haze”, an annually recurring phenomenon in the Arctic spring. Numerous plumes with significantly enhanced carbon monoxide (CO) were observed, for example on April 21, when a plume with up to 400 ppbv of CO was present between 4 and 5 km altitude (Fig. 3A). The tight correlation between acetonitrile and CO measured on this flight (Fig. 3B) indicated that these air masses had a biomass burning origin, and simulations using the FLEXPART model confirmed that the plumes originated from forest fires in the border region between Russia and China, and from agricultural fires near Kazakhstan. Biomass burning is a significant source of benzene to the atmosphere, and mixing ratios up to 300 pptv were observed in the plumes in the Arctic (Fig. 3C). Such high levels are comparable to our airborne observations just downwind from major urban areas. Work is currently underway to determine how commonly the springtime transport of biomass burning smoke into the Alaskan Arctic occurs.

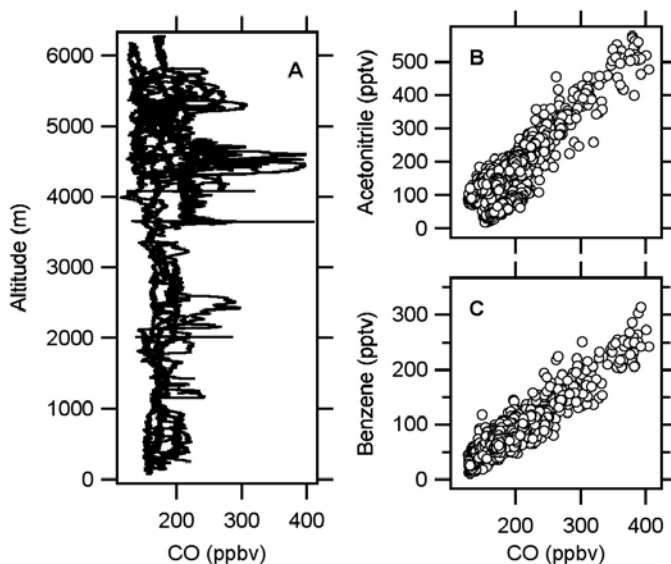


Figure 3: (A) Altitude profile of carbon monoxide measured on April 21, 2008, during a research flight over Alaska. Scatter plots of (B) acetonitrile and (C) benzene versus CO for the data from this flight.

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Mass spectrometry in Medicine

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Abstract

Diagnostic tests play an important role in medicine. Analysis of blood, urine and other body fluids is indispensable for reliable diagnostics and tailoring of therapy to the needs of the individual patient. Despite great progress in clinical chemistry information that we can get out of these tests is not sufficient, not specific or does not arrive in time. Hence, there is an increasing need of reliable and fast diagnostic tests which ideally should be non i or minimally invasive requiring only a minimum of blood or other body fluids. (Hyphenated) mass spectrometric techniques as applied in breath analysis or drug/metabolite monitoring in principle can meet these requirements. This lecture is intended to show how application of mass spectrometry can be used to set up a novel kind of diagnostic tests in medicine.

Latest developments concerning analysis of biomarkers and drugs in various biological matrices will be described. Specifically, aspects of volatile biomarker recognition and drug monitoring will be discussed in detail. Key experiments realizing clinical applications of improved sample preparation and detection techniques will be outlined.

Volatile biomarkers reflect various physiological and pathological conditions. Breath analysis in critically ill patients enables early diagnosis of ARDS (acute respiratory distress syndrome), recognition of ischemia/reperfusion injury, sepsis and SIRS (systemic inflammatory response syndrome). Concentrations of exhaled substances could be linked to inflammation and infection of lung and airways, rejection after transplantation and cancer of different organs.

Up to now, breath analysis in the ppt/V range required time consuming sample preparation, bulky equipment and excellent analytical skills. Preconcentration techniques were improved requiring now not more than a few cc of exhaled air to do the analyses (SPME), GC technology progressed in the way that enhanced and miniaturized devices yield reliable substance separation within a few minutes (fast GC, GC/GC, GC on the chip). Modern mass spectrometry (MS/MS, Ion trap, TOF) enables detection and substance identification on the ppb to ppt level, and measurements of single breath markers without relevant delay can be done by means of direct mass spectrometry, such as proton transfer reaction – MS (PTR-MS) or soft ionization flow tube – MS (SIFT-MS).

Conventional determination of important chemotherapeutics requires sophisticated and time consuming sample preparation and analysis. A new dimension of drug monitoring can be realized through in-vein solid phase micro extraction. This technique does not require withdrawal of blood any more, speeds up analysis and can be repeated frequently without any risk for the patient. Specifically coated fibres are immersed directly in to the blood(stream). After adsorption of the target compounds the fibres are withdrawn from the blood and immediately rinsed with an

appropriate solvent. Following evaporation of the solvent substances are separated and analyzed by means of LC/MS.

The diagnostic potential of these methods can further be enhanced through online and on site techniques. Improved sample preparation techniques reduce invasiveness and duration of sampling, miniaturized and optimized chromatographic separation will soon be available at the bedside, simplified and real time detection methods such as PTR-MS, SIFT-MS, IMS (ion mobility spectroscopy) will soon enable on site detection and identification of biomarkers. In addition, mass spectrometric analysis still represents the gold standard in medical trace analysis. In order to establish and evaluate any new onsite or point-of-care diagnostic method (e.g. sensor (arrays), laser spectroscopy, IMS) MS analysis remains indispensable.

Dynamic flavour analyses using direct MS

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Abstract

Analysis of food flavors and fragrances by direct mass spectrometric (MS) methods raises different analytical issues. The issues are application dependent and three examples are given as illustrations. The first application is measuring dynamic aroma release in ethanolic beverages and the difficulty in measuring the low levels of aroma compounds (typically in the $\mu\text{L/L}$ range) against a background of ethanol in the mL/L range. The MS issues will be described, along with solutions that allow measurements to be made in a consistent and reproducible way. The second example is measuring aroma release *in vivo* as people consume food or drink. MS methods to cope with the sampling frequency associated with tidal air flow, the number of compounds present and the feasibility of using quadrupole or Time of Flight analyzers will be discussed with data from the lab. The third application is the monitoring of aroma generation during thermal processing of foods. The key issues are ion suppression and ion assignment and the methods developed to address these issues will be presented. The examples above will include some comparisons of different ionization sources and mass analyzers to determine which combinations are best suited to particular applications.

Introduction

Over the last 10 years, flavor researchers have developed direct MS techniques like PTR, APCI and SIFT-MS to measure a range of dynamic flavor processes. The initial focus was on the measurement of aroma release from *in vitro* and *in vivo* systems as a function of time [1; 2]. The effects of physicochemical and physiological factors on release and on flavor perception by humans were determined [3] and have been applied to understand how to reformulate flavorings so they perform equally in different food matrices [4]. The production of volatile compounds during thermal processes as a function of time, temperature and processing conditions has also been studied to understand (and potentially control) the interconnected chemical pathways in the Maillard reaction, albeit at a fundamental, research level [see for example 5; 6].

The technique is now mature and widely used but there are some issues that limit the use of the techniques when analyzing real food systems. These are discussed along with suggestions to develop standard methods for carrying out experiments so that direct MS data can be published with the same amount of rigor as conventional GC-MS data.

Experimental Methods

APCI-MS was carried out as described previously [7]. PTR-TOF-MS used a custom built machine (KORE Technologies, Ely, UK). SIFT-MS used a Voice 100 instrument (Syft Technologies, Christchurch, New Zealand).

Results & Discussion

Monitoring aroma release from ethanolic systems.

APCI-MS was used to analyse the headspace above ethanolic solutions from 0-12% ABV [8]. The ion profile showed that the proportions of hydronium ions (monomer, dimer, trimer) generally decreased with ABV value and protonated ethanol monomer and dimer increased with ABV. The net effect was that ionization of aroma analytes was unstable and non-quantitative under these uncontrolled ionization conditions. The solution adopted was to introduce ethanol into the sweep gas of the APCI source so as to maintain protonated ethanol as the major proton transfer ion, irrespective of sample ethanol content. The conditions were chosen so that the introduction of sample at 30mL/min had an insignificant effect on the ethanol concentration in the source which was supplied with 10L/min of sweep gas. The result was a consistent, stable and quantitative analysis of aroma compounds from model ethanolic solutions [9]. Since the Proton Affinity of protonated ethanol (746 kJ/mol) is different from that of water (660 kJ/mol), some aroma compounds were not ionized under these conditions but overall sensitivity was not affected. From the flavor point of view, using ethanol as the charge transfer reagent means that methanol (754 kJ/mol), acetaldehyde (768 kJ/mol) methylsulfide (773 kJ/mol) will not be ionized and therefore will not be detected. The availability of commercial machines with the ability to switch between different reagent ions (e.g. the Voice machines from Syft Technologies) provides the potential to build in ethanol as a reagent ion for future analyses.

The ability to monitor aroma release from ethanolic systems has been applied to reformulate low alcohol beers and to study the interfacial mass transfer mechanisms that occur in the presence of ethanol under non-equilibrium conditions (similar to those observed when drinking wine or spirits). Ethanol is present at high concentrations at the air-water interface and the evaporation of ethanol causes surface cooling which then creates a stirring effect within the bulk phase as the cold liquid descends and warmer liquid rises to the surface (the Marangoni effect). The effect is to increase in aroma release compared to pure aqueous systems despite a decrease in partition coefficient. This illustrates the power of dynamic, real time measurements which allow us to better understand how systems function in applied science settings.

In vivo monitoring of aroma release

Monitoring the aroma profile that stimulates the olfactory epithelium is of great interest for flavor scientists as this profile differs considerably from the actual or headspace aroma compositions. The analytical issues are sensitivity, as the concentrations in nose are 10-100x lower than in the liquid phase in mouth, and speed, as the breath cycle is 5 seconds and sufficient points are required to define both the breathing cycle and the more frequent chewing events. With quadrupole analyses, maximum sensitivity is obtained with SIM mode but the more ions monitored, the less the frequency of monitoring. With TOF analyzers, the sampling frequency is fixed by the sampling frequency of ions into the TOF and this time is independent of the number of ions analyzed. Selecting the equipment and operating conditions depends on the analytical problem. There is a need to further improve the sensitivity of all direct MS techniques so that more aroma compounds can be detected down to their odor threshold levels. Currently the sensitivities of APCI, PTR and SIFT methods are similar and have not significantly improved for the last decade.

An example of an application where high frequency sampling was needed [3] was the monitoring of physiological events during swallowing, when aromas are transmitted from the mouth to the nose, an event that is very important in aroma perception. A person was asked to chew gum while chewing and swallowing to a set pattern. Air flow through the nose was measured, along with swallowing movements and aroma release. Figure 1 shows the relationships three measurements with time and the whole process took place over a period of 2 seconds and high frequency data collection is essential. The quadrupole MS was set in SIM mode with a dwell time of 20 ms.

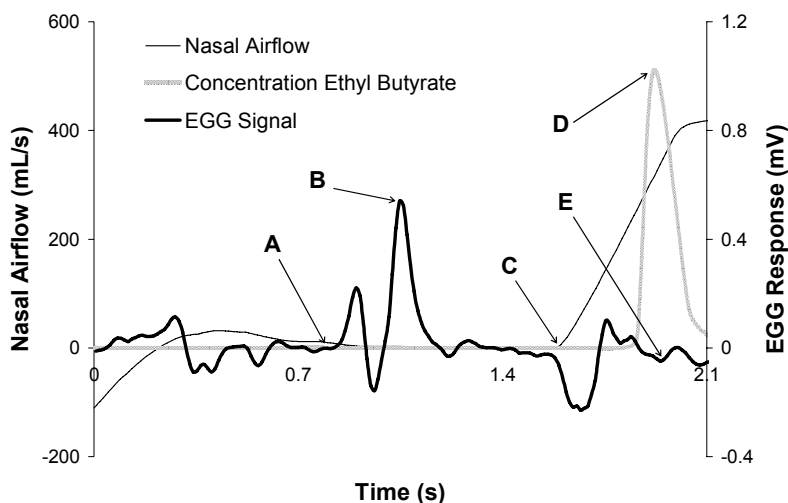


Figure 1: The relationship between the pharyngeal stage of swallowing (EGG response), nasal airflow and ethyl butyrate release (unscaled), where: A – Onset of the pharyngeal stage of swallowing, B – Vocal Fold Adduction, C – Onset of the exhalation following the swallow, D – Highest concentration of Ethyl butyrate in nose (60ppbv), E – End of pharyngeal stage of swallowing

Monitoring thermal aroma generation

Flavor generation during cooking or during processing of commercial flavors involves a complicated, interlinked series of chemical reactions and understanding how these reactions respond to changes in processing conditions (e.g. concentrations, temperature, water content and time) is of great academic and commercial interest. Classic analysis takes samples at different times and uses GC- or LC-MS analysis to identify and quantify the compounds present. However, the process is time consuming. On-line monitoring, by sampling flavor products in the gas phase, offers the potential to screen many different combinations of composition and processing conditions and to identify factors that significantly affect flavor formation.

The main analytical challenge of on-line monitoring in this application is to ensure quantitative and consistent ionization as the amounts and proportions of compounds produced vary with time and the ionization power of the source may be exceeded. It is possible that the formation of a new compound with a large delta PA value may suppress the ionization of a compound already present and confuse interpretation of the results. It is necessary to match the amount of sample to the ionizing power within the source and ensure there is always an excess.

The other issue is assigning compounds to ions in the spectrum. Soft ionization creates mainly protonated molecular ions but the presence of isobaric compounds (and fragments) makes unequivocal assignment difficult. Techniques like GC with combined APCI and EI analysis [10; 11] or the use of labeled reactants [12] can provide supporting evidence. These types of analysis need to be accepted by researchers and journals so that on-line monitoring publications meet the necessary analytical standards.

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Application of PTR MS in bioprocess monitoring for the production of recombinant proteins

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Abstract

Large-scale expression of recombinant proteins using different host/vector systems has become an important technology for various applications in the main areas of industrial biotechnology, in particular in the biopharmaceutical industry. Many processes, however, are far from being optimal due to the high complexity of bioprocesses and limited monitoring capabilities, which often results in lower total product yields and reduced product quality. Currently, key variables of cultivation processes are still beyond direct measurement in real time. Although, in the recent past a series of devices for on-line and in-situ signal acquisition, such as optical and dielectric spectroscopy, were developed the monitoring capabilities were only marginally improved, because the acquired signals do not deliver direct readable physiology relevant variables. On the other hand modern bioanalytical methods provide access to key parameters on molecular level, but their acquisition is laborious, time and cost consuming.

An opportunity to overcome this problem is the application of a chemometric approach by integrating and correlating the on-line acquired signals with a broad spectrum of off-line data to predict meaningful and highly relevant process variables in real time. In order to select the most significant signals for modeling data mining tools such as Kohonen self organising maps (SOM's) were used. The thereby reduced data sets were used to predict complex variables by application of partial least squares (PLS) and artificial neural networks (ANN), whereby ANNs showed superior performance of prediction.

The on-line acquired data sets comprise classical signals, such as base consumption and off-gas analysis and signals of higher complexity such as dielectric spectroscopy, multi-wavelength fluorescence spectroscopy and near infrared spectroscopy. Evaluation of different data sets showed that each of them contributes differently strong to the predictability of particular variables and moreover prediction could be improved by the combination thereof. By this approach the prediction of meaningful variables like nucleotides, plasmid copy number, cell dry weight, amount of recombinant protein and metabolites in real time was achieved. In this context the proton transfer reactions mass spectroscopy (PTR-MS) will largely contribute to the extended performance of the established on-line monitoring platform. The strengths of PTR-MS are the quantitative determination of VOCs whereof each is linked to specific metabolic reactions. Moreover, the quantitative knowledge of the individual compounds enables to set up a complete mass flux balance of the cultivation process. Altogether, the application of PTR-MS will significantly increase the quality and quantity of process information and will improve the level of process controllability.

The established bioprocess monitoring platform in combination with chemometric modeling complies also with the FDA's Process Analytical Technology (PAT) and Quality by Design (QbD) concept which implies a paradigm change in bioprocessing by the requirement of timely measurements to improve process knowledge. Improved understanding gained through the availability of complex parameters in real time will also accelerate the set up of upstream processes and will also facilitate the definition of the design space leading to increased robustness and process stability.

The advantages of PTR-MS integration into a monitoring platform will be shown with *E. coli* based cultivation processes.

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2. Applications in Medicine and Biotechnology

Compounds found in exhaled breath of patients suffering from lung cancer

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Abstract

The exhaled breath and inhaled room air samples from 65 lung cancer patients in different disease stages and different treatment regimes and 31 healthy controls have been analysed. Expiratory and indoor air samples were collected. Gas chromatography with mass spectrometric detection (GC-MS) and proton transfer reaction mass spectrometry (PTR-MS) were used.

Altogether 103 compounds showing at least 15% higher concentration in exhaled breath than in inhaled air were detected. All results reported here refer to concentrations being 15% higher in exhaled breath as compared with inhaled air. Among the 103 compounds detected, 84 were confirmed by determination of the GC-MS retention time using pure compounds. Around one third of the compounds detected were hydrocarbons. We found hydrocarbons, alcohols, aldehydes, ketones, esters, ethers, sulfur compounds, nitrogen-containing compounds and halogenated compounds. Acetonitrile and benzene were among 11 compounds which correlated with smoking behaviour.

As a particular example for age and gender effects, we investigated the concentration of isoprene in 205 adult volunteers by PTR-MS: there was no statistically significant difference between mean isoprene levels in the breath of males and females (GM 105.4 and 95.5 ppb, correspondingly). Aging caused decrease of the concentrations of the investigated VOC in men with an estimated slope of the regression line for the log-transformed isoprene concentrations of -0.0049, but did not influence isoprene level in women. PTR-MS analysis is very easy to be performed and therefore was used for studies including many volunteers.

A comparison of the results of cancer patients with those of 31 healthy volunteers revealed differences in the concentration patterns. Sensitivity for detection of lung cancer patients depends on the number of compounds used. Based on 8 different compounds (or 12 or 54 different compounds, respectively) not arising in exhaled breath of healthy volunteers the sensitivity was 51% (or 78% or 86%, respectively), the specificity always being 100%. Potential marker compounds are 1-propanol, 2-butanone, 3-butyn-2-ol, benzaldehyde, 2-methyl-pentane, 3-methyl-pentane, n-pentane and n-hexane.

Introduction

Analysis of exhaled breath with respect to concentrations of volatile organic compounds bears fascinating possibilities for medical diagnosis and therapeutic monitoring [1-5]. Breath samples may be obtained from babies, during operations [6, 7] and during an ergometer test or during sleep [8-10].

Here we make an attempt to determine VOCs in exhaled breath of lung cancer patients. We restrict ourselves to compounds which show at least 15% higher concentrations in exhaled breath as compared to inhaled air. In particular, we do not at all make mention of compounds which show lower concentration in exhaled breath than in inhaled air. Our experience showed that different rooms show quite different indoor air composition, which is particularly pronounced in clinical environments.

Experimental Methods

Solid phase microextraction (SPME) and gas chromatography mass spectrometry (GC-MS)

The GC/MS analysis was performed on Agilent 5975 Inert XL MSD coupled with 7890 A gas chromatograph (Agilent, Waldbronn, Germany) with split-splitless injector. The 25 m × 0.32 mm × 5 μm capillary column CP-Porabond-Q (Varian Inc., Middelburg, The Netherlands) was used. Preconcentration was done using solid phase microextraction (SPME). Carboxen/polydimethylsiloxane (CAR/PDMS) fiber of 75 μm thickness was purchased from Supelco (Bellefonte, PA, USA). The sorption and desorption of analytes have been performed automatically by means of autosampler MPS 2XL (Gerstel, Mülheim an der Ruhr, Germany).

Proton transfer reaction mass spectrometry (PTR-MS)

A high-sensitivity proton transfer reaction mass spectrometer (PTR-MS, 3 turbopumps) with Teflon rings was used for our measurements. The count rate of primary ions (H_3O^+) was around 10^7 counts per second. Dwell time was 0.5 sec for each mass-to-charge ratio measured ($m/z=21$ - $m/z=230$). The usual pressure in the drift tube was ~2.3 mbar (with slight variations). In accordance with the instructions of the manufacturer (Ionicon GesmbH, Innsbruck), we computed concentrations with using only H_3O^+ as primary ion (not considering the first water cluster $\text{H}_2\text{O} \cdot \text{H}_3\text{O}^+$).

Human subjects

A cohort of 65 patients suffering from lung cancer (at different stages and in different treatment regimes) was recruited. All individuals gave informed consent to participation in the study. The patients completed a questionnaire describing their current smoking status (active smokers, non-smokers) and the time elapsed since their last smoke. Volunteers inhaled ambient air. The classification as smoker/non-smoker/ex-smoker is based on the self-declaration of the volunteers. The cancer patients were compared with 31 healthy volunteers, who also gave informed consent to participation in the study and declared their smoking habits. The samples were collected at different daytime independent of the time of meals and were processed within 6 hours at most. The study was approved by the local ethics committee of Innsbruck Medical University.

Investigation of age and gender effects on concentration patterns in exhaled breath was performed using PTR-MS in a cohort consisting of 205 adult volunteers with different smoking background without health complaints. A particular focus in this cohort was on isoprene.

Sampling of exhaled breath

Samples of mixed breath gas were collected in Tedlar bags (SKC Inc, Eighty Four, PA) with parallel collection of ambient air (also in Tedlar bags). Breath gas samples were obtained after a ~5 minutes sitting of a volunteer. Each subject provided 1 or 2 breath samples by use of a straw. All samples were processed within 3-6 hours. We collected mixed alveolar breath. Before collection of breath, all bags were thoroughly cleaned to remove any residual contaminants by flushing with nitrogen gas (purity of 99.9999%), and then finally filled with nitrogen and heated at 85°C for more than 8 hours with a complete evacuation at the end. All compounds detected in breath were compared to the ambient air and only compounds with concentrations at least 15% higher than in ambient air concentrations were reported. For GC-MS investigation, 18 ml of gas sample were transferred to 20 ml volume evacuated glass vials, and equilibrated with nitrogen gas.

Smokers evaluation

For each compound found, we determined the proportion of persons in whose exhaled breath the compound appears, separately for smokers, exsmokers and non-smokers. Putting smokers into one class and combining exsmokers and non-smokers into one class, the respective proportions, namely $\text{proportion}_{\text{smoker}}$ and $\text{proportion}_{\text{exsmoker and non-smoker}}$ were computed for appearance of each compound. The p-value for the null hypothesis “ $\text{proportion}_{\text{smoker}} = \text{proportion}_{\text{exsmoker and non-smoker}}$ ” was computed according to the method proposed by Agresti and Caffo [11].

Results

Altogether 103 compounds showing at least 15% higher concentration in exhaled breath than in inhaled air were detected. Among those 103 compounds, 84 were confirmed by determination of the retention time using pure compounds. Around one third of the compounds detected were hydrocarbons. We found hydrocarbons, alcohols, aldehydes, ketones, esters, ethers, sulfur compounds, nitrogen-containing compounds and halogenated compounds. Acetonitrile and benzene were among 11 compounds which correlated with smoking behaviour.

The p-value for the null hypothesis “ $\text{proportion}_{\text{smoker}} = \text{proportion}_{\text{exsmoker and non-smoker}}$ ” was ≤ 0.05 for altogether 11 compounds. These 11 compounds are acetonitrile, benzene, toluene, furan, 2-methyl-furan, 2,5-dimethyl-furan, 1,3-cyclohexadiene, 1,3-cyclopentadiene, 2-methyl-1-butene, 1,4-pentadiene and p-cymene.

As a particular example for age and gender effects, we investigated the concentration of isoprene in 205 adult volunteers by PTR-MS : there was no statistically significant difference between mean isoprene levels in the breath of males and females (GM 105.4 and 95.5 ppb, correspondingly). Aging caused decrease of the concentrations of the investigated VOC in men with an estimated slope of the regression line for the log-transformed isoprene concentrations of -0.0049, but did not influence isoprene level in women [12].

Altogether 80 compounds observed in our 65 lung cancer patients which were not observed in healthy controls. Some of these compounds as being suspected to be related to hospital indoor air (like p-xylene), mouth hygiene or chewing gum (like cineole).

A comparison of the results of 65 cancer patients with those of 31 healthy volunteers revealed differences in the concentration patterns. Sensitivity for detection of lung cancer patients based on 8 different compounds (or 12 or 54 different compounds, respectively) not arising in exhaled breath of healthy volunteers was 51% (or 78% or 86%, respectively), the specificity always being 100%. Potential marker compounds are 1-propanol, 2-butanone, 3-butyn-2-ol, benzaldehyde, 2-methyl-pentane, 3-methyl-pentane, n-pentane and n-hexane.

Discussion

Our goal was the determination compounds which are exhaled by cancer patients and not yet the determination of marker compounds. Smoking behaviour has a considerable influence on the concentration pattern of many compounds and that this influence needs very careful consideration. Various groups of compounds such as: hydrocarbons, heterocycles, alcohols, aldehydes, ketones, esters, ethers, amides, sulphur compounds, acetonitrile, terpenes and halogenated compounds have been determined in human breath samples, especially for smoking cancer patients.

We are presently devising a database of volatile compounds in exhaled breath. The results which we get for the composition of exhaled breath from different patient groups and healthy volunteers will now continuously be extended and be made accessible in database of compounds. A very preliminary version of our database of compounds is accessible at the homepage of the "International Association for Breath Research" (IABR) at iabrvoc-research.at.

Acknowledgement

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The Viability of Real-Time and Non-Invasive Breath Analysis for Disease Screening

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Abstract

For endogenous volatile organic compounds (VOCs) on breath to be used as biomarkers, reproducible breath sampling procedures have to be used. In addition to the requirement for suitable sampling techniques, knowledge of the reliability and precision of breath measurements compared to those of blood is crucial. Here we present studies addressing these two aspects of breath analysis. One relates to the development of a simple-to-use rebreathing breath sampling technique, which provides reproducible results. The second study illustrates the precision of breath measurements to determine systemic endogenous VOC blood concentrations and the consistency of breath VOC concentration measurements compared to single blood measurements.

Introduction

Breath analysis is attracting growing clinical and scientific attention as a potential means for delivering non-invasive, real-time rapid screening and diagnosis of complex diseases, such as cancers and acute infections. A recent overview of the field has been published [1]. Notable are the pioneering works of Phillips, with compelling evidence being provided for the detection of molecular biomarkers in the breath related to lung and breast cancers [2-4]. Despite its many advantages, before breath analysis can be introduced into clinical practice a number of crucial technical and scientific issues need to be addressed. These include the following: (i) the use of an appropriate breath sampling method applied under carefully controlled conditions to provide reliable and reproducible results; (ii) quantification of the limits of agreement between VOCs concentrations on the breath and their corresponding values in blood; and (iii) that there is a unique biomarker, or (more-likely) specific patterns of biomarkers made up of specific and/or non-specific biomarkers, for a particular disease that can be discerned from the complex chemical environment of breath. Whilst the last issue is the most important, investigations in that area can not take place without addressing the first two issues. In this paper we present details on a rebreathing breath sampling procedure which is easy to apply and produces reproducible results [5], and a study using this sampling procedure comparing the measurements of concentrations of VOCs in the breath compared to those in peripheral venous and radial arterial blood [6]. Owing to time and funding constraints only two VOCs were selected for the blood investigation, namely acetone and isoprene. These were specifically chosen for the following reasons: they are normally present at significant concentrations in breath and blood; they have very different solubilities (and solubility is known to alter the dynamics of gas exchange [7]); and they are common biomarkers for a number of common disorders [8]. In addition to acetone and isoprene we investigated methanol in the rebreathing studies.

Experimental Methods

To develop a suitable breath sampling procedure and to analyse the breath samples in order to determine VOC concentrations a proton transfer reaction mass spectrometer (Ionicon, www.ptrms.com/products/sptrms.html) has been used. This instrument has been described in detail elsewhere in the literature [9], and therefore no review will be given here. Specific operating conditions and calibration procedures for our studies have already been published [5].

Development of Breath Sampling Method:

The subjects were six healthy volunteers; two female (F1 and F2) and four male (M1-4). The letters A and B used subsequently refer to the two repeat trials undertaken by each volunteer. None of the subjects had ever suffered from any lung disease or condition and all gave informed consent to take part in the study. The protocol for the rebreathing method has been outlined elsewhere [5] and will not be repeated here.

Use of Breath Sampling Method for Blood/Breath Comparisons:

Ten healthy volunteers - six males (M1-6) and four females (F7-10) were involved in this study. None of the subjects were smokers or suffered from any lung problems. Prior to the start of the clinical measurements all subjects performed spirometry tests to check for normal lung function. In brief, a volunteer took a deep breath and expired this into a specially designed Teflon® bag (max. volume of 5 l) maintained at a temperature of 40°C. The volunteers used this as a reservoir for 20 rebreaths (corresponding to 4 cycles of 5 rebreaths with short breaks in between the cycles). Whilst the first breath sample was being analysed, blood samples (see below) were taken from the subject followed immediately by the next breath sample using a second bag. During this time the first measurement bag was flushed three times with dry nitrogen, checked for residual VOCs, and sent back to the clinical room ready for use for the third breath sample. This cycle continued, using two bags per volunteer, until the completion of the breath measurements resulting in the analysis of 5 breath samples per volunteer.

Blood Measurements:

An attending anaesthetist inserted one cannula into the radial artery of the non-dominant wrist and another into the antecubital vein of the other arm. 4.9 ml blood samples were taken from each cannula into EDTA monovettes (Startstedt) and blood samples were then sequentially taken over a time period of approximately 2 hours for each volunteer. To determine the VOC concentrations in the blood an independent commercial analytical laboratory (Trace Laboratories, Birmingham) was used. The approach adopted by this company for the blood measurements was similar to that described in detail by Miekisch et al, who analysed arterial and venous blood samples taken from mechanically ventilated patients [10]. The blood samples were analysed by means of solid-phase microextraction and gas chromatography-mass spectrometry. Repeatability of measurement was checked by taking five samples from a mixed pool of blood and measuring the acetone and isoprene intensities, giving a standard deviation of 7% for acetone and 8% for isoprene.

Results

Rebreathing breath sampling protocol to measure VOCs in human breath:

The concentrations measured in the breath of all volunteers were found to increase with the number of rebreaths until a plateau value was reached by at least 20 rebreaths. This is illustrated in figure 1 for data obtained for volunteers F2 and M4. The average ratio of plateau concentration to single mixed expired breath concentration was found to be 1.94 ± 0.57 for isoprene and 1.30 ± 0.18 for acetone. In comparison measurements from on-line single exhalations demonstrated a positive slope in the time dependent expirograms of isoprene and acetone. The slope of the

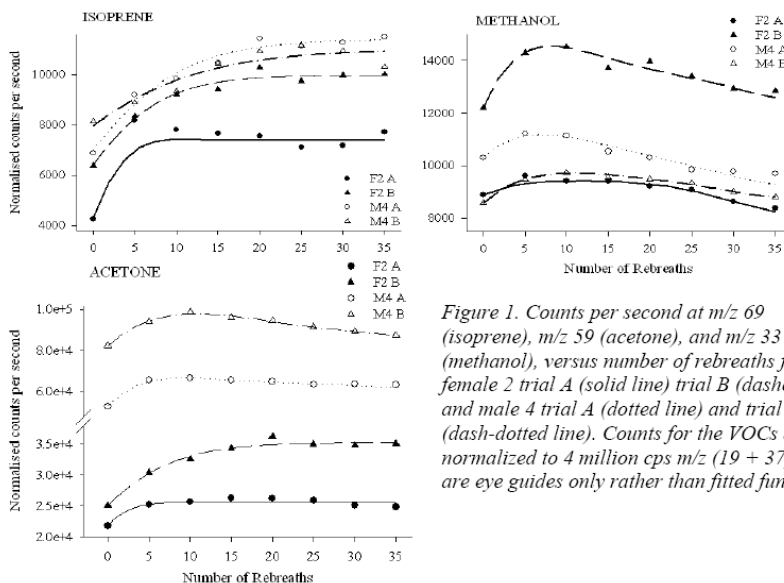


Figure 1. Counts per second at m/z 69 (isoprene), m/z 59 (acetone), and m/z 33 (methanol), versus number of rebreaths for female 2 trial A (solid line) trial B (dashed line) and male 4 trial A (dotted line) and trial B (dash-dotted line). Counts for the VOCs are normalized to 4 million cps m/z (19 + 37). Lines are eye guides only rather than fitted functions.

isoprene expirogram was found to be persistently linear. Thus the end-expired concentration of isoprene was found to be highly variable in the same subject depending on the duration of exhalation. End-expired values of acetone were found to be less sensitive to the length of exhalation, and were the same to within measurement uncertainty for any duration of exhalation for any subject. We conclude from this investigation that uncontrolled single on-line exhalations are not suitable for the reliable measurement of isoprene in the breath and that rebreathing can be the basis of an easily tolerated protocol for the reliable collection of breath samples.

Comparisons of endogenous breath and blood volatile organic compound concentrations in healthy volunteers:

In-vivo arterial blood/breath ratios were calculated for each individual and used to determine the sample mean and ranges. These are found to be 580 (320 – 860) for acetone and 0.38 (0.19 – 0.58) for isoprene. To our knowledge, this is the first report of the determination of in-vivo blood/breath ratios for endogenous VOCs in freely breathing subjects. To illustrate the type and quality of measurements obtained, figure 1 shows VOC concentrations in breath (CBr), arterial blood and venous blood (CBI) for M6 (acetone) presented as a function of the times at which the various samples were taken from the start of the trials. Error bars represent experimental uncertainties of 10%.

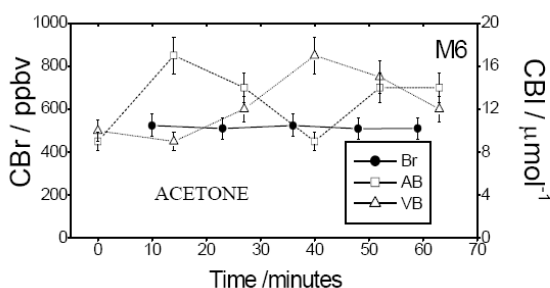


Figure 2. Concentrations of isoprene and acetone in breath (CBr) and blood (CBI) for acetone (M6) plotted as a function of time at which the samples were taken. AB refers to arterial blood and VB to venous blood. The lines drawn connecting the data points are visual guides only.

Figure 2 demonstrates that the trends in both blood and breath concentrations are reasonably flat over the sampling period. Notably, this figure demonstrates the variability in blood concentration measurements from one sample to the next for a given volunteer. Taking all samples obtained from the ten volunteers we find that, taken as a percentage of the mean, the repeatability coefficient for acetone is found to be 19% for breath, 103% for radial arterial blood and 79% for peripheral venous blood. The corresponding values for isoprene are 27% for breath, 79% for radial arterial blood and 81% for peripheral venous blood.

Discussion

The method of isothermal rebreathing allowed an equilibrium value of acetone and isoprene to be reached in the majority of cases. For the purposes of establishing a protocol for breath analysis, our data show that where a plateau was reached, it was achieved by 20 rebreaths. In some cases a plateau had been reached in a smaller number of rebreaths, presumably because of variations in the volume of the first full expired breath and the subsequent portion of breath rebreathed in each cycle. We did not attempt to control any of these volumes, as the intention was to develop a simple procedure that can be performed by the majority of subjects without extra instrumentation.

As a result of the superior repeatability of breath measurements using a rebreathing sampling technique, breath concentrations can provide an easier way of estimating systemic VOC blood concentrations than can be obtained directly from individual blood samples themselves, despite inter-individual variations in blood/breath ratio. However, the conversion of breath to blood concentrations is not necessarily crucial for screening purposes. It is the changes in the VOC

profiles which will discriminate healthy from ill patients. To monitor these requires reliable and VOC concentrations to be obtained. This study has clearly demonstrated that breath analysis using our re-breathing sampling protocol provides such measurements. In conclusion we have demonstrated that breath VOC measurements provide a more consistent measure for investigating underlying physiological function or pathology than single blood measurements.

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Factors affecting breath gas analysis

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Abstract

In the frame of a breath gas study with lung cancer patients various factors should be identified to minimize the artifacts which can influence the exhaled VOC concentration. Therefore, different biological and physiological parameters such as gender, age, BMI, food, velocity of breathing, exhaled volume, inhaled volume, breath holding and multiple exhalation were examined.

Other possible artifacts within breath gas analysis seem to be the individual day-to-day changes of the exhaled VOC pattern. Therefore, the reproducibility of consecutive breath gas measurements from 11 volunteers over 15 to 70 days was analyzed. As a result, many masses, as e.g. mass 31, showed highly variable concentrations within the same person when measured on subsequent days. Unless these phenomena are not clear, identified, and at least kept constant, many artifacts might be produced and introduced in breath gas studies.

Introduction

Identification of breath gas biomarkers for various diseases like lung cancer, COPD, diabetes etc. is a comparatively new and promising technology. Recently, several research groups claimed to have identified volatile organic compounds (VOCs) being specific biomarkers for diseases as for example lung cancer [1-5]. But altogether, it seems as if the reproducibility of the test is a major problem. The studies which have been conducted in a similar way for identification of exhaled biomarkers came out with completely new identities of VOCs each time, resulting into ambiguity of breath gas analysis technique for disease identification.

One reason for that might be the long list of variables and artifacts affecting the breath gas test itself. For this reason, the aim of this work was the systematic determination and investigation of biological and physiological parameters involved in breath gas analysis in order to increase the reliability and reproducibility of this technique. In this respect, the day-to-day variability of exhaled VOC concentrations will be in the focus of this work.

Experimental Methods

Breath gas samples were analyzed by using a proton transfer reaction-mass spectrometer (PTR-MS) with operation details as described in [6]. The data handling and corrections were performed as described in [7] with respect to breath humidity, time of storage of breath samples and transmission. The breath gas was collected from healthy volunteers. The work is divided into two parts:

a) Biological and physiological parameters affecting breath gas analysis

The effects of the biological and physiological parameters gender, age, body mass index (BMI), food, velocity of breathing, exhaled volume, inhaled volume, breath holding and multiple exhalation on the exhaled VOC concentrations were determined from 37 male and 22 female healthy volunteers. It should be pointed out, that each parameter had been investigated separately by keeping the others constant.

b) Day-to-day variability of exhaled VOC concentrations

In order to test the reliability of breath gas analysis with volunteers or patients in praxis, the reproducibility of day-to-day measurements has been investigated. Therefore, breath gas had been collected from 11 healthy volunteers in a certain time range (up to 10 times over 15 to 70 days) and measured by PTR-MS. The volunteers were asked to fill the breath gas in 3 L PTFE Teflon sample bags (SKC Inc.) at their home always at the same time at early morning before tooth brushing and breakfast. This should guarantee that the volunteers had been fasting over night before breath gas collection.

Results

a) Biological and physiological parameters affecting breath gas analysis

In this part of the study the biological and physiological parameters gender, age, BMI, food, velocity of breathing, exhaled volume, inhaled volume, breath holding and multiple exhalation on the exhaled VOC concentrations and their effect on the breath gas test have been investigated. In this respect, only some representative VOCs, i.e. oxygen, methanol, breath humidity, acetonitrile, acetaldehyde, ethanol, acetone, isoprene, benzene and toluene have been considered for evaluation.

It was found out that most of the investigated parameters have a significant influence on the concentrations of the exhaled VOCs. A detailed overview will be presented in the oral talk and poster.

b) Day-to-day variability of exhaled VOC concentrations

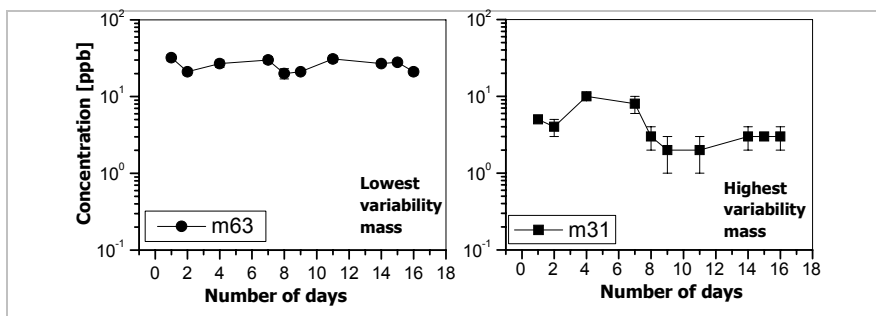


Figure 1: Comparison of exhaled volatile biomarkers in one volunteer in long term follow up study. Error bars denote standard deviation within 7 cycles per measurement and count rate variability.

In order to verify the reliability of breath gas analysis for medical application, the reproducibility of day-to-day measurements for 11 different volunteers has been investigated. Figure 1 demonstrates the test results of one volunteer and two typical masses as an example. It turned out that some VOCs (as e.g. mass 63) could be measured in quite similar concentrations. This had been expected since the breath collection was done from each healthy volunteer at the same time of the day after an over night fasting. However, there were some VOCs which showed a huge variability with regard to the detected concentrations.

In order to summarize the results from all the volunteers and masses measured, following procedure has been applied: In a first step, each of the 11 follow-up studies (one for each volunteer) has been regarded. For each single mass (above detection limit = 20 cps) the geometric mean (GM) and geometric standard deviation (GSD) of the repeatedly measured concentrations have been calculated. In a second step, the 11 follow-up studies have been averaged by calculating the median (and percentiles) of the 11 GSD values for each mass. The results are expressed as "degree of variability" and are shown in Figure 2 (In addition a correction factor was introduced since the measurement error of each VOC is influenced by its count rate).

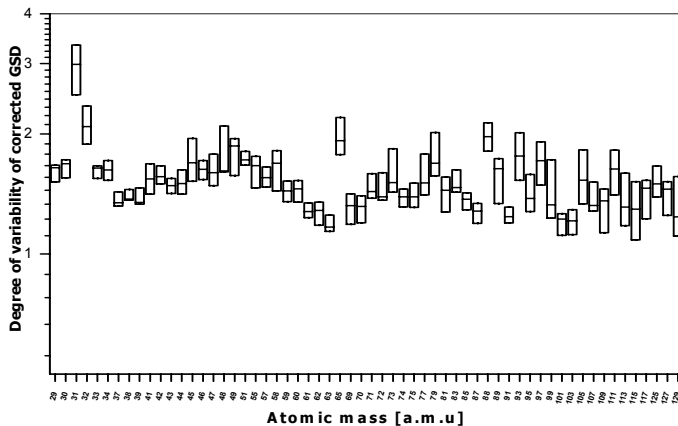


Figure 2. Degree of variability of selected masses in long term follow-up study. The results are given as median (and percentile 25 and 75) of 11 GSD values, each obtained from the statistical distribution of repeatedly measured VOC concentrations during the follow-up study for each volunteer (for details see text).

Discussion

a) Day-to-day variability of exhaled VOC concentrations

The masses like m/z 31, 33, 43, 59 and 69 were stated as biomarkers for diseases like lung cancer [1-5]. But the follow-up study presented here shows that mass 31 seems to be a highly variable VOC in breath from day to day. This might come from time-dependent physiological impacts or from other uncontrolled influences as food, beverages, etc. on the breath. Unless these phenomena

are not clear, identified, and at least kept constant, many artifacts might be produced and introduced in breath gas studies. In this context, the identification of mass 31 as a specific cancer marker should be critically reinvestigated.

On the other hand, the follow-up study also identified masses with low variability as e.g. m/z 33, 43, 59 and 69. These candidates may be much more suitable for monitoring of various diseases as they seem better reproducible in practice.

b) Biological and physiological parameters affecting breath gas analysis

The results of this work demonstrated that various biological and physiological parameters may influence the outcome of a breath gas test. Thus, it is particularly important to take into account these parameters for planning breath gas studies.

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Online breath sampling with PTR-MS – A setup for large screening studies

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Abstract

Fast response times and a high sensitivity for VOCs make PTR-MS instruments ideal tools for online breath gas analysis. Two classes of issues arise in PTR-MS online breath sampling. First, the influence of the breath gas matrix on the measurement process must be investigated for an accurate quantification of VOCs. Second, a high sample frequency is essential in order to resolve individual breath phases, which imposes limitations on the measurement process. In this paper, we summarize these difficulties and present solutions for online breath measurements and improved quantification of the data. On this basis we present a setup for online breath analysis for large scale screening studies. We have tested this setup in a clinical study and present first results.

Methods and Results

Influence of the Breath Gas Matrix onto the Measurement Process

Using a commercially available gas calibration unit (GCU-s: Ionimed Analytik, see figure 1), it is possible to determine e.g. the PTR-MS sensitivity for selected compounds for a range of sample gas humidity [3].

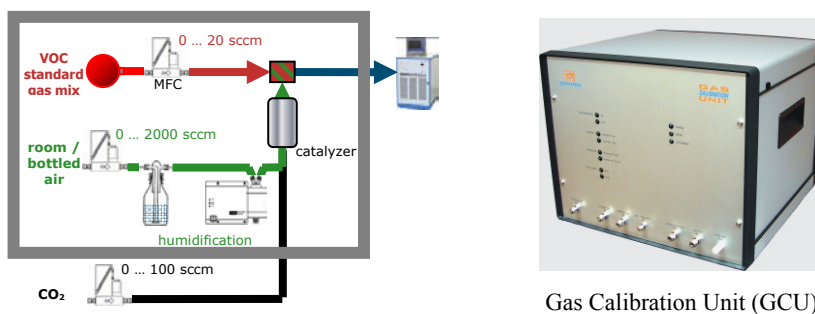


Figure 1: Setup for the calibration measurements. In the gray box, the main components that are found in GCU (which is displayed on the right) are illustrated.

We adapted this device to allow for admixing different ratios of CO_2 to investigate the effects of the breath gas matrix. We performed in total 35 calibrations at five CO_2 concentration levels and seven different sample gas humidity levels. For several compounds, such as acetone, acetonitrile (see figure 2), 2-butanone, and crotonaldehyde, we found no influence on the PTR-MS sensitivity when proper normalization is implemented. For other compounds, such as formaldehyde (see figure 2) and aromatics we found that the PTR-MS sensitivity is dependent on the gas matrix humidity. We investigated a CO_2 -dependent background signals on m/z 45 which can interfere with the measurement of acetaldehyde. Under typical operating conditions and 5% v of CO_2 , this signal is equivalent to approximately 8 ppb of acetaldehyde. We investigated the origin of this signal and suggest several measures for the reduction and/or correction of this effect.

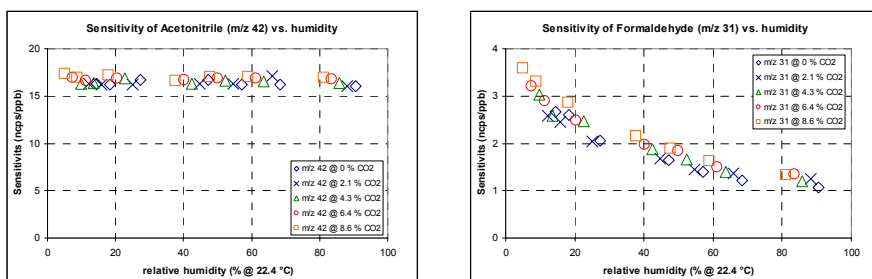


Figure 2: Sensitivity obtained for acetonitrile (left) exhibits no dependence on sample gas humidity and CO_2 concentration. (right) The most pronounced dependency of the sensitivity on sample gas humidity was found for formaldehyde.

Online Breath Gas Analysis

In online (and also offline) breath sampling an identification of the different breath phases is needed. In most applications only the end-tidal fraction is of interest, since that part resembles the alveolar concentrations most closely. An exhalation is typically characterized by the exhaled CO_2 content with the highest values corresponding to the end-tidal phase. In PTR-MS the water cluster signals (m/z 37), which is correlated with the humidity of the sample gas, is a suitable alternative measure, since it is also needed for normalization of the data. In order to resolve the individual

breath phases a sampling frequency much higher than the breathing frequency is needed. For PTR-MS instruments (and also other quadrupole MS instruments) this imposes limitations on the number of m/z that can be monitored simultaneously and the integration time per m/z . As an example, in direct online sampling [1] with an integration time of 100 ms per m/z only three m/z can be monitored in order to reach a sampling frequency of 3.3 Hz – enough to resolve breath cycles in slowly breathing test persons. When m/z 21 and m/z 37 are used as measure for normalization, only one additional m/z can be monitored. There are several ways to overcome this limitation. We tested several methods with different advantages depending on the application.

We tested one method, buffered end-tidal (BET) online sampling [1], in detail. This method facilitates the sampling of a wide range of compounds within a short period of time and also reduces the risk of hyperventilation during the breath test. Based on this method we present a setup for online breath analysis with PTR-MS, which is suitable for large screening studies. This setup has been tested in a clinical study and we have analyzed the breath of over 160 patients and controls. In a complete breath test, we measured over 140 m/z . The end-tidal value for each m/z was determined five times, each from an individual exhalation. A complete breath test takes ~ 25 minutes.

To assess our data quality we employed different biostatistical methods to determine markers for smoking and compared our results to previously published work. We found several breath markers that differed significantly between smokers and non-smokers. We could largely reproduce the breath markers found by Kushch et al. [2]. In figure 3, we plot the distributions for smokers and non-smokers of our most prominent marker m/z 42, attributed to acetonitrile, with a p -value $< 10^{-14}$. For the quantification of acetonitrile, we utilized the results obtained in the calibration experiments described above. We observed several outliers in the distribution of acetonitrile concentrations in the breath of non-smokers, which could potentially be due to passive smoking. We obtained a classification efficiency (area-under-ROC) of 96.9% for the detection of smoking behavior, which could be reproduced by several of the employed biostatistical methods. For the data plotted in the ROC (receiver-operating-characteristic) curve in figure 3, we determined a maximum Youden index of > 0.82 , which exceeds that of previously published studies [2].

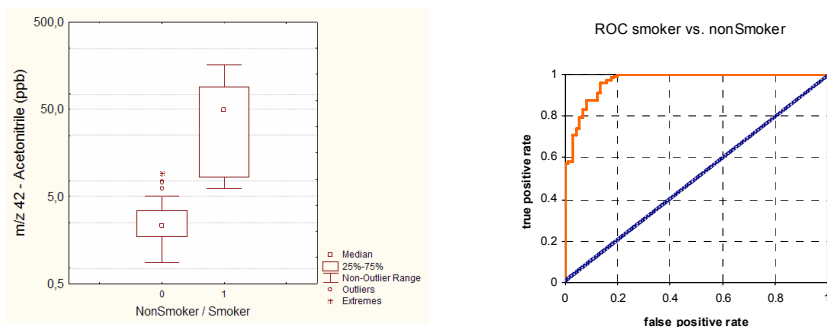


Figure 3: (Left) Box-plot (logarithmic scale) of the measured values for acetonitrile (m/z 42) for non-smokers and smokers. (Right) Receiver operating characteristic (ROC) curve of the test for smoking behavior.

Discussion and Conclusion

In our calibration experiments, we found several compounds that show a sensitivity independent of the variation of sample gas humidity and CO_2 content. The quantification of these compounds is thus straight forward, also under breath gas matrix conditions. For those compounds with a sensitivity which depends on sample gas humidity, quantification is possible by determining the sensitivity as a function of the humidity, which can be expressed through the water-cluster signal on m/z 37. Although the range of sample gas humidity addressed in this paper did not reach the levels present in breath gas, the results could still be used by extrapolating to higher humidity levels. An experimental verification is subject of further study. The next generation of gas calibration units (GCU-advanced), enabling 100% relative humidity at 37 °C and built in CO_2 admixing, is currently under development. By investigating the CO_2 dependent background on m/z 45, we can suggest several solutions to compensate or reduce this effect. Applying this knowledge can improve the quantification of PTR-MS breath-gas data. This knowledge is also valuable for other research areas employing PTR-MS for the measurement of VOCs in a variable gas matrix.

We presented first results from a large screening study where we employed buffered end-tidal sampling for online breath analysis. As a test for the data, we determined the breath markers for smoking. A comparison to a study using offline sampling techniques showed that we can largely reproduce the published markers for smoking and obtain an even greater test efficiency. We expect that our test could still be further improved if effects of passive smoking were considered. These results confirm the validity of our method for online breath analysis and the quality of the collected data.

The main objective of the presented clinical study is the detection of lung cancer by online breath analysis. A validation of the data quality and the identification of breath markers for smoking were important intermediate steps, since the pronounced characteristic of smoking in breath gas data could be a confounding variable in the detection of the supposedly weaker influence of lung cancer on exhaled breath VOCs.

Acknowledgements

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Analysis of tuberculosis using PTR-MS

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Abstract

Tuberculosis (TB) is declared a global emergency by the World Health Organization. It is estimated that about one-third of the world population is infected with *Mycobacterium tuberculosis* (*M. tub.*), the agent responsible for TB in humans. Each year, 8-9 million new cases occur, with 2-3 million deaths. The majority of these new infections and deaths occur in developing countries, where the HIV epidemic has considerably contributed to the tuberculosis problem [1]. Present available methods are too complicated, too expensive or too time-consuming to be widely used. These facts elucidate the need for new, fast, cheap and specific methods of diagnosis of TB.

Studies over the past 25 years provided consistent evidence that various microbes and mycobacteria release different quantities and types of volatile organic compounds (VOCs). In recent years, studies using an electronic nose have shown potential for the identification of *M. tub.* from headspace analysis of cultures or sputum and for diagnosing TB from sputum. These systems have shown that *M. tub.* and other species of mycobacteria produce distinctive patterns of VOCs that can be used for identification or diagnosis from sputum or breath [1]. The only study performed up to now to diagnose TB from the breath of patients has been conducted using GC-MS [2].

Proton-transfer reaction mass spectrometry (PTR-MS) has the potential of measuring online those compounds that are able to distinguish between different mycobacteria or between patients with or without TB by analyzing breath or sputum headspace. The advantage of using PTR-MS consists in the possibility to measure fast and online sputum headspace or even breath without sample preconcentration. The ultimate goal of PTR-MS analysis in this respect would be the online sampling of the breath of suspected TB patients to diagnose their status *in vivo*. Diagnosis should then be possible within a few minutes. The first logical step towards diagnosis of TB is the identification of VOCs from *M. Tub* cultures.

Here, the headspace is analyzed of three different mycobacterial species, *M. tub*, *M. avium* and *M. kansasii*. Several questions are asked: Is it possible to distinguish between the different species by the VOCs released in their headspace? Do the compounds that are produced depend also on the substrate on which the mycobacteria are grown? Would it be possible to find common masses that could indicate mycobacterial infection in a human from its breath? Can the development of cultures be followed in time to identify compounds that are produced by growing bacteria and are these compounds related to the growth stage?

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3. Applications in Food Science

Intranasal concentrations of flavors

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Abstract

The odorants emanating from the oral cavity during eating and drinking reach the olfactory mucosa via the pharynx (“retronasal olfaction”). It is unclear which variables influence the perception of intraorally applied substances. The aim of the present study was to determine the temporal profiles of volatile odor concentrations at different locations in the nasal cavity during consumption of liquid and solid custard samples using Proton Transfer Reaction Mass Spectrometry (PTR-MS). Intranasal odor concentrations were measured at least twice in 9 subjects (6 female, 3 male) at 4 nasal positions during consumption of liquid and solid custards. The low viscosity custard was swallowed earlier than the more solid one. The compounds were found to reach the nose in different concentrations. Largest maximal amplitudes were measured in the nasopharynx, whereas lowest concentrations were found in the region of the olfactory cleft. In addition, different odorants reached the different regions in the nasal cavity in varying concentrations, indicated by a significant interaction between factors “position” and “compound”. Furthermore, the compounds were found to reach the positions within the nasal cavity with different latencies. These results indicate that different volatile flavor compounds exhibit different temporal and spatial profiles in terms of their intranasal distribution.

Use of PTR-MS to predict the shelflife of crackers

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Abstract

PTR-MS has found a substantial number of applications in our research program. These applications have involved adding understanding to volatile release from dentifrices, microwavable foods, hot beverages, chewing gum, and encapsulated flavorings and their use, to name a few. In this symposium, we will present our work on using PTR-MS to monitor the flavor quality of cheese crackers during storage. This project was one of the most detailed in scope. In this study we developed the methodology to rapidly and reproducibly sample and monitor the volatile profile above crackers. Through interfacing our PTR-MS with a GC-MS, we were able to assign ions found by PTR-MS to specific compounds. The shelf life study that ensued showed that over a period of 6 months, there was a good correlation between the peak area of compounds showing an increase by GC-MS (mainly oxidation products) and their selected ions monitored by PTR-MS. A final study involved sensory and PTR-MS monitoring of flavor during storage. We found that the PTR-MS data reliably predicted the changes in scores for several sensory attributes, the best being oxidation-related notes.

Characterising human perception: when physiology deceives analytics

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Abstract

On-line breath analysis with mass spectrometric (MS) techniques, such as PTR-MS, is widely used for odorant-release studies during food consumption and for *in vivo* monitoring of release and migration patterns of volatile organic compounds (VOCs). As further progress is achieved in this area, knowledge of the number of factors potentially influencing release and transfer kinetics, as well as perceptual phenomena, is correspondingly increased. This article highlights parameters that might bias *in vivo* measurements if not carefully considered in the experimental set-up, and provides an overview of relevant literature.

Introduction

Over the years, on-line odorant monitoring using PTR-MS in the field of food chemistry research has been applied with regard to diverse investigative aspects. Early studies aimed to assess the “basic” release of compounds from model systems or complex food matrices, such as intact strawberries or pieces thereof [1,2,3]. Conventional analysis of VOCs or odour-active constituents in food has mainly been related to VOC release, and many characteristic PTR-MS mass profiles have been determined [4,5].

To mimic *in vivo* release and transfer patterns, PTR-MS has been coupled to model mouth systems, e.g. incorporating simulation of mastication and saliva [3,6]. In addition, PTR-MS has been directly applied to observe release phenomena *in vivo*. For instance, studies have been carried out on predefined odorants exhaled from the nose during food consumption [3,7], as well as on odorants specifically introduced into the oral or nasal cavity to monitor their intrapharyngeal and intranasal migration and distribution patterns [8]. Thereby, large inter-individual variations have been observed, with correlations between individual aroma release and intensity of aroma perception [9]. In several studies, individual consumption patterns were reported to be one source for inter-individual variation.

The key target of *in vivo* PTR-MS measurements has been to relate release of specific odour substances with sensory perception during eating. However, diverse physiological studies indicate that pure release and transfer of free odorants from the oral to the nasal cavity might not be the only source for inter-individual differences, and that perception might not be fully mirrored by VOC breath profiles.

Sensory evaluation

In an assessment of odour-quality alteration, panellists were asked to evaluate different aqueous 2-furfurylthiol (FFT) samples. The FFT samples (25 mL volume at 100 µg/L water) were presented to the sensory panel for ortho and retronasal evaluation, either with or without saliva addition. Samples were taken into the mouth, kept for 10 s with closed lips and closed velum, and rinsed carefully within the oral cavity, then expectorated [10]. At defined time intervals after expectoration, the perception of FFT aroma, as well as of any “changed” odour quality, was rated by the panellists by deliberately opening the velum-tongue barrier at defined times (cf. [11]). Panellists were asked to score odour intensities from 0.0 (not perceivable) to 3.0 (very intense). Results are discussed below.

Metabolic processes in perception and *in vivo* monitoring

Metabolic aspects of the human physiology are one parameter that has not yet been regarded in detail in *in vivo* monitoring of odour-active substances and sensory perception correlation. Recent data, however, indicate that there might be significant flavour changes induced upon introduction of certain odorant precursors into the oral cavity. Starkenmann et al. showed that potent odorants are generated in the mouth from odourless, non-volatile cysteine-conjugates only after several seconds due to microbial action [12]. This can lead to a delayed onset of an intense odour perception when other free odorants from the respective food material might already have declined. The authors showed that flavour perception was strongly modified by liberated thiols that were initially not present as free volatiles. Other studies have shown that malodorous compounds are generated in the mouth from food residues due to microbial action [13,14,15]. However, these processes generally extend over a relatively long time-span and are not, or only to a minor extent, perceived by the person. When considering the release process as described by Starkenmann et al., obviously the opposite is true. As release is quickly achieved, individuals likewise perceive rapid changes in retronasal aroma perception.

On the other hand, it has been shown that odorants are not only generated but also degraded upon contact with saliva, for example the *coffee-like* and *roasty* smelling FFT [16]. Huge inter-individual variation has been observed for these processes [16], as well as for the respective sensory perception: the initial aroma perception intensity, when retronasally evaluating FFT model solutions, varied significantly among panellists (cf. Fig. 1a). Accordingly, the persistence of the specific “initial” FFT sensory impression differed to a major extent between individuals (cf. Fig. 1b), with intensity rating generally correlating with the reported combined persistence of retronasal aroma impression (cf. Fig. 1a and 1b). However, it is interesting to note that in retronasal evaluation of the FFT model solution all panellists reported a shift in the sensory impression with time that could not be easily described by the panel, but exhibited a *roasty-sulphury* aroma note that clearly differed from that of the original FFT solution. This altered aroma impression was additionally recorded with regard to sensory persistence (cf. Fig. 1b), and was shown to last for some panellists for up to a few minutes.

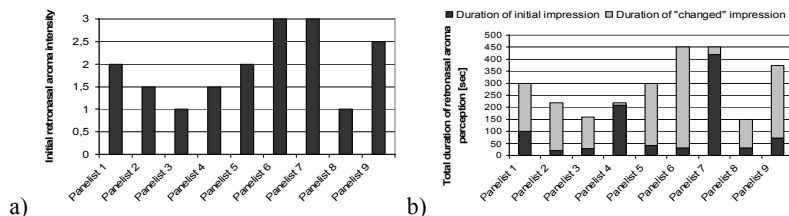


Figure 1: a) Different initial retronasal intensities for nine panellists during sensory evaluation of a FFT model solution, and b) individual persistence of the initial retronasal FFT impression and subsequent "changed" retronasal aroma perception.

Interestingly, there seemed to be a tendency that panellists perceived the "changed" impression more intensely if the initial sensation was less pronounced, and vice versa. We assume that this indicates aroma modification due to metabolic processes, as the same aroma changes were observed when spitting saliva into the FFT model solution and evaluating the changes orthonasally, but not when orthonasally evaluating the FFT model solution alone (data not shown). As in the studies of Starckenmann et al., the relevant processes could not be monitored by means of breath MS analyses as the respective odour compounds were well below the detection thresholds of the instrument. The individual variations indicate differences in the modifying systems that result in corresponding variations in the reported individual changes in retronasal flavour perception.

Previous model studies on aqueous FFT solutions with saliva addition showed that 2-FFT is not only degraded but that, to some extent, the respective odour-active disulphide is formed, with drastic differences between panellists [16]. However, formation of Bis-FFT was not the only reaction product, since not 100 % Bis-FFT product was generated. Cross-reactions with salivary constituents might have occurred and are indeed most likely. To date it is not clear whether specific salivary metabolic systems or microbial processes are involved.

Discussion

PTR-MS is a useful tool for *in vivo* monitoring of odorant release and transfer processes. Nevertheless, when trying to correlate specific release patterns and related perceptual phenomena, additional physiological aspects should be considered. According to recent studies, human physiology exhibits diverse aspects: Processes are induced by metabolic activity of saliva itself, or by microorganisms that constitute the typical oral bio flora. The proof of the presence of precursor substances in natural food systems that release odorants upon contact with saliva still needs further investigation. It might be that cysteine conjugates are not the only compounds that induce flavour release upon contact with saliva. Also, modification of thiols into the respective disulphides is most likely not the only odorant metabolism process.

When looking at metabolic or chemical interactions during mastication in the presence of more complex media such as food systems, cross-reaction products with other VOCs, odorants, or any other food ingredients might occur, thereby leading to ever-changing sensory impressions depending on the respective food matrix. Likewise, possible further metabolic processes in the

nasal cavity need to be considered. For example, Schilling et al. showed that VOCs/odorants can be further metabolised by nasal systems to form compounds with differing aromatic characteristics [17]. At times these can be monitored by MS techniques, but the full dynamics of their formation and sensory perception are still unknown. Without knowledge of these processes, *in vivo* monitoring by on-line MS systems might not focus on the relevant substances.

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Release of berry and citrus aroma compounds from chewing gum

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Abstract

The release of aroma compounds during chewing gum mastication was monitored in real time by a Proton-Transfer-Reaction Mass Spectrometer (PTR-MS). All aroma compounds were selected from fruit flavours. Their physico-chemical properties covered a broad range which allowed investigating the influence of these properties on aroma release.

The high time resolution of the PTR-MS provided detailed information about the mechanism of aroma release for compounds with different hydrophobicities. Whereas hydrophobic compounds started to be released with the very first chewing motion, the release of hydrophilic compounds only started after 30 seconds when the panellists were asked to swallow the first time. Time to reach maximum intensity of release was reduced with rising hydrophobicity of the aroma compounds until this exceeded a certain value. Compounds with hydrophobicities above this value were slowly extracted from the gum base, not reaching their maximum of release within the 8 min of chewing.

Introduction

When chewing gum, consumers look for a product that tastes good and continuously releases a pleasant flavour sensation over the total time of chewing. To achieve this, the flavour industry needs to understand how aroma compounds are released during chewing gum consumption and develop flavours accordingly. Flavours might be added to chewing gums as liquid solution in a solvent or encapsulated. This article will focus on release occurrences in chewing gums containing liquid flavour.

Experimental Methods

Materials

Non-coated chewing gums were prepared using gum base (140g), sorbitol (277g), maltitol syrup (50g), glycerine (25g), aspartame (0.2g), acesulfame K (0.2g), and triacetin (3g). Two different fruit flavours were used in two sets of experiments: a citrus flavour composed of octanal (m/z111), decanal (m/z157) and limonene (m/z81) and a berry flavour composed of 1,4-butyrolactone (m/z87), cis-3-hexenol (m/z83), benzaldehyde (m/z107), ethyl butyrate (m/z117), and butyl iso-pentanoate (m/z103). The ions which were used for monitoring by PTR-MS are indicated in the brackets.

PTR-MS measurements

Each gum was chewed and the exhalations were analyzed using a PTR-MS (Ionicon Analytik GmbH, Austria). The experiments were performed by 3 panellists with 2 repetitions each. The panellists started with 5 blank exhalations to determine the breathing background. Then, they put the chewing gum in the mouth and chewed it during 8 minutes at one chew per second swallowing every 30 seconds. The time was controlled by a timer.

Results

The selected aroma compounds in the two flavour types can be divided into a relatively hydrophilic group (logarithm of partition coefficient octanol-water $\text{Log } P < 1.8$) and a relatively hydrophobic group ($\text{Log } P > 1.8$). The time-intensity curves of both groups are shown in figure 1.

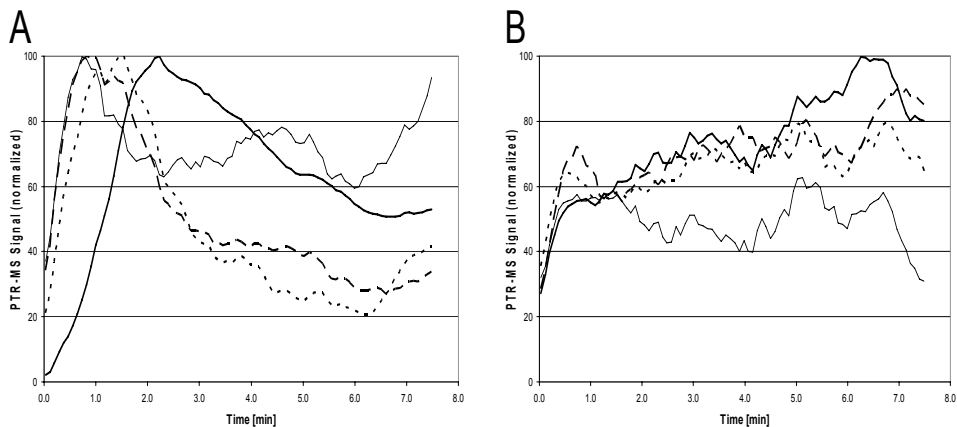


Figure 1: Aroma release from chewing gum during 8 min of mastication. Average of 3 panellists with 2 repetitions each. Signals were smoothed by taking the average of 10 data points.

A: Hydrophilic compounds. Signals normalized by setting maximum value to 100.

— 1,4-Butyrolactone
 — Benzaldehyde
 ■ ■ cis-3-Hexenol
 — Ethyl Butyrate

B: Hydrophobic compounds. Signals normalized by setting maximum value of limonene release curve to 100. For comparison all other curves were adjusted to the limonene level at 1.5 min mastication

— Limonene
 — Butyl isopentanoate
 ■ ■ Decanal
 — Octanal

For hydrophilic compounds (figure 1A) the maximum signal intensity is reached within the first 3 minutes of mastication. The time to reach this maximum seems to be correlated to $\text{Log } P$ in a reverse way: the lower $\text{Log } P$ (i.e. the more hydrophilic the compound), the later the maximum of release (table 1).

Prolonged aroma release after the maximum peak can be observed for the 2 compounds with the lowest and the highest hydrophobicity within this group (1,4-butyrolactone with LogP = - 0.76 and ethyl butyrate with Log P = 1.77).

Table 1: *Partition coefficients octanol-water and time to reach maximum intensity of release (Tmax)*

Compound	Log P ¹	Tmax
1,4-Butyrolactone	- 0.76	1.9 – 2.4 min
cis-3-Hexenol	1.61	1.3 – 1.6 min
Benzaldehyde	1.64	0.6 – 1.1 min
Ethyl butyrate	1.77	0.6 – 1.0 min
Octanal	3.03	n.d.
Butyl iso-pentanoate	3.18	n.d.
Linalool	3.28	n.d.
Decanal	4.09	n.d.
Limonene	4.45	n.d.

¹ Calculated using Advanced Chemistry Development (ACD/Labs) Software V9.04

For hydrophobic compounds (Log P > 3.0) release is increasing continuously during the 8 min chewing time (figure 1B).

In this group octanal has the lowest Log P value (3.03). Its release is constant over time but does not show the initial impact when the sugar phase is released. All other compounds with Log Ps > 3.1 have very similar curves with increasing release till the end of the mastication time at 8 min.

Comparing real time release of 1,4-butyrolactone and limonene (figure 2) reveals another difference between the release of hydrophilic and hydrophobic aroma compounds.

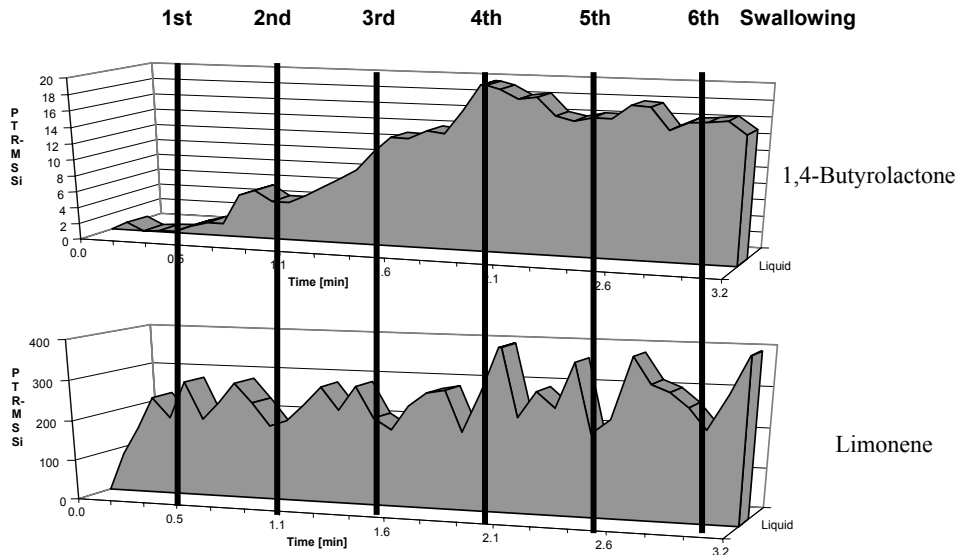


Figure 2: Aroma release of 1,4-butyrolactone and of limonene from chewing gum during the first 3 min of mastication. Average of 3 panellists with 2 repetitions each.

Whereas limonene starts to be released immediately after the gum is put into the mouth (0 min), 1,4-butyrolactone starts only to be released after the 1st swallowing after 30 seconds and its release increases continuously till the 4th swallowing after 2 minutes.

Discussion

It was supposed in the past that hydrophilic aroma compounds are released from chewing gum at the same time as taste components during the first minutes of mastication and that hydrophobic compounds on the other hand are slowly extracted from the gum base [1]. Several “in-vivo” measurements of aroma release from chewing gum were performed (e.g. [2]-[4]) but real time aroma release monitoring by PTR-MS allowed us now to understand better what exactly the influences on an aroma compound with given physico-chemical properties are and how they will affect its release.

Interestingly the most hydrophilic molecule (1,4-butyrolactone) reaches its maximum of intensity later than all other compounds within the group of relatively hydrophilic aroma components. Its release is also more prolonged compared to molecules which are less water soluble (benzaldehyde and hexenol). These observations contradict the hypothesis that all hydrophilic aroma compounds will be released at the same time together with hydrophilic taste molecules like sugar or acids and that the most hydrophilic compounds will disappear first. A possible explanation can be found in figure 2. Unlike limonene which is released together with the very first chewing motion, butyrolactone is only present in the breath after several swallowing events explaining the late time

of maximum intensity. Obviously butyrolactone is not released in the mouth from the gum base but from a saliva layer in the throat which is formed after swallowing as it was postulated for liquid and semi-liquid foods [5]. Due to its hydrophilic nature it stays in this saliva layer several minutes, generating the prolonged release over time. With rising hydrophobicity compounds won't stay in the watery layer that long, so their release is less prolonged (see benzaldehyde and hexenol). When hydrophobicity exceeds a certain level the compound starts to be dissolved in the hydrophobic gum-base and it will be released from there over a prolonged time (see ethyl butyrate). Compounds with high hydrophobicity are only released from the gum base and do not reach maximum intensity within 8 min mastication.

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Coffee Roasting: Exploring the Impact of the Time-Temperature Profile on the Formation Kinetics of Volatile Organic Compounds by PTR-MS

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Abstract

Green coffee was roasted in a laboratory scale fluidized bed roaster to a fixed end point (dark roast) using different roasting temperatures (isothermal roasting) - 228°C, 238°C, 248°C and 258°C. The formation kinetics of selected ion masses were monitored on-line by PTR-MS. Strong differences in the formation kinetics of compounds underlying the ion masses were observed, as a function of roasting temperature.

Introduction

The flavor of a freshly prepared cup of coffee is the final expression and perceptible result of a long chain of transformations, which link the seed to the cup. The first prerequisite of a high cup quality is that the green coffee be of highest quality. This depends on many factors, including botanical variety, geographical origin, soil conditions, climate and methods of preparation.

Green beans contain all the ingredients necessary for the later development of a typical coffee aroma. Yet, they convey neither the characteristic smell nor the taste of a cup of coffee. To reveal its characteristic flavor, coffee has to be roasted. They also become brown and take on a certain brittleness so that they can be ground and extracted. The overall roasting process can be divided into three phases: (i) Drying: During this first stage, the beans lose a good deal of their moisture. Once roasting, beans weigh about 15% less than at the start. (ii) Roasting: This is the main stage of flavor formation. Dry beans are heated over a 3 to 20 minute period, with the temperature starting at 100°C and rising to between 220°C and 250°C. A multitude of chemical processes are initiated inside the beans, leading to the formation of the typical coffee aroma. (iii) Quenching: To end the roasting progress, the coffee is cooled to ambient temperature.

Transforming the freshly roasted beans into a cup of coffee is the last part of the long journey of coffee. Here again, a range of experts are involved in order to deliver the best quality in the cup. Indeed, even the freshest and finest roasted coffee can be spoiled if not properly processed and carefully extracted and served, a responsibility that extends all the way to the Barista.

The sensory qualities of coffee, so much appreciated around the globe, paired with physiological virtues related to caffeine and other ingredients, made it become the second most important commodity of world trade, behind crude oil. Its world production in 2007 was 119 million 60kg-bags [1]. Relative to green beans, roasting yields an added value of 100-300%.

Considering the economic importance of roasting as well as its key role in the flavor formation process, we have extensively explored, over the last years, the coffee roasting process from various angles. One angle was the free-radical processes and formation kinetics in green and roasted coffee and during roasting, under various conditions [2, 3]. A second axes was the chemistry of flavor and acrylamide formation during roasting [4, 5]. Finally, we have devoted ample efforts in developing advanced analytical technologies capable to examine on-line, with high sensitivity and time resolution the formation kinetics of volatile organic compounds (VOC) during roasting (this will be the focus of the paper). We have chosen to look at VOCs, in contrast to color, weight loss, water content, temperature or other process parameters, since these are closely related to the flavor of the coffee. Furthermore, we believe that characterization at the start- and end-point of the process (e.g. color, and weight-loss), as is the widespread practice today, only allows an assessment of the flavor profile within narrow process conditions. A more rigorous management of quality requires monitoring process parameters that reflect roast history.

Until now, we have developed two state-of-the-art on-line technologies of VOC analysis for process control and for the study of the complex chemical processes taking place during coffee roasting. Both are based on direct injection of roast gas into a mass spectrometer, following soft ionization. This assures little ionization-induced fragmentation, high sensitivity and sub-second time resolution. The first approach uses lasers for ionization either in resonant (two UV photons) or alternatively in a non-resonant (one VUV photons) mode. Application of laser ionization to coffee roast-gas is documented in a series of publications [6-11]. The second approach that we developed is PTR-MS [12-14]. In recent papers, we outlined the advantages and limitations of both soft ionization approaches [8, 15-18].

The study presented here examines the formation kinetics of VOC by PTR-MS for different time-temperature profiles during roasting, a process parameter that has been intensely studied since the early history of coffee. While the first generations of roasters achieved low heat transfer rates to the beans and therefore long roasting times (30 minutes) later generations improved the efficiency of heat transfer and reduced the roasting time. A concept, introduced in the US in the early 1970's and later abandoned due to unsatisfactory sensory characteristics, was the so-called "high yield" coffee [19, 20]. It was observed that a high-temperature short-time (HTST) roasting profile (as short as 2 minutes) yielded roasted coffee beans of larger volume, lower density and higher extraction yield, while reducing costs due to better utilization of the roasting equipment. Therefore, some manufacturers recommended using only 80-85% of the usual amount of coffee for the same quality of brew when using HTST roasted. Yet, it turned out that such a brew was lacking in body and was organoleptically less satisfactory. Using a normal dosage of coffee, the cup prepared from a "high yield" coffee was bitter/burnt and astringent. Although this approach was abandoned in the 1980's, roast times still strongly vary among different roasters in the range 4 minutes to more than 20 minutes, illustrating an important characteristic of roasting: flavor development during roasting depends on the time-temperature history to which the beans are subjected, and not just on physical properties at the end-point such a color, or weight loss.

Experimental Methods

Temp. roast-gas [°C]	roast loss [%]	CTN	roast time [s]
228	17.85	67	1500
228	17.65	65	1500
238	17.3	65	820
248	17.67	62	550
258	17.75	60	400

Table 1: Roasting conditions. Batches of 200 gram Arabica coffee from Columbia were roasted to a fixed roast degree (approx. 65 CTN; dark roast). Besides the isothermal roasting temperature, the weight loss during roasting, roast level (CTN) and roasting time are given.

Roasting, under the conditions shown in table 1, was performed in a fluidized bed laboratory scale roaster of a capacity of 1 kg, while batches were each 200 gram. The formation kinetics of VOC generated and released during the roasting process was analyzed on-line by sampling the roaster off-gas in the exit line of the roaster by PTR-MS. The setup is show in figure 1.

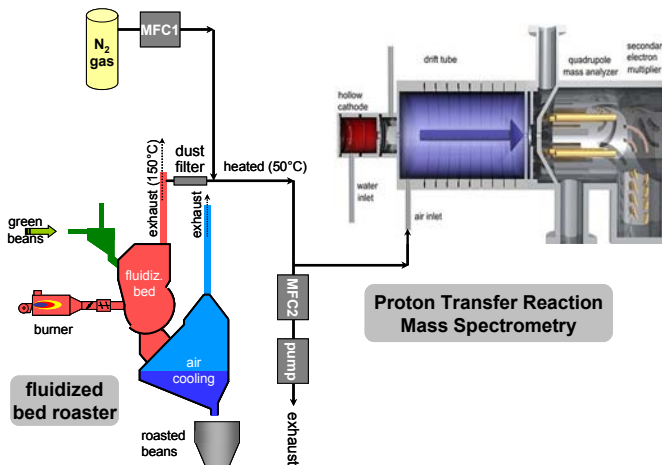


Figure 1: The off-gas of a roaster was sampled through a dust filter, at constant flow rate (adjusted via mass flow controller MFC1 and a pump in the exhaust line). Immediately after the dust filter the gas is diluted/cooled with N_2 gas at a constant flow (MFC2).

Results

The formation kinetics of a series of ion masses was monitored on-line by PTR-MS for the various isothermal roasting processes, four of which are shown in figure 2. To tentatively assign the ion traces, one can apply the outcome of a recent study where PTR-MS was coupled to GC/MS [25-27]; e.g. m/z 111 is superposition of 70% 5-methylfurfural and 30% 2-acetylfuran [21-23]. Applying this approach, we expect to assign the ion traces observed during roasting.

Discussion

This project aimed at developing an on-line analytical tool to monitor the formation kinetics of VOC in the off-gas of a roaster, using PTR-MS. The conditions during coffee roasting - high temperatures, air flow rates, dust load - required developing novel interfacing solutions between the process and the PTR-MS. The results in figure 2 show that the approach taken here delivers detailed information on the time-intensity profiles of VOCs and allow exploring the impact of time-temperature roast profile on the formation kinetics of selected VOCs.

In particular, a very strong impact on the time-intensity profiles is observed with changing time-temperature profile. Under HTST conditions, intensities of most VOC strongly increase towards the end of the roasting circle, and often show a bimodal shape with a maximum in the formation/release curve early on in the roasting process followed by an intermediate decrease and finally a steep increase before the roasting process is stopped. In contrast, the LTLT roasting process shows release/formation curves which reach early on in the roasting process a maximum and subsequently decrease gradually towards the end of the roasting process. Further detailed experimental studies are needed at this point to link the time-intensity traces and their dependence on roast temperature to the underlying chemistry.

Besides the chemical identification, it is also important to link the chemical profile, as analyzed by PTR-MS, to the sensory profile of the coffee. In a recent publication, we have presented a novel methodology to correlate sensory data with PTR-MS ion-mass profiles [24]. Combining these various approaches, we believe that we have developed and validated the critical technical ingredients needed to acquire a new and better understanding of the flavor formation process of coffee aroma during roasting. In this context, PTR-MS has proven to be a critical technology.

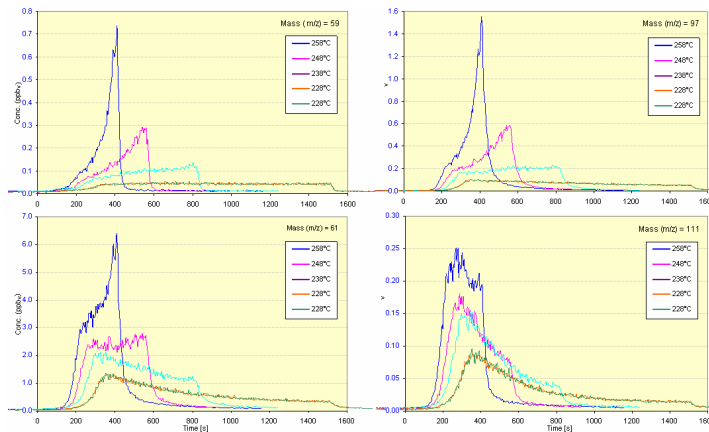


Figure 2: Time-Intensity traces of four selected ion masses ($m/z = 59, 61, 97, 111$) under four different time-temperature roasting conditions. Each frame shows one mass. To illustrate the high reproducibility of the isothermal roasting, the profiles at 228°C are shown in duplicate.

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PTR-MS in food authentication

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Abstract

The main aim of food authenticity studies is to detect when foods are not what they claim to be and thereby prevent economic fraud or possible damage to health. Food fraud can generate significant amounts of money for unscrupulous traders so the risk of adulteration is real [1]. The development of new and increasingly sophisticated techniques for the authentication of food products continues apace with increasing consumer awareness of food safety and authenticity issues. Food authentication is of course also of concern of food processors that do not wish to be subjected to unfair competition from processors who would gain an economic advantage from the misrepresentation of the food they are selling. The rights of consumers and genuine food processors in terms of food adulteration and fraudulent or deceptive practices in food processing are set out in a European Regulation regarding food safety and traceability (EU regulation 178/2002D). The definitive authentication of food products requires the use of highly sophisticated analytical techniques, as the perpetrators of this type of fraud employ methods of adulteration and misrepresentation that are increasingly difficult to detect. Important techniques are spectroscopic techniques, such as MIR and NIR, RAMAN, NMR, SNIF-NMR and IRMS, fluorescent, UV-vis spectroscopy. Other techniques include GC, HPLC, electronic nose, DNA, ELISA and thermal analysis [2]. Over the last decade the consumers' interest in regional foods has increased substantially. The EU has recognized and supported the potential of differentiating quality products on a regional basis [3]. An integrated framework for the protection of geographical indications and designations of origin for agricultural products and foodstuffs was introduced in 1992. The scheme allows the application of special geographical indications to a food product. The use of geographical indications allows producers to obtain market recognition and often a premium price. False use of geographical indications by unauthorized parties is detrimental to consumers and legitimate producers [4]. Organic foods is also produce gaining consumers' interest. The organic farming movement started almost a century ago. During the past two decades, organic farming and organic food markets became large enough to call for legislation order to organize farming procedures and marketing routes. The EU has regulated the organic products market in a set of laws such as EC Regulation No. 2092/91, 1804/99, 834/2007. The EU recognizes the dual societal role of organic farming: (a) organic land management generating public benefits; and (b) organic food as a direct response to consumer concerns relating to quality, safety, health and animal welfare. Fraud in organic farming may become an increasing concern as the sector experiences rapid year-on-year growth. Therefore, for both producer (fair competition) and consumer (reassurance) there is an urgent need for scientific independent technologies for organic produce authentication [5].

From this point of view, the development of new techniques for determining the authenticity of agricultural products is highly desirable. Despite the wide array of analytical techniques, most either suffer drawbacks of being too time-consuming to be practicable for analyses of many

samples, or they lack the sensitivity to provide distinguishable features between different samples. PTR-MS, being a fast and sensitive technique for volatile organic compound (VOC) detection and enables many samples to be analyzed within a short period [6]. For mass spectral fingerprint comparison a multivariate statistical approach is generally useful. The present paper will show the application of PTR-MS in food authentication: butter quality prediction; fat authenticity; and verification of geographical origin and production systems (organic vs. non-organic).

Acknowledgements

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Chemometrics in food research: A new concept of untargeted chemometric profiling of coffee

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Abstract

Chemometrics is a powerful mathematical and statistical approach when dealing with multivariate chemical data being too complex to be used for approximations by first principal models. Analytical methods like fast on-line techniques (PTR-MS), LC-MS, GC-MS, GC-PTR-MS and NIR allow obtaining rich chemical fingerprints of complex food matrices. When searching for new chemical compounds and investigating their impact on selected quality descriptors, the application of the untargeted approach might be promising. However, the interpretation of the obtained datasets is often complicated when properly aligning individual markers within a multitude of different samples or methods to manage huge data files containing a lot of redundant information. The aim of this presentation is to show methods allowing for an untargeted data treatment of GC and LC datasets by alignment and clustering techniques prior to the application of multi variate analysis. After interpretation of the minimalistic approach a targeted data treatment leads to possibilities to develop models using datasets obtained by on-line techniques known to be much quicker available than data obtained by chromatographic separation. As an example the data treatment of GC-MS and GC-PTR-MS analysis of roasted coffee is shown by alignment and clustering methods.

Introduction

In the early 1970s already the introduction of chemometrics has led to the development of statistical and mathematical methods to process multivariate data sets obtained by chemical analysis [1,2]. The application of “omic” approaches to investigate the formation of metabolites in the human body related to diverse diseases accelerated the development of mathematical, statistical and instrumental development. Minimalistic approaches with the aim to project a multidimensional space down to low dimensional planes or hyper-planes with typically two to ten dimensions (components) as principal component analysis (PCA) and partial least squares (PLS) and their extensions such as orthogonal-PLS (OPLS), hierarchical PCA, PLS and OPLS are frequently used to manage complex problems. Nowadays we can use a pool of software tools and sophisticated instrumentation that allows for obtaining rich chemical fingerprints at a high throughput and in a reproducible manner and to manage the data analysis by means of multivariate methods. The main advantage of “data driven” methods is that they are not based on

fundamental chemical theories and therefore can be applied to reproducible unbiased data without the need of a complete understanding of the process in its full complexity.

The application of chemometrics to coffee is interesting not only because of the complexity of the phenomenon “coffee perception” and aroma formation during the roasting process of coffee but as well because of its outstanding advantages in the reproducible characterization of quality differences and their related aroma compounds and precursors. The way from the seed to the cup implies a multitude of stages (see Fig. 1) which all of them need to be optimized to obtain a highly appreciated coffee.

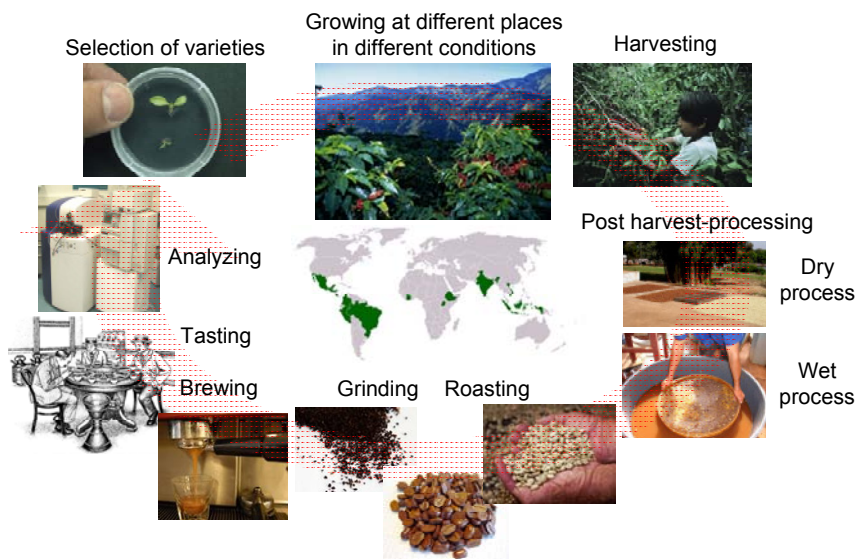


Figure 1: Overview of the process of coffee production as a step by step approach

Different sets of data can be obtained like genetic fingerprints, agricultural information, meteorological data during the growing period, chemical fingerprints and sensory profiles. Some of the data can directly be compared between different samples (like the number of days of sunshine or the growing region) while others need to be preprocessed prior to use in comparative studies. Especially GC or LC data need to be preprocessed in a way that eluted peaks can be recognized and aligned in order to compensate for slight shifts in the retention time. A targeted approach includes the necessity to identify all compounds of interest before applying multivariate analysis and reduces the information flow from the very beginning. An untargeted approach can overcome these limitations but requires a more sophisticated preprocessing of the data including baseline correction, peak picking, alignment and centrotyping of raw data sets. After multivariate analysis of the data, a selection can be made based on significance tests. Selected markers can finally be identified whereas this represents a major part of needed resources when dealing with plant materials.

The characterization of aroma compounds and their precursors, directly influencing the cup quality, and in further extent the link to genetic fingerprints might have a strong impact on the selection of green coffee. The optimization of processing stages in the production of R&G coffee is key to finally unfold the full potential of an individual coffee. The application of on-line techniques like PTR-MS is a powerful way to analyze aroma compounds in real time and by applying statistical models the characterization of the quality of coffee is possible.

Experimental Methods

For the investigation of the differences in the aroma composition related to the cup quality evaluated by trained coffee panelists, a set of 65 coffee varieties grown in well defined conditions were obtained. GC-MS and GC-PTR-MS measurements of volatile organic compounds released from coffee extracts were performed by Tenax trapping during two minutes of dynamic headspace release from a headspace sampling cell [3]. Online headspace data were interpreted by using the GC-PTR-MS datasets in combination with identification based on GC-MS datasets. With this technique the molecular contribution to single ion traces monitored on-line could be quantified and the dynamic changes in the contribution over time interpolated. The results of the application of a developed sensory predictive model [4], was applied using online PTR-MS measurements of coffee headspace. For the sake of simplicity, the sensory predictive tool is focusing on Robusta samples showing most significant individual differences.

GC-MS and GC-PTR-MS raw data were processed by using the Metalign software (www.metalign.nl). The software includes base line correction, peak picking respecting a limitation in signal to noise ratio, and alignment of the detected peaks through all samples by an algorithm which compensates for slight shifts in retention time for single mass signals. Due to the rich fragmentation patterns obtained by electron impact ionization in the used MS, single compounds are represented by typically 10 mass signals and more. In order to reduce the data volume and eliminate redundant information a “centrotyping” program was applied [5]. This program correlates the intensity profiles of individual mass signals across all samples within a predefined retention time window which can be adjusted according to the shifts in retention time caused by the limitation of instrumental accuracy. Correlating mass signals are clustered and expressed as single centroid since they are expected to belong to one compound (see Fig. 2). This reduces the data volume by an important factor without any losses of information since the individual fragmentation pattern of each centroid is stored. In a further extent this information can directly be used for comparison with databases for identification.

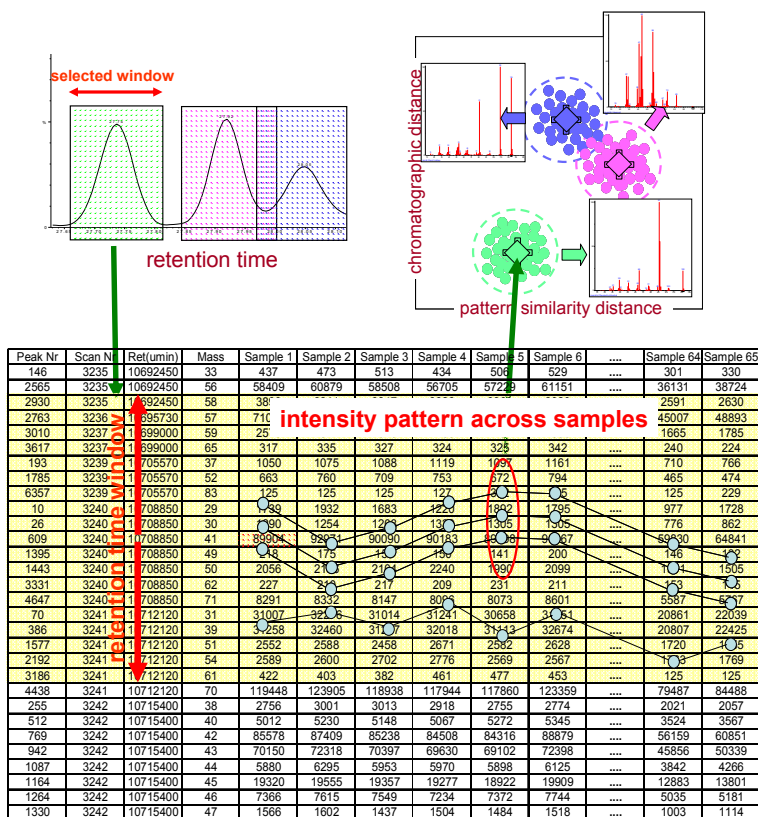


Figure 2: Example of eluted peaks selected within a defined retention time window. Three mass peaks correlate strongly through all samples and therefore most probably belong to the same compound. A fourth mass peak intensity shows a different pattern and therefore belongs to another compound.

LC-MS measurements of powder from roasted beans were obtained by LC-PDA-QTOF MS analyses in ESI positive mode [6]. After processing the raw data with Metalign and the centroid program, the obtained results were fused with the GC-MS and GC-PTR-MS datasets. Cluster analysis and correlation maps were obtained to visualize correlations between sensory data, volatile compounds and precursors. The correlation maps are created by plotting all centroids in 2D while keeping the distances between them intact. In a next step, correlation coefficients (Pearson product moment correlation) are calculated between all centroids. Only centroids that have a significant correlation (corrected for the false discovery rate) are selected and those that have at least a correlation of 0.9 (or any other value) are connected by a line. In this way a network is obtained that shows which centroids are closely related to each other, since they are connected through a series of lines. This type of analysis can detect groups of functionally related centroids and show the interrelatedness. In a consequence, highly correlated centroids can be

identified by comparing the fragmentation pattern with databases or further applying techniques to fractionate the compounds from the eluent.

Results and Discussion

A model was developed for Robusta samples selected by their highest diversity in terms of genetic pattern by using on-line PTR-MS headspace data. The difference between the sensory evaluation data and the results obtained from the model are in the range of the least significant difference evaluated for each sensory attribute. It was further deduced that the attributes coffee odour, roasted and chemical were predicted most accurately by the instrumental data which can be explained by the large differences for these attributes between the used samples. The sensory profiles including the model results are shown in Figure 3 as spider diagrams for a selection of three coffees. The interpretation of the GC-MS and GC-PTR-MS datasets together with the on-line datasets of PTR-MS headspace measurements allows for a precise interpretation of the impact of individual compounds on the coffee quality descriptors. In a further extent the causality can be validated by the application of olfactometric studies.

A correlation map including non-volatile compounds, volatile aroma compounds and sensory data for all analyzed coffees was calculated. By zooming into the correlation map, a network of pyrazines can be shown to be highly correlating (Figure 4). Further data analysis of individual correlations leads to a characterization of quality differences depending on the coffee variety, roasting degree or coffee extraction (data not shown). It could be demonstrated that the application of chemometric methods on coffee is possible and offers new opportunities for a deeper understanding of chemical markers correlating to quality attributes. By investigating changes in the chemical composition of volatiles and non-volatiles and testing their impact on predicted or evaluated sensory profiles, a robust method can be developed for selecting coffee varieties with the highest potential of in-cup quality by chemical markers.

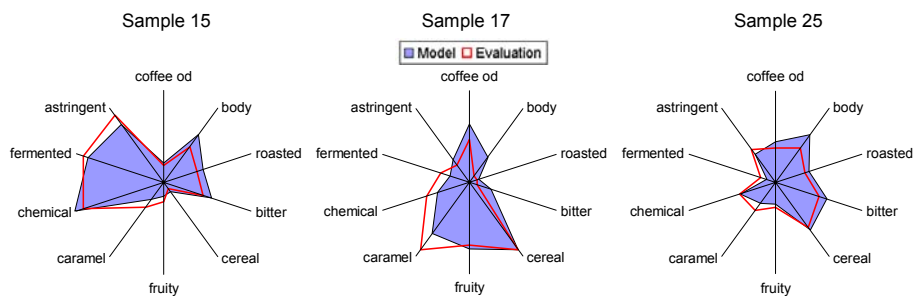


Figure 3: Sensory profiles including the model results for four different Robusta samples.

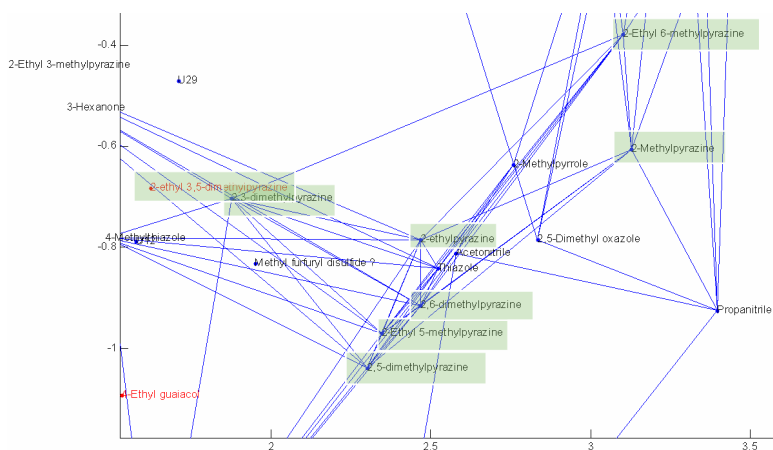


Figure 4: A network of pyrazines can be shown to be highly correlated when zooming into the correlation map (for better readability LC-MS data is removed).

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Unilever Perspective on Implementation and Application of PTR-MS in Food Research

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Abstract

Direct ionisation mass spectrometry techniques, like APCI, SIFT or PTR-MS, have now been applied in food science for more than a decade and are gradually becoming standard research instruments to monitor aroma release. Although Unilever has pioneered the application of dynamic aroma release measurements with APCI-MS in the mid 90's, a high sensitivity PTR-MS instrument was acquired only in 2006 for food flavour analysis. This paper will first present how PTR-MS was implemented within Unilever food research and will be followed by various application examples.

The instrumental set-up needed adjustments to meet dynamic aroma release requirements: the design of the sampling interface was modified to obtain an instantaneous response to changes in air concentration, at a flow rate low enough to be suitable both for static headspace and breath-by-breath measurement.

The accurate control on ionisation conditions was utilised to find the best compromise between maximal sensitivity, minimal fragmentation and no dependence to water clusters, as some of our applications were operating at high amount of water vapours.

Calibration and day-to-day variation in sensitivity were addressed with the design of a simple system delivering a controlled concentration of a desired aroma compound in air.

Applications of the so-modified PTR-MS system are then illustrated through various examples of aroma measurement:

- The instrument ability for in-vivo measurements is presented with in-nose aroma release of Unilever products.
- The performance of aroma encapsulates was monitored during food preparation above water of 80°C. The relative humidity in this situation posed challenges on water clusters distribution in the ionisation chamber.
- Finally, we have also applied PTR-MS for static headspace measurement as a fast and simple method for high through-put screening of large sample sets.

Application of PTR-MS to measure perceived freshness of model cakes varying in different sweetener, fat types and shelf-life

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Abstract

Model cake samples (n=24) were produced using a 4 x 3 factorial design, with four sweeteners (sucrose, glucose, xylitol, isomaltose) and three fat types (butter, margarine, shortening). Cakes were analysed at two different time points; fresh, or day one, and 15 days from time of baking. The sensory properties and volatile composition of all cakes were analysed by descriptive sensory analysis, and by Proton Transfer Reaction-Mass Spectrometry (PTR-MS), respectively. As a next step, cakes (n=12) with the largest sensory and volatile variations were evaluated for perceived freshness by consumers (n = 120). Partial Least Squares Regression (PLSR) was applied to determine relationships between sensory attributes, volatile composition and perceived freshness. A balance of twelve mass ions measured by PTR-MS were found to relate well with descriptive sensory characteristics intensity, and with perceived freshness intensity, leading to the development of a robust predictive model for perceived freshness of cakes in relation to variations in sweetener type, fat type, and time from baking.

Introduction

The distinctive aromas and flavours of freshly baked cakes result from a balance of volatile compounds present at low concentrations [1]. Considering the large number of aroma compounds produced during baking, the strong and varied influence of ingredients, and changes in composition that occur with time from baking, it can be time-consuming and costly to determine the influence of volatile composition on sensory character and consumer perceptions of quality. PTR-MS enables a rapid analysis and quantification of volatile compounds in the headspace of foods, and has been used successfully to determine correlations between volatile “finger-prints” and sensory characteristics of odour and flavour [2-4]. The objective of this work was to establish a rapid and practical method using PTR-MS to measure the sensory quality of baked foods.

Experimental Methods

Cake flour, butter, sucrose, fresh eggs, baking powder and salt were purchased locally. Sweetener substitute xylitol (*xilisorb200*) was sourced from Danisco NZ Limited, Auckland, while isomaltose (*Isomalt PF*) and glucose were purchased from Invita NZ Limited, Auckland. Margarine (*Morah Cake Continental 1575*) and shortening (*Hi-mix Cake Shortening 1583*) were obtained from Bakels Ltd., Auckland. Cake variations were prepared by total substitution of sucrose by one of the following: xylitol, glucose or isomaltose, whilst total fat content was varied by addition of butter, margarine or shortening. Duplicate cakes from each formulation were prepared to represent two different batches. All cakes were analysed by descriptive sensory analysis and PTR-MS as fresh (day one), and after 15 days of storage. Twelve of the cake samples that varied most in sensory and volatile composition were selected for perceived freshness evaluation by representative consumers ($n=120$). For PTR-MS measurements, cake samples in triplicate were cut into $30 \times 30 \times 20 \text{ mm}^3$ and weighed (100 g) into 1 L Schott Duran bottles and allowed to equilibrate at room temperature for 1 h. Bottles were connected to the PTR-MS inlet flow using Teflon 1/16 tubing and headspace was sampled at a flow rate of 50 ml/min. The headspace air was replaced by an equal flow of pure air (BOC, New Zealand; purity; oxygen 21.999 %, nitrogen 77.999 %) Data was collected over a mass range of m/z 20 to 180 using a dwell time of 0.2 s per mass. Sample measurements were performed in 6 cycles, and the means of cycles 2 to 6 were represented in further analysis. Mass ion intensities were recorded in concentration ppbv. PLSR1 was applied to determine the mass ions that were correlated best with individual sensory characteristics and with perceived freshness. Mass ions that contributed little information of value were removed. Optimum models were selected based on root mean square error of prediction (RMSEP).

In addition, models were applied to predict perceived freshness of the twelve cake samples analysed by descriptive sensory analysis and PTR-MS, but not evaluated by consumers.

Results & Discussion

Successful regression models for perceived freshness were developed for both PTR-MS and descriptive sensory measures. Calibration coefficients and validation coefficients (ability to predict new samples) for PTR-MS and sensory models were both 0.97 and 0.92, respectively, whilst RMSEP values were 8.83 – 8.52 (on a scale from 1 – 150 mm) (Table 1) Cakes perceived as most fresh contained high concentrations of mass ions m/z 47, 74, 84, 86, 87, 97, 100, 102, 124, and were described by the sensory characteristics “sweet”, “buttery”, “caramel” and “eggy” flavour. In contrast, cakes perceived as least fresh contained high concentrations of the mass ions m/z 53, 95, 156, and were described by the sensory characteristics “fatty” odour, “sour”, “bitter”, “doughy” flavour, and “bitter”, “sour” after-flavour (Table 1). Models measured and explained the influence of baking ingredients and time from baking on perceived freshness of cakes.

Table 1 – Results of PLSI regression between the PTR-MS mass ion intensities and sensory descriptive characteristics (X-variables) with consumer freshness perceptions (Y-variables) of the twelve cake samples evaluated by consumers.

Measurement	Positive correlations	Negative correlations	Calibration	Validation	RMSEP*
PTR-MS measurements	47, 74, 84, 86, 87, 97, 100, 102, 124	53, 95, 156	0.97	0.92	8.83
Sensory characteristics	F-sweet, buttery, caramel, F-eggy	F- O-fatty, F-sour, F-bitter, AF-bitter, AF-sour	0.97	0.92	8.52

*RMSEP, root mean squares of prediction; *O*, odour, *F*, flavour, *AF*, after flavour

In addition, by relating both sample volatile and descriptive sensory measures simultaneously

The ‘one model’ approach was distinguished from the previous separate PTR-MS and sensory characteristics models presented in Table 1, by retaining additional mass ions m/z 34, 42, 56, 72, 93, 94, 98, 110, 126, and the additional sensory characteristics “buttery”, “dusty”, “musty”, “rancid” odour and “sweet” after-flavour. . Optimum model performance was determined by the calibration coefficient of 0.99, validation coefficient of 0.97, and RMSEP value of 5.66. Figure 1(a) showed that the cakes perceived as most fresh were evaluated on the day of baking, and contained margarine and sucrose, or butter and sucrose. The cakes perceived as least fresh were evaluated after 15 days of storage and contained shortening and isomaltose, or butter and isomaltose. In addition, interactions between ingredients and time from baking were determined. Figure 1(b) showed that cakes perceived as most fresh contained relatively high concentrations of the mass ions m/z 124, 74, 97, 93, and relatively high intensity of the sensory characteristics, “buttery” odour, and “buttery”, “eggy” flavour. Cakes perceived as least fresh contained relatively high concentrations of the mass ions m/z 110, 95, and relatively high intensity of the sensory characteristics, “rancid”, “dusty” and “fatty” odour. In addition, the PLS loadings plot illustrated correlations between X-variables. For example, the sensory characteristics of “caramel”, “sweet” flavour and “sweet” after-flavour were positively correlated with the mass ions m/z 126 and 56, whilst “sour” after-flavour, “musty” odour and “sour” flavour were positively correlated with the mass ions m/z 53 and 156. Models ability to predict perceived freshness of samples not evaluated by consumers was also good (data not shown). Taken collectively, this approach illustrates how PTR-MS can be applied to predict sensory attributes that determine perceived freshness of cakes that vary based upon formulation and shelf-life.

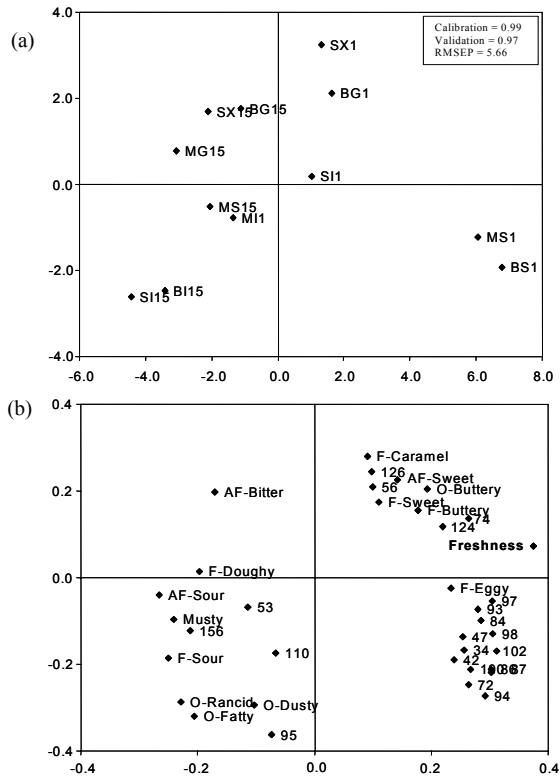


Figure 1: Partial Least Squares Regression 1 (PLSR1) showing scores plot of the twelve cake samples presented to consumers for freshness testing (a). Fat types are distinguished by: Butter (B), Margarine (M), Shortening (S), Sweetener types were recognised by: Sucrose (S), Isomaltose (I), Xylitol (X), Glucose (G). Time from baking was distinguished by: fresh evaluated on the day of baking (Day 1) and after fifteen days of Storage (Day 15). Correlations loading plot, showing relationships between mass ions, sensory characteristics and perceived freshness scores, with calibration, validation coefficients and RMSEP values (b). Sensory attributes are identified by: Odour (O), Flavour (F), Afterflavour (AF).

Conclusion

Relationships between PTR-MS spectra, sensory characteristics and consumer perceived freshness were determined for a range of cakes that varied in ingredients and time from baking. The influence of ingredients and time from baking, and their interaction on cake quality could be determined. Volatile compound analysis using PTR-MS can be applied as a rapid technique for determining perceived freshness of cakes that vary in formulation and shelf-life.

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Real-Time Insights into Complex Phenomena in Food Science by PTR-MS – Formation of Furan

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Abstract

Proton transfer reaction mass spectrometry (PTR-MS) was applied to on-line monitoring of furan generated by Maillard-type reactions and lipid oxidation. Unambiguous identification and quantitation in the headspace was achieved by PTR-MS/GC-MS coupling. Ascorbic acid showed the highest potential to generate furan (10 mmol/mol), followed by glyceryl trilinolenate. The furan yields from ascorbic acid were lowered in an oxygen-free atmosphere (-30%) or in the presence of reducing agents (e.g. sulfite, -60%), indicating the important role of oxidation steps in the furan formation pathway. Furthermore, already simple binary mixtures of ascorbic acid and amino acids, sugars or lipids reduced furan by 50-95%. These data suggest that more complex reaction systems result in much lower furan amounts compared to the individual precursors.

Introduction

PTR-MS has been shown to be a suitable method for rapid and on-line measurement of volatile organic compounds (VOCs) with major applications in environment, food and medicine [1-3]. In food science, PTR-MS has widely been employed to analyse the headspace composition of foods and beverages as well as during consumption (*in-vivo*) [4-6]. Interestingly, PTR-MS has received only limited attention when studying dynamic processes generating VOCs.

In the 1st PTR-MS symposium [7], we reported on the formation of Maillard-reaction products in model reactions containing various precursors (e.g. reducing sugars, amino acids). Many of the odour-active components could be detected by PTR-MS, e.g. furaneol, acetic acid, and diacetyl. The kinetic curves gave a particular insight into aroma generation, adding a new dimension to aroma research. In addition, important volatile intermediates were detected, which represent major pathways in the Maillard reaction cascade.

In the 2nd PTR-MS symposium [8], we presented results on the formation of vinylogous compounds and odour-active Strecker aldehydes from amino acids. Acrylamide was the major vinylogous compound found followed by styrene. On the contrary, methylpropanal was the most abundant Strecker aldehyde followed by phenylacetaldehyde, whereas 3-oxopropanamide could not unequivocally be identified. These data indicated that the decomposition of amino acids to Strecker aldehydes and/or vinylogous compounds cannot be generalized and hardly predicted.

In the 3rd PTR-MS symposium [9], we have investigated the feasibility of modelling the release kinetics of aroma compounds from dry roast and ground coffee. Accurate modelling of aroma release kinetics from coffee allowed discriminating fine variations in aroma release and determining the release mechanisms involved. In this paper, we use PTR-MS for quantitative on-line analysis of furan, a well known food constituent that may lead to undesirable effects.

Experimental Methods

Equimolar amounts of precursors were mixed in the dry state and homogenized in the reaction vessel. In some samples, additives were used such as antioxidants (1.2%, w/w) and ferric sulfate added (Fe^{3+} ions, 1%, w/w) to study their effects on furan formation. The samples were distributed inside the reaction vessel with the highest possible surface contact. More experimental data are given in [10].

The detailed description of the release-setup used and the instrumentation applied has recently been described for the analysis of acrylamide [11]. The reaction vessel (100 mL) was placed inside the oven and heated up to 220 °C with a heating rate of 4 °C/min. The purge flow, consisting of dry air/nitrogen was maintained at 600 sccm and the dilution flow at 5190 sccm. Detailed experimental data are given in [10].

Results and Discussion

Selected mass traces obtained by heating ascorbic acid were monitored by PTR-MS (Fig. 1). The major signals were represented by the ions at m/z 69, 47 and 97, followed by many other ion traces. The ions at m/z 69 and m/z 47 may correspond to furan and formic acid, respectively.

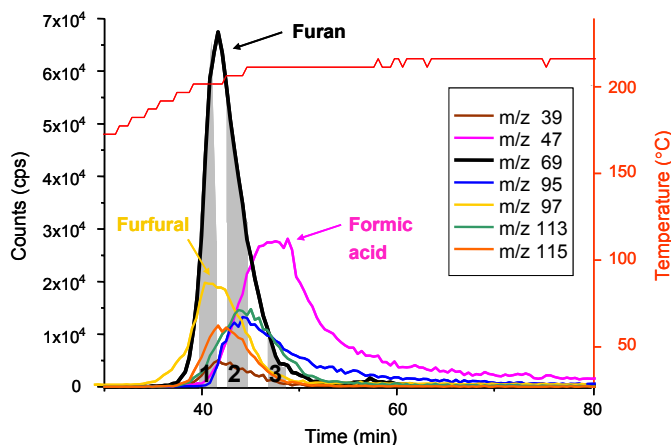


Figure 1: PTR-MS traces of selected ions obtained by heating ascorbic acid. Some of these traces were identified by coupling with GC-MS after trapping the headspace on 3 Tenax tubes as indicated by the gray zones.

As one ion trace may represent various compounds, the recently described PTR-MS/GC-MS coupling [12] was employed to elucidate the composition of various ion traces with particular interest in m/z 69. Aliquots of the headspace were trapped on three Tenax cartridges and analysed off-line by GC-MS. As shown in Figure 2A, thermal decomposition of ascorbic acid led to many VOCs. The compound with the retention time at $t_R = 8.9$ min was identified as furan by comparing its EI mass spectrum with that of the reference compound. Furthermore, only one volatile constituent was found by GC-MS with the mass at m/z 68 (Figure 2B), thus confirming that this mass trace was homogeneous, solely represented by furan, and not contaminated by further ions originating from other VOCs. All reaction systems were characterized by the combined PTR-MS/GC-MS method to define the purity of the ion traces at m/z 69 for furan.

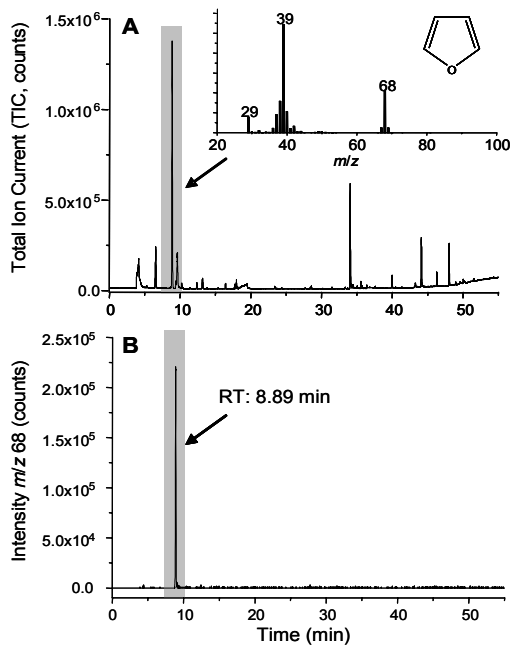


Figure 2: (A) The GC total ion count signal of a Tenax trap obtained from the ascorbic acid experiment showing the fragmentation pattern of furan. (B) Only one volatile compound was found with m/z 68 eluting at 8.89 min, which was identical to the injected reference compound furan.

Ascorbic acid was formed in the narrow temperature range of 180-210 °C ($T_{\max} = 195$ °C), which lasted for about 10 min (Figure 3A). Interestingly, the furan formation profile was different in lipid reaction systems based on linolenic acid, in which furan was generated and released at lower temperatures (110-220 °C, $T_{\max} = 180$ °C) and on a broader time scale of about 70 min (Figure 3B). While the ion trace at m/z 69 was homogeneous in the ascorbic acid sample (94-100% purity), it only represented partially furan in the lipid-based systems with a purity of about 10-25%, determined by Tenax trapping and GC-MS analysis. As the ion trace composition was

changing with time, different percentage contributions during the respective time windows had to be considered for analysis.

The PTR-MS signals (cps) of furan were converted into concentrations (μg of released quantity of furan per μmol of precursor) using the PTR rate constants of $2.28 \times 10^{-9} \text{ cm}^3/\text{s}$ determined by experimental measurements. Direct quantitation of furan was possible in the ascorbic acid samples due to high purity of the signal at $m/z=69$ representing 100% furan in almost all traps obtained from model systems containing only ascorbic acid as furan precursor. In all other reaction systems, the relative composition of the ion trace at $m/z=69$ was determined by GC-MS, which was used to correct the PTR-MS signal intensities accordingly prior to the calculation of concentrations. The cumulated data obtained allow a fair comparison of the trends observed in the various reaction systems. As shown in Table 1, highest furan amounts were obtained from ascorbic acid (10 mmol/mol) and trilinolenin (4.8 mmol/mol).

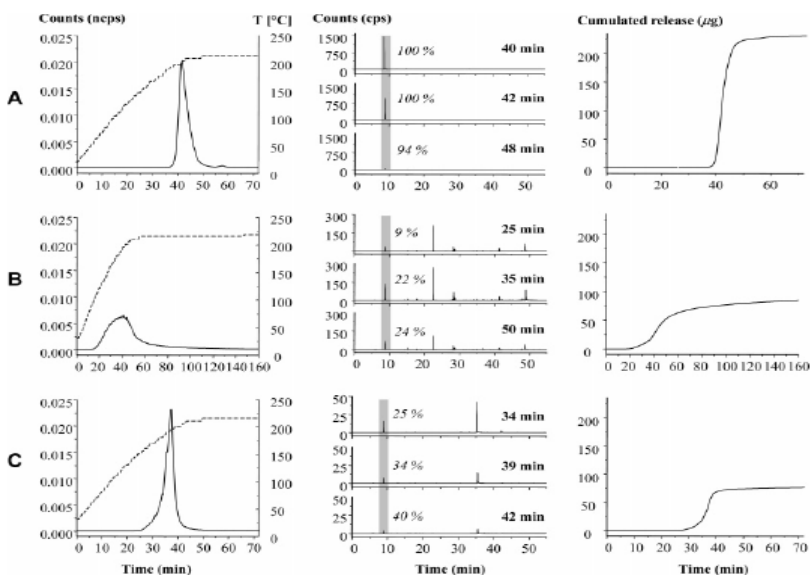


Figure 3: Release curves (m/z 69) obtained by on-line PTR-MS in normalized counts per seconds (ncps) towards primary ions (1st column), PTR-MS signal (m/z 69) of the trapped volatiles after GC separation in counts per seconds (cps) with the highlighted peaks corresponding to furan (2nd column), and the total amount of furan in μg released into the headspace (3rd column). All plots versus time in minutes: (A) ascorbic acid, (B) linolenic acid, and (C) ascorbic acid in the presence of sodium sulfite (1.2%, w/w).

	Model system	Furan ($\mu\text{mol/mol}$)	VC ^b (%)
1	Ascorbic acid	9950	7.8
2	Dehydroascorbic acid	270	3.9
3	Erythrose	1674	8.6
4	Glucose + alanine + threonine	749	11.3
5	Linoleic acid (C18:2; 9,12)	681	26.2
6	Trilinolein	1727	21.5
7	Linolenic acid (C18:3; 9,12,15)	3270	0.9
8	Trilinolenin	4747	23.2

^a Quantitative data refer to concentrations in headspace. ^b Variation coefficient.

Table 1: Formation of Furan from various reaction systems based on ascorbic acids, Maillard-type precursors, and lipids^a.

It should also be noted that, PTR-MS data only indicate the concentration in the headspace, but not in the actual sample. However, as recently confirmed for acrylamide [11], PTR-MS data, indeed, reflect well the trends in the formation of VOCs compared to those obtained by liquid extraction.

The furan yields from ascorbic acid were lowered in an oxygen-free atmosphere (-30%) or in the presence of reducing agents (e.g. sulfite, -60%), indicating the important role of oxidation steps in the furan formation pathway [10]. Furthermore, already simple binary mixtures of ascorbic acid and amino acids, sugars or lipids reduced furan by 50-95%. These data suggest that more complex reaction systems result in much lower furan amounts compared to the individual precursors, most likely due to competing reaction pathways.

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4. Applications in Environmental Science

PTR-MS in smog chamber research - a review

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Abstract

Proton Transfer Reaction – Mass Spectrometry (PTR-MS) is increasingly being used as an analytical technique in smog chamber experiments. The reference section of this abstract is a list of the pertinent literature based on a Web of Science search [1-33]. The first PTR-MS smog chamber measurements were performed in February 2000 at the former Environment Institute of the Joint Research Centre of the European Commission in Ispra [29]. Since then, most of the major smog chamber facilities (e.g. in Irvine, Jülich, Pasadena, Riverside, Valencia, Villigen) have been permanently or at least temporarily equipped with PTR-MS instruments.

Experiments in smog chambers (also denominated atmosphere simulation chambers or environmental chambers) are used to generate kinetic and mechanistic data on gas phase and aerosol reactions of atmospheric relevance and to validate photochemical reaction mechanisms used in computer models. The experiments involve adding known amounts of representative (primary) pollutants to large enclosures, and measuring changes in reactant concentrations as well as secondary pollutants (gases and particles) formed under the action of typical atmospheric oxidants (OH, O₃, NO₃) and/or light. The chemistry of photochemical air pollution can thus be studied under controlled conditions in which emissions and meteorology are not complicating factors. In such experiments, PTR-MS instruments have been used to:

- i) measure the reactive decay of the primary pollutants [e.g. 24]
- ii) measure the formation and yield of selected (targeted) gas-phase products [e.g. 2, 12, 27, 29]
- iii) comprehensively measure all reaction products (including analytically challenging species with carbonyl, hydroxy, nitrooxy, or nitroperoxy functional groups) to establish carbon closure (i.e. account for the ultimate fate of each initial carbon atom that reacts) [e.g. 5, 23]
- iv) measure the concentrations of HONO [17] and N₂O₅ [23] to better constrain the densities of OH and NO₃ radicals, respectively
- v) measure the formation of selected particulate-phase products [11]

In my talk, I will review the PTR-MS related work in this field, outline the benefits of using PTR-MS as an analytical technique in smog chamber experiments, identify analytical difficulties and limitations and give a brief overview on recent work focused on overcoming known deficiencies.

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Recent PTR-MS activities at NIES: Instrumentation and field measurements

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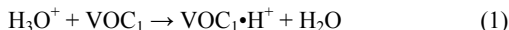
Abstract

We developed a two-stage ion source for proton transfer reaction (PTR) ionization to achieve more selective mass spectrometric (MS) detection of selected volatile organic compounds (VOCs) than that achieved with commonly used PTR-MS instruments, which are based on single-step PTR ionization with H_3O^+ . The two-stage PTR ion source generated reagent ions other than H_3O^+ by an initial PTR between H_3O^+ and a selected VOC, and then a second PTR ionization occurred only for VOCs with proton affinities larger than the affinity of the reagent VOC. Acetone and acetonitrile were useful as reagent VOCs because they provided dominant peaks as a protonated form. Using two-stage PTR-MS, we differentiated isomeric VOCs by means of differences in their proton affinities. The PTR-MS-derived concentrations agreed quantitatively with those independently determined by Fourier transform infrared spectroscopy (FT-IR). This two-stage PTR ionization may be useful for distinguishing various isomeric species, including aldehydes and ketones.

Introduction

The hydronium ion (H_3O^+) is commonly used as a primary ion because it undergoes proton transfer reaction (PTR) with most VOCs but not with the major components of the atmosphere (such as N_2 , O_2 , and CO_2). If an appropriate reagent ion other than H_3O^+ is chosen for PTR ionization, designated chemical species can be detected selectively [1]. However, little research has been done on this technique, probably because few methods are available for the selective production of other reagent ions.

In this study, we developed a new ion source for PTR-MS in which designated reagent ions other than H_3O^+ are produced through protonation by H_3O^+ ions. In the first stage, PTR between H_3O^+ and a designated VOC (VOC_1) selectively produces reagent ions $\text{VOC}_1\cdot\text{H}^+$; when a second VOC (VOC_2) with a PA larger than that of VOC_1 is present in a sample, a second PTR ionization occurs to produce $\text{VOC}_2\cdot\text{H}^+$ ions:



Note that soft ionization is used for the production of the reagent ion, as well as for the ionization of the VOCs in the sample. The performance of this ion source was tested with several VOC₁ candidates. The ion source was used to differentiate isomeric VOCs (VOC₂); we identified the isomeric VOCs from the mass signals of their parent ions (i.e., the protonated VOCs). Therefore, this technique is unlike the collision-induced dissociation (CID) technique in PTR ion trap mass spectrometry and the CI-MS technique that uses O₂⁺ and NO⁺ as reagent ions, both of which make use of fragment ions for the identification of isomeric VOCs.

Experimental Methods

The instrument used in this study was a custom-built proton transfer reaction time-of-flight mass spectrometer (PTR-TOFMS). The details of the instrumental setup have been described elsewhere [2-4]. In the two-stage mode, a reagent VOC (VOC₁) (e.g., acetonitrile, toluene, acetone, or diethyl ketone) was introduced from the port between the ED3 and ED4 electrodes (the VOC₁ port), and the sample gas was introduced from the newly inserted port between the ED4 and ED5 electrodes (the sampling port). In the one-stage mode, in which the reagent ion was H₃O⁺, nitrogen gas was introduced from the VOC₁ port by means of a three-way ball valve. Switching between the one-stage and two-stage modes did not affect the discharge between the ED1 and ED3 electrodes. Experiments were carried out at a drift-tube field strength of 100 Td.

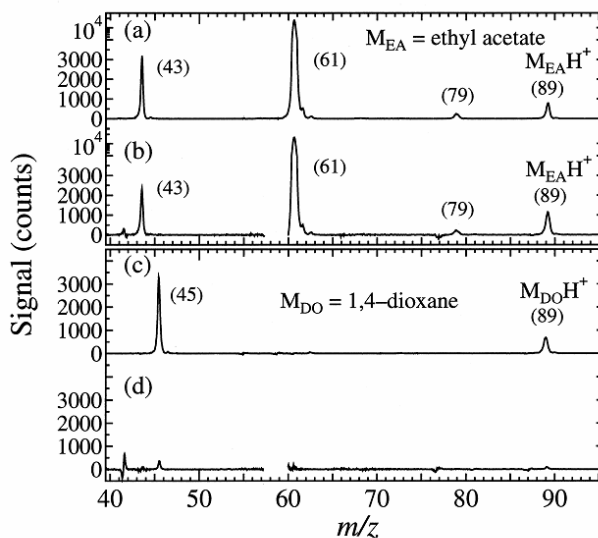


Figure 1: Mass spectra of ethyl acetate (MEA, mass 88) and 1,4-dioxane (MDO, mass 88) obtained in the one-stage mode ((a) and (c), respectively) and the two-stage mode ((b) and (d), respectively). VOC₁ = acetone.

Results and Discussion

In the mass spectrum of ethyl acetate, with the background mass spectrum subtracted, in the one-stage mode (Figure 1a), signals of protonated ethyl acetate and fragment ions assigned to CH₃CO⁺, CH₃C(O)OH•H⁺, and CH₃C(O)OH•H₃O⁺ were observed at m/z 89, 43, 61, and 79, respectively. A similar mass spectrum was obtained in the two-stage mode (Figure 1b). (Ion signals between m/z 58 and 60 were masked because they were scattered by subtraction of the background mass spectrum.) Signals for protonated 1,4-dioxane and a fragment ion assigned as C₂H₅O⁺ were observed at m/z 89 and 45, respectively, in the one-stage mode (Figure 1c), but these signals were not observed in the two-stage mode (Figure 1d). These results clearly show that, as a result of the difference in the PAs of the two VOC₂ samples, a second PTR ionization occurred for ethyl acetate but not for 1,4-dioxane.

The ethyl acetate and 1,4-dioxane concentrations determined from the two-stage PTR-TOFMS were compared with those obtained by FT-IR (Figure 2). Note that the concentrations for both ethyl acetate and 1,4-dioxane were determined independently; PTR-TOFMS was based on gravimetrically prepared gas standards, and FT-IR was based on absorption photometry. These results generally agreed within their measurement uncertainties, which suggests that by switching the reagent ions, we can differentiate isomeric VOCs by means of the mass signals of their protonated molecules.

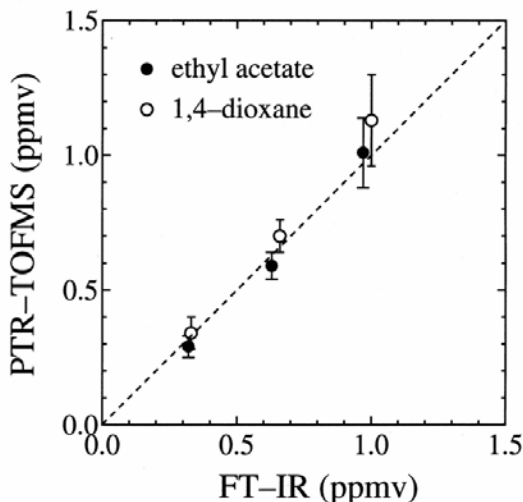


Figure 2: Comparison of ethyl acetate and 1,4-dioxane concentrations determined by two-stage PTR with those obtained by FTIR. The dashed line represents an $x = y$ line. $VOC_1 = \text{acetone}$.

Summary

A two-stage PTR ionization source was developed to generate reagent ions other than H_3O^+ , and the feasibility of the source for selectively detecting VOCs was examined. We tested protonation reactions of H_3O^+ with acetone and acetonitrile and found that both reagents gave a single strong peak, which corresponded to the protonated target VOCs. By switching reagent ions between H_3O^+ and the designated $\text{VOC}_1 \cdot \text{H}^+$, we could differentiate isomeric VOCs with the same molecular weight but different PAs by monitoring the ion signals of their protonated molecules. This two-stage PTR ionization approach is also applicable to other isomeric species including aldehydes and ketones if the appropriate reagent ions for those pairs is selected [5,6].

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PTR-TOF performance during the icebreaker based ASCOS campaign in the high Arctic

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Abstract

The polar regions are the primary global heat sinks and the central Arctic Ocean and its sea ice are major elements in the global climate system because of the importance of the ice in controlling mass and energy fluxes at the surface. The Arctic is changing dramatically with an increase of the average surface temperature over the last fifty years that was twice as rapid as the global warming and an ever faster decreasing sea ice extent particularly pronounced in the summer minimum ice concentration (IPCC Report, 2007). There is still a lack of understanding of the strong feedback mechanisms within the polar climate system involving ice, clouds (in particular, frequent and persistent low-altitude clouds and fogs), and radiation that is adequate to predict changes in the system under anthropogenic forcing (ASCOS – Science and Implementation Plan). The Arctic Summer Cloud Ocean Study (ASCOS) was one effort to collect *in-situ* data that provides a better comprehension of the complex interaction mechanisms involved.

With an integrated study from the sea-ice interface to the cloud-topped boundary layer ASCOS was designed to understand controlling factors of the low-level cloud system and heat balance over the Arctic pack ice and thus provide pressing scientific data for the unexplained rapid-melt of permanent ice floes. ASCOS was a six weeks field study and involved an international party of 33 scientists. A distinct feature of ASCOS is its necessarily interdisciplinary nature, which includes marine biochemistry, atmospheric chemistry, aerosol and cloud chemistry/physics, and meteorology. The remoteness and inhospitality of the studied area requires substantial logistic efforts and puts strain on experimental equipment and scientists. The Swedish icebreaker Oden was platform and vehicle for ASCOS. The installation of sensitive scientific equipment on an icebreaker needs considerable precaution to protect instruments from mechanic stress and damage.

A recently developed high sensitivity high mass resolution PTR-TOF was employed to characterize the organic trace gas composition of the atmosphere during ASCOS. The PTR-TOF was run without problems even under harsh conditions in the open water and during ice breaking. Continuous time-series of full mass spectra with a one minute time resolution were recorded on-line throughout the campaign between August 2nd and September 7th 2008 running up to a net VOC data set of 745 hours. Over 370 mass peaks have been separated, about 340 show signal intensities above the 30 minute detection limit. Additionally we analyzed samples from nine helicopter based soundings up to 2970 m.a.s.l. providing vertical VOC profiles.

The mass resolution of 5000 (FWHM) and a mass accuracy below 10ppm allow the separation of isobaric analyte ions and the identification of their atomic composition. These features are important to monitor for instance Dimethyl sulfide (DMS; CH_3SCH_3) which is one key compound in the marine troposphere as its photo-oxidation products are involved in the formation of cloud condensation nuclei (CCN). With the PTR-TOF the peak of the protonated DMS ($m/z=63.0268$ Th) can be separated from its neighboring peaks at $m/z=63.0082$ Th (CH_3O_3^+) and $m/z=63.0446$ Th ($\text{C}_2\text{H}_7\text{O}_2^+$) present as variable chemical background. At DMS concentrations in the low pptv range as experienced at times in the high Arctic atmosphere the background peaks dominate the mass spectrum at nominal mass 63 and would therefore mask the DMS signal in a mass spectrometer that cannot separate those isobars with drastic consequences for the detection limit for DMS.

During the ice-drift period where Oden was moored to an ice-floe ($>86^\circ\text{N}$) highest DMS concentrations were ~ 90 pptv typically they were in the range of a few to several tens of pptv. Vertical DMS profiles support model results that show advection of DMS from source areas around the pack ice into the high Arctic (Lundén et al. 2007).

Acknowledgment

ASCOS is an acknowledged International SOLAS project. It is part of DAMOCLES (EU 6th Framework Programme) and strongly linked with OASIS. The ASCOS expedition was arranged by the Swedish Polar Research Secretariat (SPRS) and was an effort within the framework of SWEDARCTIC 2008. For more information on ASCOS see <http://ascos.se/>.

We thank the all ASCOS participants, the SPRS and the Oden crew for the excellent team work.

We thank Armin Wisthaler for sharing his experience he gained in a previous Arctic campaign (AOE 2001) and for his assistance in planning and preparations.

The TOF-MS system was funded by the University of Innsbruck („Uni Infrastruktur 2004“ Programm, GZ.10.220/2-VII/2004). The PTR-TOF was developed in collaboration with Ionicon Analytik GmbH and with assistance from TOFWERK AG. The development project is financially supported by the Austrian Research Funding Association (FFG; Basisprogramm – Brückenschlag 1, P.-Nr. 810074).

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Measurement of Aviation Engine Exhaust Emissions using PTR-MS

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Abstract

Measurements of selected gas phase volatile organic compounds (VOCs) in jet aircraft engine exhaust using the PTR-MS technique have provided important quantitative information on the chemical composition of the exhaust. A PTR-MS has been deployed as part of a series of aircraft engine exhaust emission studies to examine how engine design, power setting, fuel composition and ambient conditions influence the composition and quantity of the emissions. VOC emissions show a strong dependence on engine power setting and are highest at low thrust and decrease dramatically at high power. Surprisingly, the VOC composition within the exhaust remains essentially constant and independent of engine design, power setting and fuel composition. A review of the measurement methods employed and interpretation of the results is provided.

Introduction

Prior to 2004 very few speciated VOC measurements of jet engine exhaust had been conducted and all of analyses had been restricted to the collection of grab samples.[1-2] These speciated measurements are important to understanding the impact that air travel has on global climate change and local air quality. Because of the limited number of engines examined in these previous studies there was concern about how well the data represented modern commercial aircraft engines. Starting in 2004 a series of aircraft exhaust emission field studies were initiated. For the first time instrumentation was deployed that permitted on-line real-time measurement of the gas phase and aerosol emission products. Three of these studies were conducted using on-wing engines from staged aircraft and are referred to as Aircraft Particle Emissions eXperiments (APEX).[3] APEX 1 examined the influence fuel composition. Engine to engine variability within a single engine type was studied during APEX2 and the affect of engine design was examined at APEX3. Two sensitive on-line methods, a PTR-MS and a tunable infrared laser differential absorption spectroscopy (TILDAS), were used to measure a variety of VOC emissions within the engine exhaust and the results from these instruments is presented here.

Experimental Methods

The PTR-MS and TILDAS instruments were housed within the Aerodyne Mobile Laboratory (AML). Gaseous samples from the different sample probes were delivered to the main AML sample inlet where the flow was split into two separate streams - one for the particle instruments and the other for the gas phase instruments. Engine exhaust sampling was accomplished via a series of sample extraction probes mounted on stands that were located at 1 meter, 30 meters and 43 meters behind the engine exit. Sampling at 1 meter was done using a sample rake that

contained a series of gas and particle sampling tips. Samples were extracted using dilution probes where the samples were diluted by adding nitrogen within the probe tip and transported via unheated lines. The sample was typically diluted by a factor of 10:1. Downstream samples are naturally diluted and were not further diluted.

Table 1: Compounds monitored within the exhaust emission matrix. Their proton transfer reaction products and relative abundance. The ion used for quantification with the relevant branching fraction or the calibration compound, shown in parentheses. Calibration factors and reaction rate coefficients used for quantification.

Compound	Ions formed (abundance)	Ion quantified (branching fraction)	S_R	X_R	$k^{(a)}$ (mL/s) $\times 10^9$
Methanol	33 (100%)	33 (methanol)	0.51	0.3	2.33
Propene	41 (23%), 43 (77%)	43 (0.77)	-	-	1.58
Acetaldehyde	45 (100%)	45 (acetald.)	0.85	0.3	3.36
Butenes	57 (100%)	57 (note b)	-	-	1.73
Acrolein	57 (100%)	57 (note b)	-	-	3.35
Acetone	43 (3%) 59 (97%)	59 (note c)	1.34	0.5	3.00
Propanal	59 (100%)	59 (note c)	1.54	0.5	3.44
Glyoxal	59 (100%)	59 (note c)	0.60	0.5	1.34
Acetic acid	43 (30%), 61(70%)	61 (0.70)	-	-	2.27
Benzene	79 (100%)	79 (benzene)	0.73	-0.4	1.97
Toluene	93 (100%)	93 (toluene)	1.05	-0.1	2.12
Phenol	95 (100%)	95 (1.0)	-	-	2.52
Styrene	105 (100%)	105 (1.0)	-	-	2.33
o,m,p-xylene	07 (100%)	107 (p-xylene)	1.20	0.15	2.27
Ethylbenzene	79 (30%) 107 (70%)	107 (p-xylene)	-	-	2.25
Benzaldehyde	107 (100%)	107 (p-xylene)	-	-	4.12
C3-benzenes	121 (100%) propylbenzene 79 (70%) 121 (30%)	121 (1,2,4 trimethylbenzene)	1.30	0.25	2.4
Naphthalene	129 (100%)	129 (1.0)	-	-	2.59
C4-benzenes	135 isomer specific	135 (1.0)	-	-	2.5
Methylnaphthalenes	143 (100%)	143 (1.0)	-	-	2.71
C5-benzenes	149 isomer specific	149 (1.0)	-	-	2.6 ^(d)
Dimethylnaphthalenes	157 (100%)	157 (1.0)	-	-	2.9 ^(d)

(a) Reaction rate coefficients are taken from Zhao et al.[6]

(b) Assumes a weighted average reaction rate coefficient ($k = 2.6 \times 10^{-9}$ ml/s) based on a distribution of 45% butenes and 55% acrolein, see Knighton et al.[5]

(c) Assumes a weight average sensitivity factor ($S_R = 0.79$) see Knighton et al.[5]

(d) Estimated reaction rate coefficient.

Formaldehyde (HCHO) and CO were measured using two TILDAS instruments. Concentrations for these species were determined by fitting the measured rotational-vibrational transmission spectra using a Voight line shape model as described by Herndon et al.[4] Carbon dioxide (CO₂) was measured using a commercial non-dispersive infrared instrument, Licor 6262.

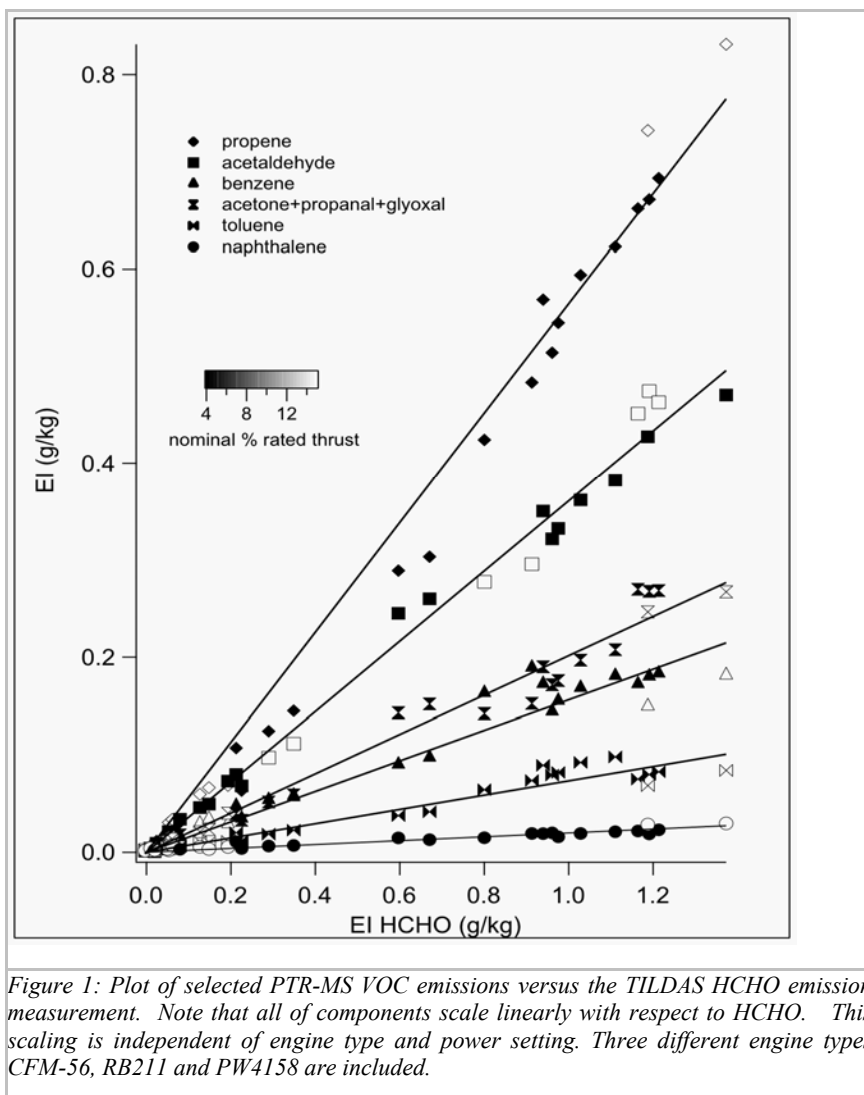
The PTR-MS (Ionicon Analytic GMBH) is a chemical ionization based mass spectrometer that utilizes H₃O⁺ as a reagent ion. This instrument has been described previously,[5] so only the relevant details are provided here. The mass spectrometer monitored a selected set of 32 ions at 0.2 seconds per mass, along with the drift tube pressure and temperature, which yielded a cycle measurement time of approximately 8 seconds. The ions monitored included the reagent ions H₃O⁺ (m/z 21 O-18 isotope) and H₃O⁺(H₂O) (m/z 39 O-18 isotope), the diagnostic ions NO⁺ (m/z 31) and O₂⁺ (m/z 32). The concentrations of the VOC emission components are deduced from the measured ion signals using relationships derived either from calibrated response factors (when standards were available) or simple reaction kinetics.[5] Concentrations quantified from calibrated response factors are assumed to be accurate within +/- 20%. Concentrations determined using reaction kinetics typically agree to within +/- 25% of the values derived from calibration standards and the stated accuracy of these measurements is estimated to be +/- 40%.[5] Table 1 provides a summary of the proton transfer reaction rate coefficients and the calibration factors used in this study. The limits of detection for most compounds are on the order of 0.3 - 1.0 ppbv for the 0.2 second integration times.

Results

Aircraft turbine engine exhaust contains a complex mixture of VOCs. Spicer et al. [2] have identified 57 different organic compounds in the exhaust of which 45 are expected to ionized and detected by the PTR-MS. Many of these compounds do not provide unique ion signatures in the PTR-MS and thus are not distinguishable or identifiable on the basis of mass alone. Using the chemical detail provided in the Spicer et al. [2] study, it has been shown that quantitative information for the compounds listed in Table 1 can be derived using a PTR-MS.[5] Results obtained using a gas chromatograph coupled to a PTR-MS provide additional support to the interpretation of the PTR-MS responses.

The most important parameter controlling the magnitude of the VOC emissions is engine power. It is well known that the VOC emissions exhibit a strong non-linear dependence with engine power.[7] VOC emissions are highest at the lowest engine powers (4% rated thrust) and decrease typically by several orders of magnitude as the engine power is increased to 30% rated thrust. Ambient temperature also has a significant influence on the magnitude the VOC emissions where lower ambient temperatures lead to higher VOC emissions [8].

During the analysis it was observed that the ratio of any two VOCs in the engine exhaust is approximately constant and independent of engine design, power setting, fuel composition and ambient temperature. This result is demonstrated in Figure 1 where a selected series of VOCs measured with the PTR-MS have been plotted versus formaldehyde measured with TILDAS. Similar results to those illustrated in Figure 1 have been observed for 14 different aircraft using 6 different engine designs and different fuels under a variety of operating conditions. This result seems to hold for all compounds whether they are combustion byproducts or fuel components.



Discussion

The PTR-MS technique has been shown to be a powerful and sensitive technique for quantifying VOCs emission components in jet aircraft engine exhaust. The measurements provided by the PTR-MS have revealed that VOC chemical composition of turbine engine exhaust does not

change significantly with engine type or operating condition. This result is valuable to airport modelers since it allows them to use one chemical speciation profile for all aircraft.

Measurement challenges using PTR-MS to quantify selected VOC components still exist. The complex nature of engine exhaust results in the formation of isobaric ions that can't be resolved using quadrupole mass spectrometers, which has necessitated the use of alternative reagent ion systems and the addition of gas chromatographs to provide quantitative information on specific components. While these auxiliary methods have provided solutions to specific molecules, high-resolution measurements that are available with new PTR-MS instruments using Time of Flight mass spectrometers should shape the future. The high mass spectral resolution inherent to time of flight mass spectrometers will allow the ion signatures from compounds such as protonated butene ($C_4H_8H^+$) and protonated acrolein ($C_3H_4OH^+$) directly.

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Analysis of secondary organic aerosols by PTR-MS

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Abstract

Many oxygenated volatile organic compounds are produced during photooxidation of volatile organic compounds and contribute to both the gas phase and secondary organic aerosols (SOA). The inlet system of the PTRMS instrument was modified to allow also for the measurement of the particulate phase of an aerosol. The new inlet consists mainly of a denuder to strip off the gas phase, and a heater to vaporize the aerosol particles. This inlet system was tested with pinonic acid particles generated with a nebulizer and SOA particles formed during the photooxidation of α -pinene with NO_x in a smog chamber. The performance of this new technique is discussed. Partitioning coefficients for some oxidation products are estimated and also compared to measurements with ion-chromatography-MS.

Introduction

Ambient aerosol particles have a variety of important impacts, including adverse health effects, visibility reduction, and influence on climate. Organic species are a major fraction of the total aerosol mass and account for 20-90% of the total fine particle mass concentration in a wide variety of atmospheric environments [1]. The formation of secondary organic aerosols (SOA) has recently received much attention. Even in urban areas, more than 50% of the total organic aerosol mass can be attributed to SOA [2]. The chemical mechanisms associated with SOA formation are also still poorly understood. One of the main reasons for this is that traditional methods for organic particle analysis, such as filter sampling with subsequent solvent extraction and gas chromatography/mass spectrometry (GC-MS), are very time-consuming and are unable to provide the sensitivity and time resolution needed to study aerosol formation in detail. In addition, these methods are known to suffer from sampling artifacts [3]. Furthermore, the high polarity and low levels of polar oxygenated organic compounds, which are the primary components of secondary organic aerosol, preclude direct GC-MS analysis. Progress in this area requires the development of online techniques for rapid, real-time measurements of particle chemical composition, with increased sensitivity and time resolution, and minimal sample handling procedures. Several real-time mass spectrometric methods have been developed and applied to aerosol analysis in recent years [4,5]. Laser or thermal sample volatilization and various ionization methods have been employed including electron impact, laser photoionization, and atmospheric pressure chemical ionization (APCI). Current shortcomings are the lack of quantitation and detailed chemical speciation and the limitation to particles larger than about 100 nm.

Proton-transfer reaction mass spectrometry (PTR-MS) has the advantage of a low detection limit and low fragmentation for many compounds due to relatively soft chemical ionization. Therefore

PTR-MS has the potential to measure quantitatively oxygenated organic compounds not only in the gas phase but also in the particle phase. We modified the inlet system of the PTR-MS instrument to be able to measure the chemical composition of both phases online with the same technique. The system has been applied to smog chamber experiments and partitioning between gas and particle phase has been determined and compared with other techniques.

Experimental Methods

To measure molecular constituents of particles in an aerosol we modified the inlet system of the commercially available high-sensitivity PTR-MS instrument (IONICON Analytic GmbH, Innsbruck, Austria). Figure 1 shows a simplified scheme of the modified inlet system. The pressure was reduced to 300 mbar by a silco steel capillary and kept constant by a pressure controller. A Teflon capillary then reduced the pressure to the instrumentally required 2.1 mbar. These capillaries were heated to 100 °C to avoid back condensation of the semivolatile compounds onto walls. The drift tube was kept at 80 °C.

Before entering the capillaries the sample flow passed an 18 cm tube (i.d. 10 mm), heated to 150 °C to evaporate the particle phase. The system in front of the capillaries and heater consists of three sampling lines; one for particles, one for background, and one for gas + aerosol. The aerosol are sampled through a charcoal denuder to strip off the gas phase. To determine the background of the particle measurements a Teflon filter was introduced in front of the denuder to remove the particles. The gas + aerosol inlet line was heated to 120 °C.

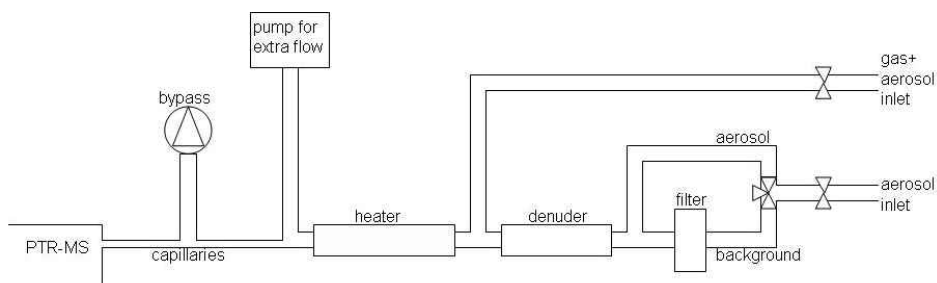


Figure 1: Diagram of the PTR-MS inlet to measure gas and particle phase.

Results and Discussion

The performance of the PTR-MS was checked with a test aerosol containing cis-pinonic acid which was simultaneously measured with an aerosol particle sizer (APS). A good correlation between the aerosol mass concentration measured with the APS and PTR-MS was obtained. We also evaluated the derivation of partitioning coefficients by our PTR-MS system on the example of cis-pinonic acid. A Tedlar bag was flushed with nebulized cis-pinonic acid. Assuming the SOA mass was pure pinonic acid a K_p value of $7.55 \cdot 10^{-4} \text{ m}^3 \mu\text{g}^{-1}$ was derived. This value agrees well with literature data [6,7].

The photochemical degradation of α -pinene was measured in the smog chamber. Figure 2 shows the evolution of the aerosol mass measured with the SMPS as well as the mixing ratios of α -pinene and a fragment (m/z 167) of pinonic acid. The precursor α -pinene is steadily consumed by the reaction with OH radicals and ozone. Pinonic acid appears from the beginning and reaches the maximum after 200 minutes simultaneously with the maximum in particle mass. In these smog chamber experiments the sum of mass concentration of all masses measured in the particle phase reached $25 \pm 4\%$ of the total aerosol mass concentration measured with the SMPS. The partitioning coefficient K_p of pinonic acid in these photooxidation experiments was found to be $5.1 \cdot 10^{-4} \text{ m}^3 \mu\text{g}^{-1}$ which is similar as in the pure pinonic acid aerosol.

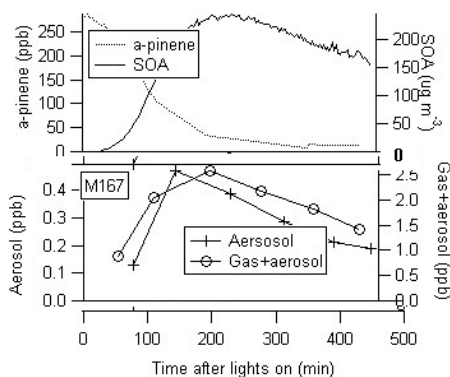


Figure 2: Temporal evolution of the aerosol mass, α -pinene and pinonic acid (m/z 167) in the particle and gas+aerosol phase.

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A lightweight, high-sensitivity PTRMS for aircraft platforms

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Abstract

For the new German research aircraft HALO (High-Altitude and Long-range Aircraft, Gulfstream GV-550), an extremely lightweight, high-sensitivity, quadrupole PTRMS was developed. The total weight including zero-air generator and calibration system is ~50 kg (without aircraft rack). The instrument is completely automated and controls all instrument components depending on flight altitude (pressure) and flight phase. It is aircraft-certified, e.g. is equipped with EMI filters, mechanical shock absorbers for sensible parts and contains negligible amounts of flammable materials. A sophisticated heating concept allows rapid high-sensitivity measurements shortly after aircraft launch. The first deployment is envisaged for the campaign “Oxidation Mechanism Observations (OMO)” in August 2009.

Introduction

The most crucial confinement for the deployment of instruments for atmospheric research onboard aircraft is – besides measurement accuracy – instrument weight and size. Up to now four PTRMS were deployed onboard research [1, table 4] and passenger aircraft [2]. Their weight including calibration system is about 110-130 kg. All these instruments are modified commercially available PTRMS from IONICON which contain some heavy components such as a stainless steel tube system, a large turbo pump and a control and data processing hardware from Inficon (Liechtenstein).

As of May 2005 our institute deploys a modified PTRMS from IONICON onboard the passenger aircraft CARIBIC. Once per month for four consecutive long-distance flights from Frankfurt (Germany) to North/South America, Southern India or the Philippines the PTRMS measures acetone, acetonitrile, methanol, and acetaldehyde at 9-12 km altitude, which in the meantime led to the largest available dataset of these compounds in this altitude range.

The broad experience collected during the CARIBIC flights and with a custom-made PTRMS for laboratory use allowed us to develop a new airborne instrument where all components are optimized in terms of weight, size and function for the deployment onboard aircraft.

Experimental Methods

Mechanical Setup

The arrangement of the detection system (hollow-cathode ion source, ion source drift region, drift tube, sequential pumping system, quadrupole mass spectrometer) was inherited from the

IONICON instrument. The (originally stainless steel) tube system holding the turbo pumps is made up of aluminium with non-standard flanges sealed with Indium wire and weights 2.5 kg, only. Three small turbo pumps (Pfeifer Vacuum, HiPace 80, 2.4 kg each) are used. All pieces above (hollow-cathode ion source, ion source drift region, drift tube) are optimized in terms of weight and are additionally sealed by O-rings to prevent leakage. The air flow into the system is controlled by a PFA proportional valve that (together with the new turbo pumps) allowed us to use a light-weight diaphragm pump (Vacuubrand, MD1 Vario, 4.1 kg). Particular care was exercised to guarantee an excellent alignment of all ion orifices.

The entire inlet tube system is made of PFA and is controlled to 30°C. A by-pass line (1 SLM, standard liter per minute) guarantees effective flushing of the inlet tube between aircraft inlet system and the sample air entrance at the drift tube. The drift tube pressure (~2.8 hPa) is controlled using the PFA proportional valve. A light-weight Pt VOC scrubber (0.73 kg) produces VOC-free sample air for background measurements. An additional PAN scrubber (not used until now) shall allow distinguishing between PAN, PPN, and MPAN, and other isobaric species on the relevant masses. The calibration is based on a permeation device containing four organic compounds, i.e. acetone, methanol, acetonitrile, and acetaldehyde.

Electrical Setup

The system is powered with 115VAC (400 Hz). An AC/DC converter with input and output filter converts the primary voltage to 24VDC and limits the maximum possible output power to 500W. The entire 19" control module from Inficon and all high-voltage supplies (for the ion source, drift tube, and the quadrupole MS itself) were exchanged with small custom-made Eurocard modules. A control computer (with DSP board) reads the counts from the CP400 preamplifier attached to the SEV, diverse pressure and temperatures, control valves heating wires/plates, pumps, relays etc, i.e. controls the entire instrument.

Results and Discussion

The first laboratory tests have started in the first half of November, so that the performance of the instrument was not known during submission of this abstract. Results and a discussion will be presented during the PTRMS conference in February 2009.

Due to the optimization of some components, it's expected a sensitivity at least comparable with the "High-Sensitivity PTR-MS" from IONICON.

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Laboratory and Field Measurements of Sesquiterpenes by PTRMS

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Abstract

Analytical characteristics of sesquiterpenes (SQT), an important class of semi-volatile compounds (SVOC) were investigated using a high sensitivity PTR-MS (IONICON). Due to the mass discrimination of a quadrupole mass filter (QMS), the quantification of fragmentation patterns is necessary to apply PTR-MS for the measurement of SVOC (e.g. >150 amu). In this context, a series of experiments, injecting a wide mass range ($m/z \sim 79^+ - 205^+$) of aromatic compounds was conducted to obtain an empirical transmission curve. The curve is applied to assess the degree of fragmentation of the parent ion ($m/z = 205^+$) of SQT, generated from the capillary diffusion system. The exercise indicates that the parent ion ($m/z = 205^+$) and the most pronounced fragment ($m/z = 149^+$) ion compose more than ~70% of the total counts of SQTs. Calculated mixing ratios of standard samples based on the total ion count and proton-transfer-rate constants show reasonable agreement (20% of difference) with measured mixing ratios by GC-FID. The lower limit of detection of SQT is calculated as ~20 pptv for a ten minute integration time. This suggest that PTR-MS can be a viable technique to measure SQTs in forest canopies where some studies have suggested that SQT mixing ratios can be as much as a few hundreds of pptv during the summer season. Results from a recent field deployment will be used as an example to evaluate the usefulness of PTRMS for SQT measurements.

Introduction

Biogenic semi-volatile organic compounds (SVOC) are thought to play a significant role for photochemistry and secondary organic aerosol (SOA) formation in the troposphere. Yet, the measurement, of SVOC in the atmosphere has been particularly limited due to technical challenges resulting from their high reactivity (e.g. ozonolysis) and vulnerability to wall losses during conventional sampling procedures which involve pre-concentration stages. . These technical difficulties can be overcome by using in-situ techniques such as proton-transfer-reaction mass spectrometry (PTR-MS), which has been established as a standard CIMS technique for VOC measurement [1,2,3]. This study synthesizes a series of laboratory and field experiments

over the last year, exploring the possibility to quantify sesquiterpenes (SQT) with PTR-MS. SQT along with other terpenes are an important class of compounds released from the biosphere which have received a lot of attention as they are suspected to comprise a large fraction of unidentified VOCs from ambient and enclosure samples in forest canopies.

Experimental Methods

A high-sensitivity PTR-MS (IONICON) was used for this study. Seven different SQT species, generated by a capillary diffusion system [2] were applied to examine fragmentation patterns. An experimental transmission curve of the QMS was obtained by injecting an aromatic gas standard (Matheson TriGas, USA) covering a mass range between m/z 79+ and 181+ at 117 Td. In addition, the instrument sensitivity of 1,3,5-triisopropylbenzene (TIPB), available from the capillary diffusion system is used to specifically assess the transmission efficiency at m/z 205+. Rigorous calibration experiments obtained from laboratory measurements are put into context of previously published work on SQTs measured by SIFT and PTRMS [4] and are applied to data obtained from branch enclosures and ambient air measurements in a forested Southern Rocky Mountain area, Colorado USA.

Results and Discussion

The mass spectrum of α -caryophyllene, indicated the most pronounced fragmentation (at 117 Td) of all SQTs investigated in this work and is shown in Figure 1. To examine contributions of the parent ion (m/z 205+) to each fragmented ion, the empirical mass discrimination curve (Figure 2) is applied to deduce the transmission corrected mass spectrum as shown in Figure 3. The results indicate that two major product ions (m/z 149+ and 205+) account for more than 80% of total product ion counts for α -caryophyllene and 95% of those for cadinene, the least fragmented SQT observed in this study. The calculated mixing ratios with the total counts of all product ions and proton transfer rate constants agree with measured mixing ratios from GC-FID within 20 %. In addition, the lower limit of detection is estimated as ~ 20 pptv for a 10-minute integration period. This is well below the speculated summer time SQT mixing ratios of SQT in the forest canopy, e.g. ~ 100 pptv during the summer season.

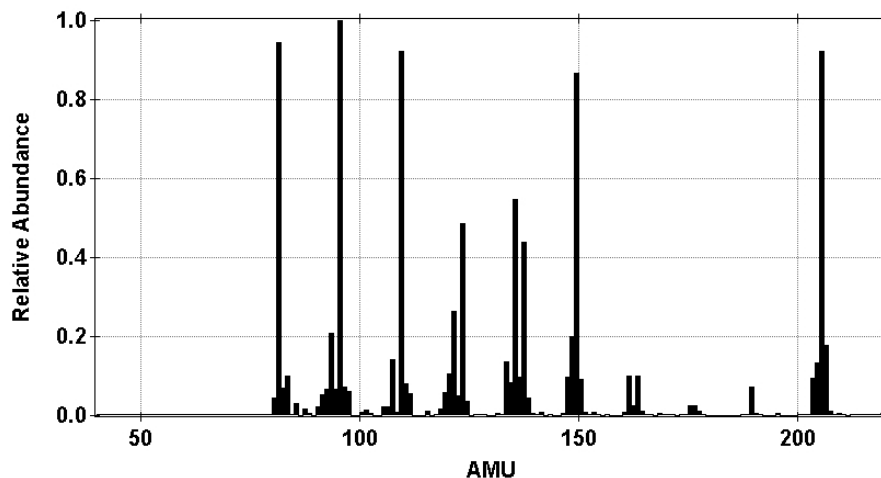


Figure 1: The mass spectrum of β -caryophyllene that is taken by PTR-MS

For test purposes, we deployed a PTR-MS system to measure emissions from branch enclosures of Ponderosa Pine and ambient SQT in the forest canopy. We will discuss the emission patterns and some aspects of photochemistry of SQTs and other BVOC in this presentation.

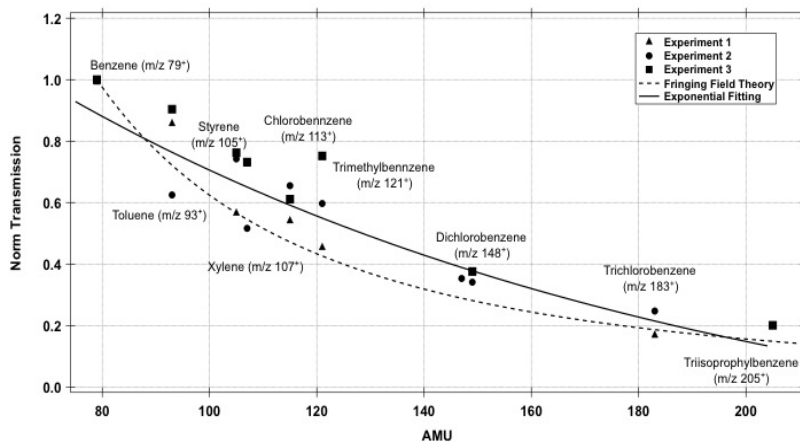


Figure 2: Empirical transmission curve of the PTR-MS system, used for this study. Each data point represents a denoted aromatic compound, a mass range from m/z 79⁺ to m/z 205⁺

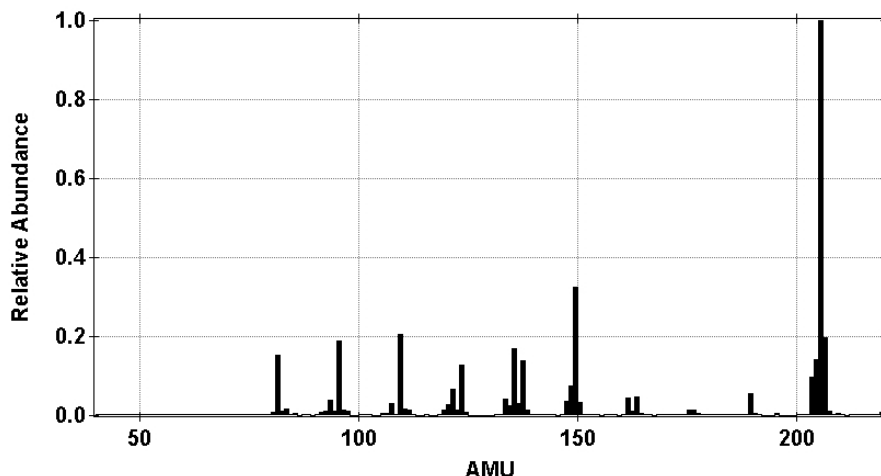


Figure 3: The transmission-corrected mass spectrum of β -caryophyllene

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PTR-MS measurements of concentrations and fluxes of biogenic VOCs in the humid tropics - rain forest vs. oil palm plantation

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Abstract

For the first time, concentrations and eddy covariance fluxes of several of the most abundant biogenic volatile organic compounds (VOCs) have been measured by PTR-MS in SE Asia (Malaysian Borneo) at two locations: at 75 m on a GAW tower over secondary rain forest and above an oil palm canopy. The operation of the instrument at both sites and the results are discussed in respect of the extremely high ambient humidity levels, which are not generally encountered outside of the humid tropics. Despite the challenging conditions, smooth operation could be achieved through instrument optimisation and anticondensation protection of the instrument set-up generally. The most abundant VOC was isoprene, with peak emissions of 4.7 mg m⁻² h⁻¹ above the rainforest and 30 mg m⁻² h⁻¹ above the oil palms. For most VOCs good sensitivities were obtained, but some of the strongly cluster-dependent OVOC species suffered a reduction in their detection limits. The results from both sites are presented, focussing on the effect of the different ambient humidity, which affected the normalised signal. At standard drift conditions the proton transfer reaction is known to dominate over the reaction with water clusters. However, at high humidities, water clusters appear to play a more significant role in the ultimate detection efficiencies. Nevertheless, PTR-MS has been proven to be capable of measuring various VOCs even in extreme tropical environments. In addition, some ideas for future instrument adjustments to facilitate tropical measurements are proposed.

Introduction

For more than a decade Proton Transfer Reaction Mass Spectrometry (PTR-MS) has been serving as an enormously useful analytical tool among others in atmospheric sciences. Facing environmental issues such as Global Warming or Global Climate Change, it becomes necessary to better understand atmospheric processes. However, since many important atmospheric constituents are present in low concentrations PTR-MS requires appropriate optimisation to come up with the desired detection limit. Both ambient humidity and the level of relative H₂O clusters in the reaction chamber impact the optimal detection limit that can be achieved. Thus, adaptation of PTR-MS technique to measurements in very humid environment can be essential for the precise quantification of volatile organic compounds (VOCs) emitted to the atmosphere. This, in turn, is needed for elaboration of dynamic models predicting regional and global climate changes.

PTR-MS was indeed a breakthrough invention allowing for its combination with most direct and accurate micrometeorological methods such as eddy covariance for obtaining VOC fluxes at ecosystem and regional scales. It is also possible to measure water vapour fluxes by PTR-MS, but in order to obtain absolute values calibration against a sensor of absolute humidity is necessary.

Not a long time ago it was observed that plants emit vast amounts of volatile substances to the atmosphere. These include for example compounds like isoprene, monoterpenes, methanol, acetaldehyde or acetone as well as a variety of other metabolic, stress, or signalling compounds. According to recent estimates [1], biogenic sources globally far exceed those of an anthropogenic origin, which on the other hand can dominate locally around large cities. There have been numerous studies already involving PTR-MS of the polluted urban environment, as well as focussing on emissions from forests and other vegetation types. There is still a limited number of VOC flux measurements over tropical regions apart from some data from the Amazon. In the collaborative projects of OP3 and ACES ground and aircraft measurements were carried out for the first time in Borneo in 2008. Some of these results are presented here. Although the PTR-MS technique is now quite well known, at some conditions running the instrument can be quite difficult, for example at extremely high specific humidity levels and high temperature. When sampling the air along a sampling inlet line (e.g. PTFE), one needs to prevent water condensation, which could potentially result in a loss of the flux data and flood the instrument. One of the widely used methods is heating the line, although some groups limit the heated segment to inside the building only and some heat the whole length of the tubing. Another way is to decrease the pressure in the line by using a high flow vacuum pump for the inlet line. A combination of those methods was used at the rainforest and the oil palm sites and specific optimisation of the PTR-MS led to its steady operation.

Experimental Methods

Rainforest

The VOC analyzer was the PTR-MS from CEH Edinburgh, corresponding to the high sensitivity (HS) type, and described elsewhere in detail (e.g.). The fluxes were measured by the virtual Disjunct Eddy Covariance (vDEC) technique, which was introduced by Thomas Karl in 2002 [2]. The schematic of the setup is presented in Fig. 1a. The air was drawn from 76 m above the ground level through ½'' PTFE tubing, of which the inlet was connected in the vicinity of the Windmaster Pro sonic anemometer. The flow rate in the sample line was kept constant at 56 l/min (measured at the inlet), so as to sustain the turbulence and to prevent water condensation. For the latter reason, the segments of the tube at the ground and inside the building were additionally heated to approx. 40 °C by a heating tape wrapped around the tube and were further protected by enclosure in an insulation sleeve. Following previous studies of dew point influence on sensitivity according to the E/N ratio, the PTR-MS system was optimized for operation at relatively high E/N ratio of 130 Td for the purpose of preventing excessive clustering. In order to keep the drift pressure at a constant value (fluctuation free) when connected to the low pressure line it was lowered from 200 Pa (normally used) to 178 Pa, and the drift voltage was adjusted to 500 V and the temperature to 45 °C.

Oil palm plantation

The same PTR-MS was used at the oil plantation and the same flux technique was used. The pictures of the site set up are presented in Fig. 2. The E/N ratio was kept constant at 140 Td by adjusting drift tube parameters of pressure to 160 Pa, temperature to 45 °C and the drift voltage to 485 V. The sampling inlet and the 20Hz R3 sonic anemometer were placed at about 15 m (5 m above the canopy level). The instrument and tubing were protected against water condensation by heating above the ambient temperature (approx. 50 °C). The inlet pressure was reduced by maintaining the constant flow in the sampling line of 35 l/min.

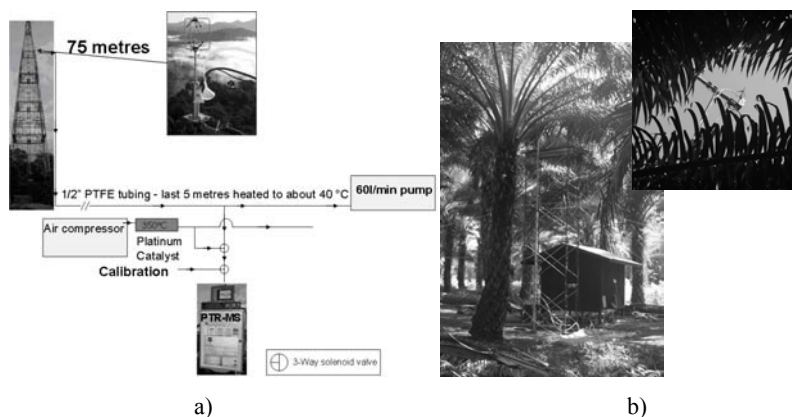


Figure 1: Setup a) rainforest b) oil palm plantation

Results and discussion

The PTR-MS technique was coupled to virtual disjunct eddy covariance at both the rainforest and the oil palm sites. The concentrations and fluxes of several key compounds were obtained as well as the scans of the whole spectrum of m/z of the ambient air. For several VOCs (i.e. methanol, acetone, acetaldehyde, isoprene, monoterpenes and acetonitrile) external calibration was used in deriving their sensitivities, while remaining compounds had their sensitivity approximated from the relationship between calibration and transmission curves. The calibrated sensitivities for the compounds contained in the standard were dependent on the level of water clusters at m/z 37, which was different at the two sites. The air sampled from 15 meters at the plantation was usually completely saturated with water and its specific humidity exceeded the one sampled at the rainforest site. Thus generally higher normalised sensitivities were obtained at the rainforest for m/z susceptible to clustering. Sensitivities for isoprene were similar. The clear water vapour flux could be derived from m/z 37. Figure 2 shows the diurnal patterns of water vapour fluxes measured at the oil palm plantation by PTR-MS and Li-COR (infra-red gas analyser).

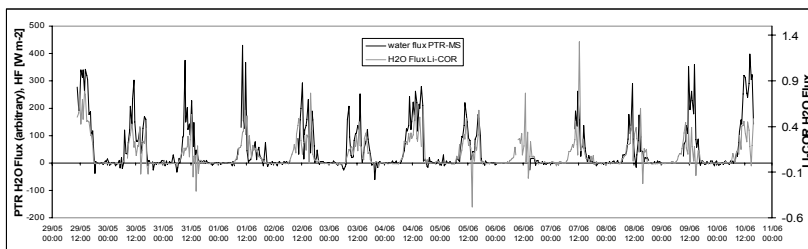


Figure 2: The timeseries of water fluxes at oil palm plantation derived from PTR-MS (black) and contrasted with the same data from Li-COR (grey).

At both sites the most abundant VOC was isoprene, with peak emissions of $4.7 \text{ mg m}^{-2} \text{ h}^{-1}$ above the rainforest and $30 \text{ mg m}^{-2} \text{ h}^{-1}$ above the oil palms. The averaged diurnal fluxes throughout the campaigns are shown in Figure 3.

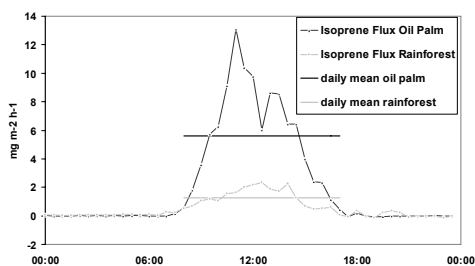


Figure 3: Isoprene flux comparison between the sites based on diurnal averages. Horizontal lines denote means of the whole periods.

Six times higher isoprene emissions from oil palm can have implications for regional zone, for example in terms of ozone and secondary organic aerosol formation as well as radical chemistry in troposphere. In addition, a range of other VOCs were measured and examples are presented as diurnal averages in Figure 4.

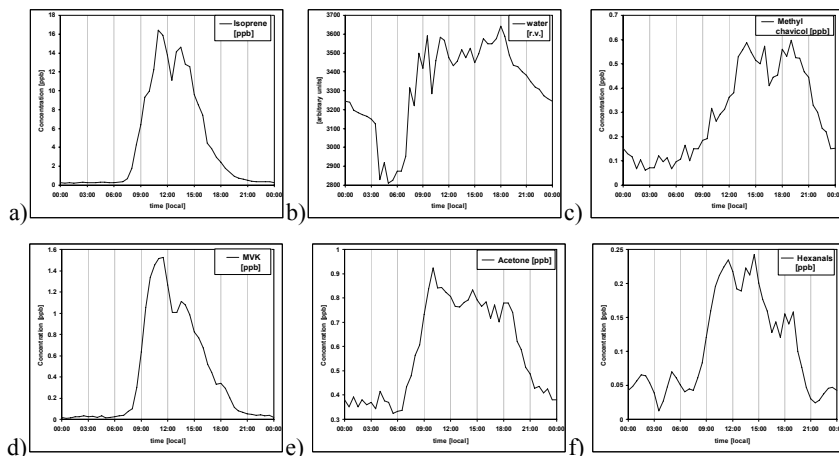


Figure 4: Diurnal averages of concentrations of selected VOCs at the oil palm site. a) isoprene; b) water vapour c) methyl chavicol d) methyl vinyl ketone / methacroleine e) acetone f) hexanals

A similar trend of isoprene and its oxidation products (MVK/MACR) as well as acetone and hexanals was observed. Processing and analysis of the results from both campaigns are ongoing.

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Chemical Flux Measurements Using PTRMS

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Abstract

In recent years PTR-MS technology has played a significant role for quantitative analysis of Volatile Organic Compounds (VOC) in environmental sciences. Due to its rapid sample analysis capability PTRMS has been particularly useful in combination with micrometeorological measurement strategies. Recent airborne and ground based measurement campaigns will be reviewed and put into context of future experiments.

Introduction

Volatile organic compounds (VOCs) critically influence the composition of the Earth's atmosphere by fueling tropospheric chemistry [1], thereby modulating its oxidation capacity and providing condensable material for organic aerosol formation [2]. More recently their importance for the organic aerosol budget in the atmosphere has been demonstrated in the field (e.g. ref [3]). On a global scale the emission strength of biogenic VOCs ($\sim 1000 \pm 600$ Tg/y) dominates the annual VOC budget, accounting for up to 90% of the reduced carbon flux entering the atmosphere. The tropics are of particular importance because more than 70 % of the global total biogenic VOC flux occurs there. Yet, only a few direct measurements are available from tropical regions to constrain global bottom-up emission models and estimates. The first part of this presentation will describe recent airborne and ground based flux measurements in the Amazon basin. These flux measurements allow assessing uncertainties associated with emissions, chemistry, and vertical transport of reactive biogenic VOC.

The second part of this presentation will focus on emission inventories for developing Megacities which are considered particularly uncertain. Air pollution management relies on accurate predictions of VOC to NO_x ratios in order to determine effective ozone reduction strategies (e.g. ref [4]). The sensitivity of modeled ozone concentrations to VOC emission inputs has also been demonstrated by adjusting biogenic emission maps in the US. For example, the difference between two biogenic emission inventories (BEIS1 and BEIS 2) almost doubled the frequency of modeled ozone exceedances (e.g. mixing ratios >80 ppbv) in the Eastern US [5]. As a consequence the assessment of emission inventories has important implications for policy decisions. It is common practice to use measured VOC concentrations as one important constraint for chemistry and transport (CT) models. The atmospheric concentration of a reactive compound however can be seen as balance between emission, deposition, transport and chemistry. With so many degrees of freedom, concentration measurements alone make it hard to diagnose uncertainties in CT models. In order to disentangle surface exchange from other processes effecting the distribution of reactive trace gases, direct flux measurements can add one important additional constraint on the atmospheric cycle of VOCs and help lessen uncertainties of emission inventories. Airborne flux measurements using PTRMS over a Megacity will be used as an

example to demonstrate how top-down constraints can significantly improve our ability to model VOC emissions on spatial scales that are representative for large scale regional and global CT models.

Experimental Methods

A modified high sensitivity PTR-MS system (Figure 1) was deployed during 2 recent aircraft campaigns. The PTR-MS instrument was operated at standard conditions (2 mbar, 110 Td).

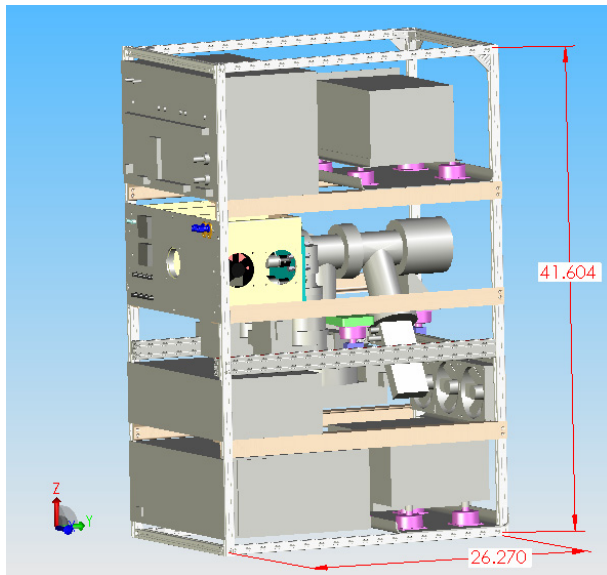


Figure 1: Redesigned high sensitivity PTR-MS instrument.

Results and Discussion

Example 1: Biogenic flux measurements in the Amazon Basin

To test biogenic emission models in tropical regions regional isoprene fluxes were measured based on the mixed layer gradient technique (Figure 2) and, for the first time, the mixed layer variance technique. Comparison with ground based eddy covariance measurements showed reasonable agreement. Average noon time isoprene and monoterpenes fluxes were (7.8 ± 2.3) $\text{mg/m}^2/\text{h}$ and (1.2 ± 0.5) $\text{mg/m}^2/\text{h}$ respectively. Isoprene and monoterpene fluxes corrected to standard conditions (30°C , 1000 PAR) on the ground were 5.9 ± 0.1 $\text{mg/m}^2/\text{h}$ and 1.2 ± 0.1 $\text{mg/m}^2/\text{h}$ respectively.

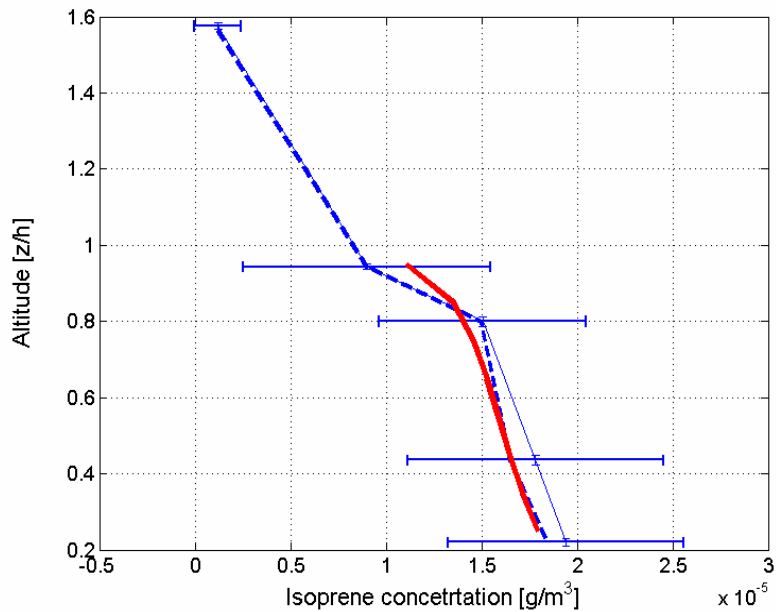


Figure 2: Isoprene concentrations measured over pristine tropical rain forest (blue). The red line represents predicted concentrations based on the mixed layer gradient method.

Example 2: Airborne eddy covariance measurements over Mexico City

Toluene and benzene are used for assessing the ability to measure disjunct eddy covariance (DEC) fluxes of Volatile Organic Compounds (VOC) using Proton Transfer Reaction Mass Spectrometry (PTR-MS) on aircraft. Wavelet analysis of instantaneous toluene and benzene measurements during city overpasses is tested as a tool to assess surface emission heterogeneity. High toluene to benzene flux ratios (Figure 3) above an industrial district (e.g. 10-15 g/g) including the International airport (e.g. 3-5 g/g) and a mean flux (concentration) ratio of 3.2 ± 0.5 g/g (3.9 ± 0.3 g/g) across Mexico City indicate that evaporative fuel and industrial emissions play an important role for the prevalence of aromatic compounds.

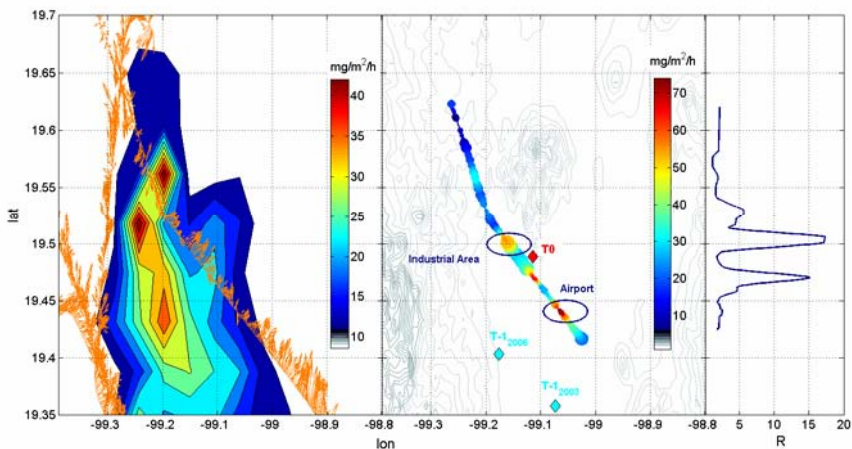


Figure 3: Comparison between an anthropogenic emission map (left panel) with flux measurements across Mexico City (middle panel). The feather plot on top of the emission map (left panel) depicts the C-130 flight track across the city and shows the instantaneous horizontal wind speed vector. The middle panel shows toluene fluxes across MC on top of an elevation map. The right panel shows the ratio between instantaneous toluene to benzene fluxes measured across the city.

Acknowledgements

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Quantitative long-term measurements: challenges and results from boreal forest

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Abstract

We will present measurement results from Scots pine shoot and canopy scale emissions, as well as ambient air volume mixing ratios. We will discuss solutions for problems arising in the quantitative long term field measurements.

Introduction

The VOCs are a key player in many biosphere - atmosphere processes that are essential for understanding of the climate system. Therefore high quality, long term, field measurements of VOCs are needed with comprehensive additional measurements of environmental and other important parameters.

Experimental Methods

The measurements were carried out at the SMEAR II measurement station [1] in Hyytiälä, southern Finland (61°51'N, 24°17'E, 180 m above sea level). Fig. 1 presents the measurement set up that was used for quantitative long term measurements of ambient volume mixing ratios [2, 3], above canopy emissions [4, 5] and Scots pine shoot emission measurements [6, 7].

Measurement time of the PTR-MS was divided among the measurements in three hour cycles: The first hour was dedicated to the ambient volume mixing ratio measurements from three heights below, in and above canopy at 4, 14 and 22 m heights. The second hour was dedicated to background signal measurements and for ecosystem scale flux measurements with micrometeorological disjunct eddy covariance method. The third hour was dedicated for shoot scale emission measurements from automated chambers.

The measurements were continuous and automated. Separate computers were used for the chamber system, meteorological measurements and the PTR-MS.

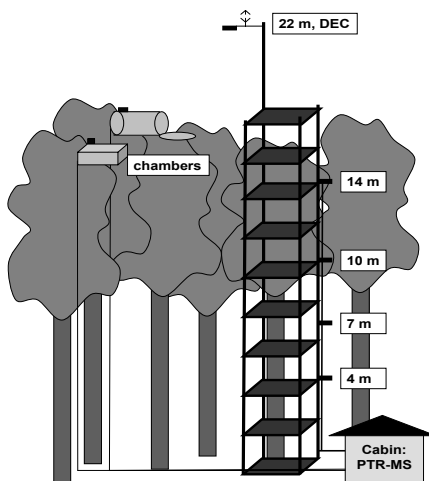


Figure 1: Schematic presentation of measurement set-up.

Results and discussion

Simultaneously measured methanol emissions from Scots pine shoot and canopy, as well as ambient volume mixing ratios inside canopy are presented in Figure 2.

Methanol emissions exhibited clear diurnal cycles with maxima around noon and minima after midnight. The diurnal pattern was more profound in the shoot scale chamber measurements. The disjunct eddy covariance method requires sufficient vertical wind speed and at night time turbulence is suppressed and the above canopy flux are either not available or highly unreliable. The measured methanol emission, up-scaled from shoot to canopy scale, was of similar magnitude as the methanol flux, measured above canopy. The influence of local biogenic emission of methanol was seen in the ambient volume mixing ratio and its diurnal cycle.

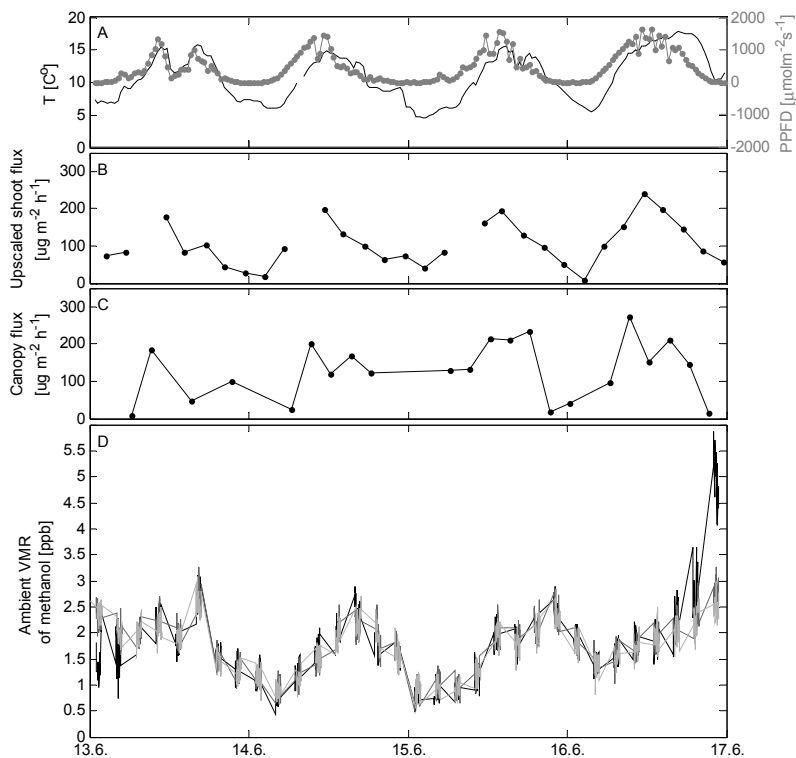


Figure 2: Simultaneous field measurements at SMEAR II station in 2007 of A) T and PPFD, methanol emissions from B) shoot and C) canopy, and D) inside canopy ambient volume mixing ratio.

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VOC measurements within a boreal forest during spring 2005: the role of monoterpenes in selected intense nucleation events

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Abstract

Several clear aerosol “nucleation events” were observed during the BACCI4-QUEST campaign at Hyytiälä Forestry Field Station in 2005. Some of these events are synchronized with and strong increases in monoterpenes, while others seemed to correlate more strongly with sulphuric acid. In this study, we elucidate these two distinct forms of aerosol production at the Hyytiälä using the measurements and information from back trajectories.

Introduction

Boreal coniferous forests have been shown to profoundly influence regional atmospheric chemistry through the emission of reactive trace gases such as monoterpenes and their oxidation products e.g. [1,2]. The role of these organics in new particle formation has been the subject of considerable research activity in recent years e.g. [3,4] but is not fully understood. The composition of newly formed ultrafine atmospheric aerosols is not known with certainty. A small number of field studies on newly formed particles (3–5 nm) e.g. [5,6] have concluded that they were primarily composed of high molecular weight oxidised organic species. However, sulphate/organic mixtures have been suggested [7].

In this study we present measurements of trace gases and aerosol made in a Boreal forest during the “Biosphere – Aerosol – Cloud – Climate Interactions – Quantification of Aerosol Nucleation in the European Boundary Layer (BACCI4-QUEST) intensive field campaign in Hyytiälä, Finland in April-May, 2005 [8,9]. The measurement period marked the transition from cold winter temperatures to more temperate spring temperatures. This is a particularly interesting period to examine and to compare with previous measurements, since most previous measurements have been taken in the summer e.g. [10,11].

Experimental Methods

At the SMEAR II station in Hyytiälä, long-term measurements of meteorological, physical, and chemical parameters as a function of height (up to 74m) are available. A more detailed description of the site is given in [12]. VOC, aerosol particle, and gas phase sulphuric acid

measurements, were made within a 100 m radius from the SMEAR II tower and therefore are considered to be co-located.

VOCs were quantified using a Proton Transfer Reaction Mass Spectrometer (PTR-MS) [9], a Thermo Desorption - Gas Chromatography Mass Spectrometer (TD-GC-MS) [13] and a Methane & Total Non Methane Hydrocarbon analyser [14]. Gas phase sulphuric acid was quantified using a flow reactor CIMS instrument [15] and the number of aerosol particles in the diameter range 3 nm to 850 nm was measured by a Differential Mobility Particle Sizer (DMPS) [16].

Results and discussion

Generally higher levels of VOCs (e.g. acetone, methanol and monoterpenes) and aerosols were observed during the slightly warmer periods of this campaign when airmasses came from the south. The diel profiles of the monoterpenes, show a distinct cycle with higher mixing ratios at night when the site was influenced by a shallow nocturnal boundary layer. Increases in the nocturnal VOC mixing ratios have been observed before at Hyytiälä [17]. Although monoterpene emissions have been previously shown to be a function of the ambient temperature, with higher monoterpene emissions during the day e.g. [2,18]. For the Hyytiälä site in spring the monoterpene mixing ratios are clearly more controlled by inversion height than by temperature related emission rate changes. Generally good agreement was found between the higher mixing ratios of monoterpenes and the potential temperature inversions in the forest. The highest short term peaks in the monoterpenes did not occur during the strongest gradient. These very high monoterpene mixing ratios correlated with compounds whose emission is not normally associated with temperature or biogenic emissions such as toluene, again suggesting that these monoterpenes are associated with an unnatural event.

In this study, a daytime and a night-time aerosol event exhibiting high background particle concentration, were scrutinized for possible correlation with meteorological parameters as well as with organic and inorganic trace gases. Figure 1 shows that Event 1 (daytime; April, 30) was characterised by a five fold increase in the total aerosol number concentration ($dN/d\log D_p$) lasting several hours and Event 2 (night-time, April, 27-28) was of short duration with 11 fold increase in $dN/d\log D_p$.

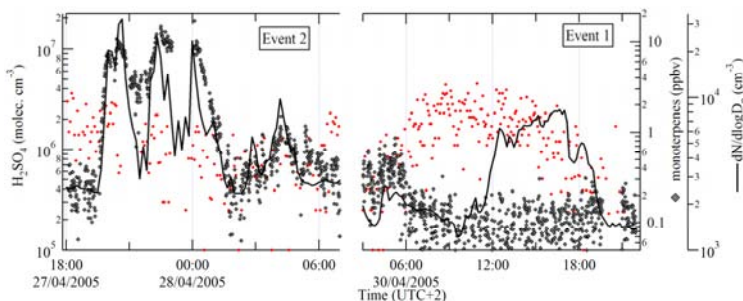


Figure 1: Total aerosol number concentration in relation to monoterpenes and H_2SO_4 measured near SMEAR II for two selected events.

The analyses suggest that both events correlated with anthropogenic air pollution markers. However, the anthropogenic contributions to the occurrences of these events were of a different kind for each case.

No evidence was found that monoterpenes or other volatile organic compounds measured were involved in the growth of particles during nucleation Event 1 which occurred under elevated ozone. Rough fetch calculations conjecture that the SO₂ emissions from Jyväskylä, a city 88 km from Hyytiälä were in the footprint of the measurements. H₂SO₄ and intermediates from the photo-oxidation of SO₂ play a role in atmospheric new particle formation which is generally thought to occur due to homogeneous or ion-induced nucleation of sulphuric acid [19,20].

During Event 2, SO₂ mixing ratios and sulphuric acid concentrations were found to be fairly low and both declined during mysterious intensive night-time bursts in aerosol particles occurring before midnight. Based on compiled data, more toluene was released relative to benzene and more methanol relative to acetone. This is on top of a biomass-burning background where massive amounts of aerosol particles were released along with monoterpenes and NO₂. This has lead us to the nearby Korkeakoski sawmill as the likely source.

Additional source identification besides the rough fetch calculations is obtained from the detailed analyses of the monoterpenes up to the enantiomeric level by TD-GC-MS which have shown that (+)- α -pinene, Δ^3 -carene, (-)- α -pinene and (-)- β -pinene were most abundant above the boreal forest. Daytime enantiomeric ratios of [(+)- α -pinene]/[(-)- α -pinene] varied between 1.6 and 2 during this campaign, generally increasing from the break-up to the onset of the nocturnal boundary layer. This enantiomeric ratio was higher than 2 during nocturnal monoterpene peaks. Such peaks on the 26th and 27th were believed to be of similar kind as monitored by the PTR-MS during Event 2, with much higher α -pinene enantiomeric ratios of 3-5. TD-GC-MS and Solid Phase Micro Extraction-GC-MS (SPME-GC-MS) data using cuvettes also sheds light on the interpretation of the α -pinene enantiomeric ratio. Damaged leaves emit (+)- α -pinene and (-)- α -pinene in a ratio, similar to those observed during the on-line measurements (Yassaa, N., personal communications).

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PTR-MS measurements of VOCs over Russia

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Abstract

First VOCs measurements over Russia along the Trans-Siberian railroad (37°E - 132°E) are presented. It was carried out in TROICA-12 project (TRanscontinental Observations Into the Chemistry of the Atmosphere) using PTR-MS instrument. In this project eighteen different organic species were measured. For all these species spatial profiles were built. Analysis of spatial variability was done. It was confirmed that isoprene has mostly biogenic origin. Mean isoprene concentration on Russian territory covered by forest through which the Trans-Siberian railroad goes is 0.4 ppb. At the same time it was confirmed that benzene has mostly anthropogenic origin. In unpolluted area benzene concentration is near zero. In general, VOCs have both anthropogenic and biogenic sources. In the report factors defining characteristics of spatial distribution and daily VOC variations are discussed.

Introduction

In the last two decades VOC measurements became very important due to recent atmospheric composition changes and the necessity to evaluate total organic carbon in the atmosphere [1]. Russia occupies one seventh of global continental territory. That is why it is very important to know VOC concentrations over Russia to solve upper mentioned problems. Up to now, there have been done some studies of VOC over Russia in TROICA experiments using flasks [1], at Zotino station (Central Siberia) and around Moscow. But there is no atmospheric stations which would be able to carry out regular observations of VOC in Russia.

Experiments TROICA are based on a moving platform and allow performing atmospheric composition measurements over large territory of Russia. This platform is installed at the head of the passenger train going from Moscow to Vladivostok and back. These experiments have been conducted since 1995, mainly through the transcontinental Russia. The laboratory is equipped with instruments allowing measurements of O₃, NO, NO₂, CO, CO₂, CH₄, SO₂, NH₃, THC, 222Rn, and aerosol parameters (including soot). Since 1995, twelve TROICA experiments have been performed. Here some results of VOC concentrations measurements obtained in TROICA-12 (July 21 – August 4, 2008) are presented.

Experimental Methods

TROICA-12 project took place 21 July – 4 August 2008. During TROICA-12 concentrations of eighteen organic species were measured with 10 sec time resolution using PTR-MS, purchased from Ionicon Corp.

Next species were measured using PTR-MS: acetylene (27), methanol (33), acetonitrile (42), acetaldehyde (45), ethanol (47), 1,3-butadiene (55), butane (57), acetone (59), isoprene (69), methyl vinyl ketone + methacrolein (MVK + MACR) (71), benzene (79), terpenes (81), toluene (93), phenol (95), styrene (105), C8-aromatics (107), C9-aromatics (121), C12-aromatics (163).

Results and Discussion

Here we present spatial distributions only for two VOC: isoprene and benzene. Profiles for other organic species and results of their analysis will be presented at the conference.

Spatial profiles of isoprene (Fig. 1) obtained in both directions don't show any significant changes while crossing cities and industrial regions. There is no strong connection between isoprene concentrations and NO and CO concentrations. The main variabilities are connected with land cover features and meteorological conditions. The Trans-Siberian railroad crosses the territory of Russia covered by forest. Mean isoprene concentration during eastern transect is very high – 0.49 ppb. The highest values are observed in eastern part of continent in Amur valley and in Ussurijsk Taiga.

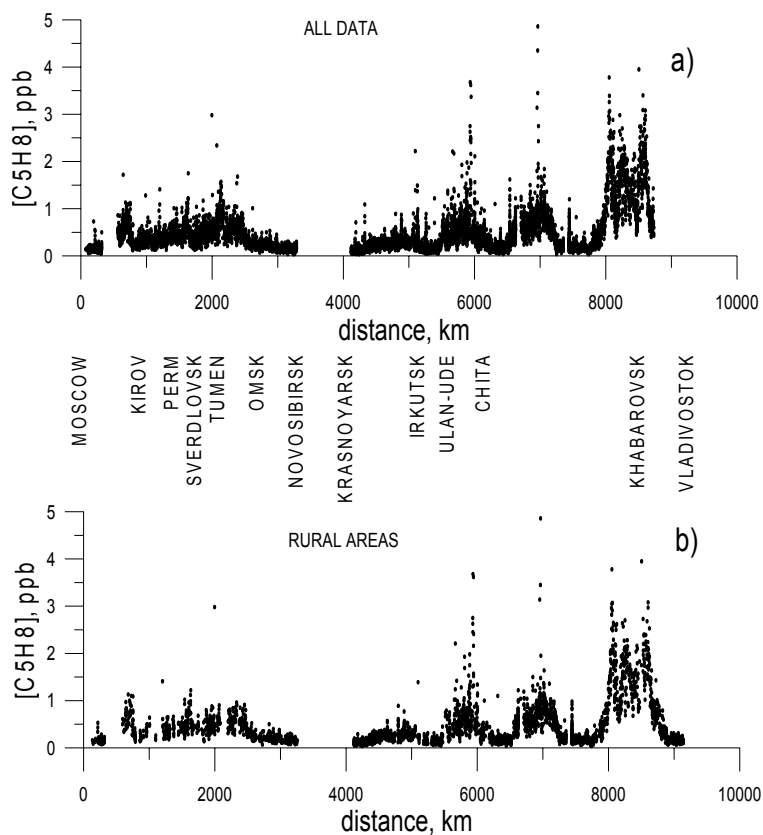


Figure 1: Isoprene concentrations between Moscow and Vladivostok (TROICA-12, eastern transect, 21-28 July, 2008): a) all isoprene data; b) selected data for rural areas only ($[NO] < 0.4$ ppb)

In Table 1 there are mean isoprene concentrations for TROICA-12 (21 July – 4 August, 2008); mean data derived in TROICA-6 (6 April – 25 June, 2000) on the way Murmansk-Kislovodsk crossing European territory of Russia from North to South; Kislovodsk high mountain station data (9 – 12 June, 2000); Hohenpeissenberg station for the same period of year 2007 (21 July – 4 August, 2007).

Table 1: Isoprene concentrations for TROICA-12 (21 July – 4 August, 2008), both eastern and western transects; TROICA-6 (6 April – 25 June, 2000); Kislovodsk high mountain station (9 – 12 June, 2000); Hohenpeissenberg station (21 July – 4 August, 2007)

<u>Experiment</u>	<u>[C5H8], ppb</u>
TROICA-12, eastern transcontinental transect	0,49
TROICA-12, western transcontinental transect	0,31
TROICA 6, meridional European transect	0,37
Kislovodsk high mountain station (2000 m.a.s.l.)	0,06
Hohenpeissenberg (985 m.a.s.l.)	0,08

From Table 1 one can see that mean isoprene concentrations are similar for different TROICA. Moreover, isoprene concentrations measured at Russian high-located station are low and similar to those measured at German high-located station.

Benzene profiles shown on Figure 2 are opposite to isoprene ones. Near-zero concentrations are observed in background conditions. High values correspond to large industrial areas. Maximum benzene concentrations were observed near Bratsk and Irkutsk – the most polluted area located along the Trans-Siberian railroad. Other measured VOCs mostly have both anthropogenic and biogenic sources. Their profiles between Moscow and Vladivostok have more complicated structure. Its features display influence of meteorological processes, which have impact on vertical mixing, advective transport, presence of cloudiness, precipitation.

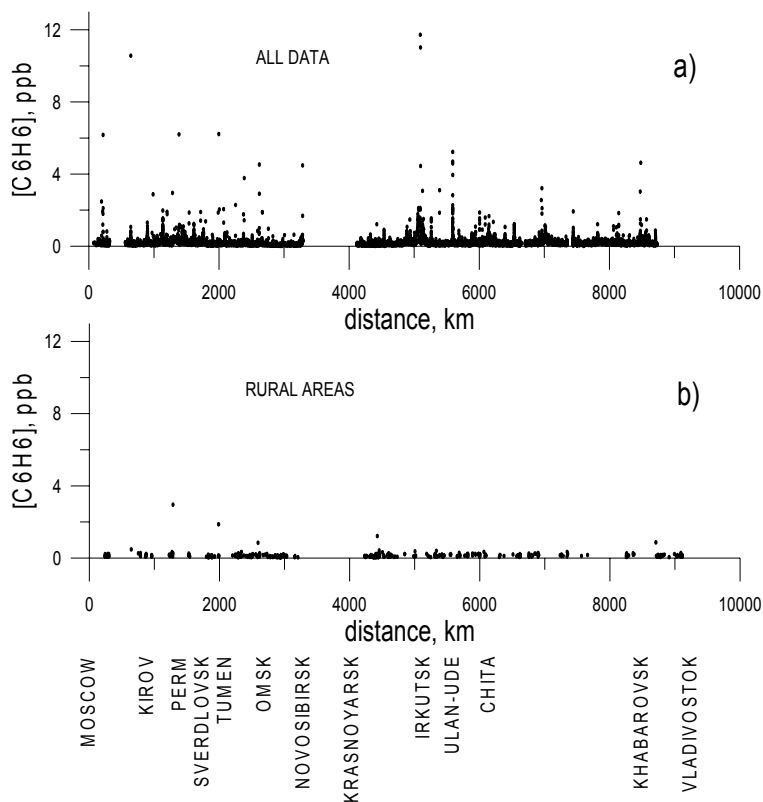


Figure 2: Benzene concentrations between Moscow and Vladivostok (TROICA-12, eastern transect, 21-28 July, 2008): a) all benzene data; b) selected data for unpolluted rural areas only ($[NO] < 0.2$ ppb, $[CO] < 0.2$ ppm)

Acknowledgements

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Induced emission of isoprenoids: ecophysiological considerations and modelling approaches

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Abstract

Constitutive emissions of isoprene and monoterpenes have been extensively studied. Understanding biosynthesis of these emitted compounds has allowed their parameterization, and the release of process-based emission models. However, volatile isoprenoid emission may also be induced. I will review some of the experimental evidences showing that the emission of isoprenoids may be induced by environmental stresses, namely drought and ozone, and by herbivore feeding. Constitutive and induced emissions often, but not always, share the same biochemical pathway. I will show that plants subjected to heavy but transient drought stress continue to emit isoprene even when photosynthesis is totally inhibited. However, this photosynthesis-independent emission does not contribute to the stimulation of isoprene emission that is often transiently observed upon recovering from drought or other oxidative stresses. I will also show evidences that herbivore-induced monoterpene emissions may be generated using the same carbon constitutively forming isoprene in the same leaves, arguing that this is a compelling evidence for opportunistic emission of isoprenoids. Finally, the question whether current modelling of isoprenoid emissions should and could also incorporate induced emissions will be posed. Current parameterizations and modelling may not be able to capture the suite of emissions induced in nature by plants facing abiotic and biotic constraints.

5. Instruments & Technology

Analysis of volatiles emitted by plants using PTR-MS and PIT-MS

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Abstract

As an alternative energy source, the use of biofuels is gaining wide attention and several grass species are possible candidates for biofuel production. However, for this purpose large areas of grass crops would have to be grown. This brings up an important question: what are the biogenic volatile organic compounds (VOCs) emitted by those crops, and how do they affect regional air quality and climate? If there are significant environmental impacts, do they outweigh the positive effect of a reduction in net CO₂ emissions? To answer such questions we have performed some preliminary experiments on maiden grass (*Miscanthus sin. variegatus*), and other crops that are being considered as candidates for biofuel production. VOC emissions during growth and post-harvesting/drying have been measured, using both PTR-MS and PIT-MS (Proton-transfer-reaction Ion Trap Mass Spectrometry). Results will be presented in the talk.

In addition we studied the response of plants under attack by root feeding insects. In the past, herbivore induced responses have been reported to occur in over 100 different plant species [1]. However the induction by and against belowground feeding herbivores has received little attention [2]. It may be expected, however, that belowground induced responses are as common as aboveground induced responses. Consequently, it is expected that there are volatile markers compounds that differentiate a non infested plant from an infested one. We have studied the emissions induced in the roots of Black Mustard (*Brassica nigra*) by the cabbage root fly (*Delia radicum*) larvae. From our measurements, we can conclude that there is specific marker that would differentiate a non infested plant from an infested one (Figure 1). Further GC-MS analysis will reveal the exact nature of this compound.

The next question to be addressed is: are those markers specific for this interaction, or could we classify them, similar to C6 compound emitted after leaf wounding as “root wound compounds”?

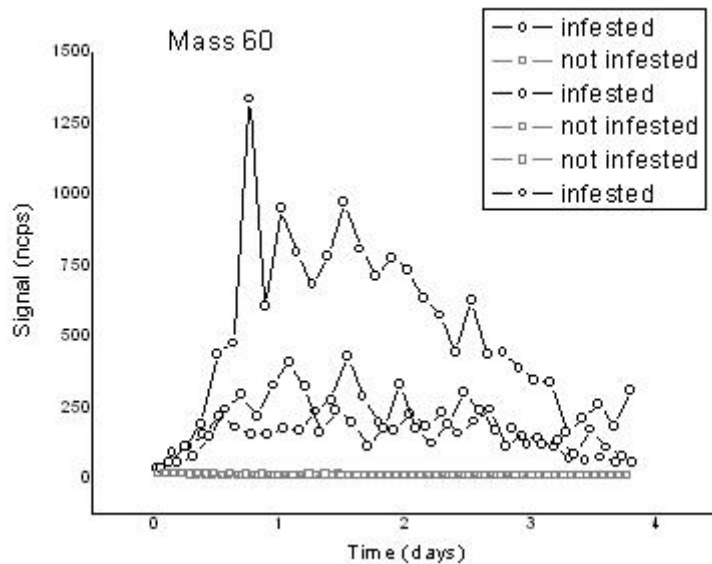


Figure 1: Evolution of the root emissions for m60 in *Brassica nigra*; a clear difference between the plants infested with *Delia radicum* and the non infested ones is observed.

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MS/MS studies of biogenic VOCs using a PTR-Linear Ion Trap and a Townsend Discharge Triple Quad MS

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Abstract

Proton Transfer Reaction Mass Spectrometry (PTR-MS) allows for quantitative analysis of VOCs in real time at low concentrations, but cannot differentiate isomers or isobaric molecules. The problem for separating isobaric molecules is solved for a lot of compounds by coupling the PTR source to a high mass resolution Time of Flight MS (PTR-TOF, [1]). Recently, the ability to conduct MS/MS experiments to separate even isomers using the PTR source has been developed. Prazeller et al. [2] and Warnecke et al. [3] described the development of a PTR Ion Trap Mass Spectrometer (PIT-MS), enabling a CID-based detection of isomeric VOCs. Here we present first results of a triple quadrupole MS (QqQ-MS) and a linear quadrupole ion trap (PTR-LIT, [4]) in combination with H₂O chemical ionization to provide the advantages of identification capability from MS/MS for investigation of biogenic volatile organic compounds (BVOC).

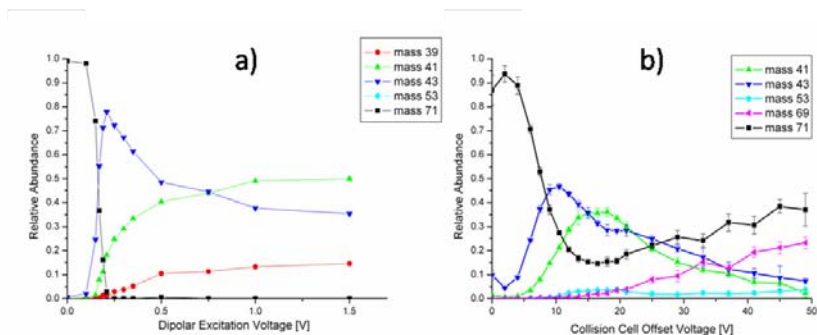


Figure 1: CID fragmentation pattern of protonated methacrolein ($C_4H_7O^+$) measured a) with the PTR-LIT, b) with the QqQ-MS.

The PTR-LIT instrument couples a PTR ion source to a linear ion trap using helium as a trapping and collision induced dissociation (CID) gas. Protonated volatile organic compounds are accumulated in the trap up to a few seconds. After the filling time, ions are isolated and

manipulated using the technique of dipolar excitation. With the PTR-LIT, multi MS (MS^n) is possible with almost unit trapping efficiency. Therefore fragment ions can be isolated and fragmented further with almost no loss in sensitivity.

The Varian MS-320 QqQ-MS was configured with a H_2O Townsend discharge ion source as a source of ions. The QqQ-MS has two quadrupole mass filters connected by a 180° curved collision cell filled with argon as a CID gas. The first quadrupole is used for preselecting the ions. Afterwards, these ions are fragmented in the collision cell. The resulting fragmentation pattern is recorded using the last quadrupole mass filter.

During laboratory comparison of the two instruments CID patterns of six monoterpenes, two sesquiterpenes, methyl vinyl ketone (MVK) and methacrolein (MACR, figure 1) were analyzed. QqQ-MS results will be compared with the PTR-LIT instrument. Characterizations of both instruments such as limits of detection, sensitivities and identification abilities will be discussed.

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Chemical Ionisation in Compact FTICR Mass Spectrometers

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Abstract

COV detection in small FTICR instruments

FTICR instruments based on permanent magnets have a mass range (10 to 300, extendable to 500) well adapted to COV detection. Their resolution (100.000 @ 100 Dalton) enables the differentiation of compounds with the same nominal mass but differing by their molecular formula (such as butene C₄H₈/acrolein C₃H₄O).

Up to now we have mainly been working with instruments where the magnet assembly is an Halbach cylinder, producing in its center a magnetic field perpendicular to the cylindrical magnet assembly main axis. All the different events are performed in the ICR cell : formation of the ionic precursor, mass selection, reaction with the sample, detection and ejection of all the ions before the next sequence.

Gases are pulsed in the main chamber housing the ICR cell, reaching a maximum pressure adjustable between 10⁻⁷ and 10⁻⁴ mbar. The degree of conversion of the ionic precursor reacting with the sample is controlled either by the pressure or the pulsed valve opening time.

The timing is mainly limited by the gas pumping time, with a pressure decay time constant of 200 ms. The typical duration of a sequence is one second.

Compared to flow tube instruments compact FTICR mass spectrometers have a more limited sensitivity in direct injection mode, starting at about 1 ppm. However it is possible to measure concentrations up to 100% by adjusting the reaction time in order to maintain the conversion of the precursor ion in a range where secondary reactions can be neglected. Traces in the ppb range are accessible when using on-line concentration with membranes (at the expense of a somewhat longer time for the analysis). Analysis can be switched from one sequence to another, from a direct injection to a membrane concentration line or from the use of one precursor to another.

From traces to 100%

The detection dynamic, defined as the ratio of the highest peak intensity to the lowest detectable peak in a given mass spectrum is inherently limited in a low field Penning Trap to about 1000 (and somewhat higher when averaging many spectra). The limitation comes from the maximum number of ions that can be stored in the trap before distortions due to space charge begin to occur (about 10⁵) and the number of ions at a given mass necessary to give a detectable image current (about 100).

This limited dynamic makes the detection of trace compounds impossible when using electron ionization since the huge amount of ions produced from the main components hides the detection of those in small concentration.

We use selective chemical ionization, thus ionizing only the species we want to quantify and identify. The lowest detectable concentration limit is related to the number of non reactive collisions possible in the ICR cell without losing the ions by diffusion. With reaction rate constants of the order of $10^{-9} \text{ cm}^3 \text{ s}^{-1}$ this corresponds to about 1ppm. There is merely no high concentration limit since it is possible to change the reaction time (which in our case is controlled by the pulsed valve opening time). The reaction time is always set so as to keep the conversion of the precursor lower than 30%. In this way secondary reactions can be neglected when calculating the concentrations of the VOCs present.

Switching between different precursors

Fast switching between different precursors is very easily realized. For example it is possible to use in successive sequences chemical ionization by proton transfer with precursors of different proton affinity, with H_3O^+ for broad range VOC detection and protonated dimethyl ether for more selective detection of aldehydes in the presence of aromatic compounds in high concentrations.

Detection of alkanes and CFCs

Alkanes, Chloro Fluoro Carbons (CFCs) and Hydrogenated Chloro Fluoro Carbons (HCFCs) cannot be detected by proton transfer from H_3O^+ ions. As an example of precursor ions allowing their detection we will show results obtained with CF_3^+ and CF_2H^+ . These precursors can easily be formed from CF_4 or CF_3H gases. As they do not react with the main components of air they are potential precursors for selective chemical ionization in air. Their reactivity with alkanes and CFCs and HCFCs have been studied.

A comparison will be made between the use of O_2^+ for the detection of CFCs.

The reactivity of alkanes from C1 to C8 with both precursors CF_3^+ and CF_2H^+ will also be discussed.

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Measurements of volatile reduced nitrogen compounds with PTR-MS

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Abstract

A PTR-MS was operated using O_2^+ as primary ion with the aim to measure ammonia and selected amines. Tests in the laboratory documented that fast concentration changes of ammonia could not adequately be resolved by the PTR-MS instrument. In addition, atmospheric humidity affected both the level and the temporal behavior of the ammonia signal at m/z 17. A new drift tube recently developed by IONICON was installed and its performance with respect to ammonia is currently being investigated. First results indicate that the time response could not significantly be improved but part of the previously observed humidity interferences have disappeared.

Introduction

The major part of nitrogen transfer between the atmosphere and the biosphere occurs through the exchange of reactive nitrogen compounds. Among them are alkaline gases like ammonia and amines. Atmospheric ammonia deposition represents an important fraction of nitrogen input for terrestrial ecosystems and ammonia plays an important role in aerosol formation. Despite this environmental relevance, many uncertainties on the sources and the behavior of atmospheric ammonia persist, partly because of the challenging nature of ammonia measurements. Measurements of reduced nitrogen compounds other than ammonia are still scarce, although recent observations of aerosol compositions suggest a potentially important role of amines in new particle formation events.

As part of the EU's sixth framework research project NitroEurope (www.nitroeuropa.eu), methods are being developed to quantify the exchange fluxes of various forms of reactive nitrogen compounds over selected ecosystems. Within the experimental activities at the NitroEurope super site Oensingen (CH), we investigate the potential of PTR-MS for measurements of ammonia and selected amines. While the capability of PTR-MS to measure ammonia has recently been demonstrated [1, 2], we conducted several experiments to explore the performance of PTR-MS for simultaneous measurements of ammonia, dimethyl- and trimethylamine at high sensitivity and time resolution.

Experimental Methods

A PTR-MS was operated using O_2^+ as primary ion applying the same conditions as described by Norman et al. [1], i.e. at 450V drift tube voltage ($E/N \sim 110$ Td). A permeation source was used to generate constant concentrations of ammonia and trimethylamine in the order of tens of ppb at varying levels of humidity. The laboratory setup also allowed switching instantaneously from humid to dry air while keeping the ammonia concentration constant.

Results and Discussion

Ammonia was detected as m/z 17, major product ions of trimethylamine (molecular mass 59 g/mole) were m/z 59 and m/z 58, consistent with previous findings of Spanel and Smith [3] in SIFT experiments. Fast concentration changes of ammonia and trimethylamine under dry conditions could not perfectly be resolved by the PTR-MS instrument (Figure 1) and instrumental time response was negatively affected by humidity. Various inlet designs were tested without significant effects on the time response. A slight improvement could be achieved by increased heating of the drift tube, suggesting that a major damping of the time response occurred through adsorption effects inside the drift tube.

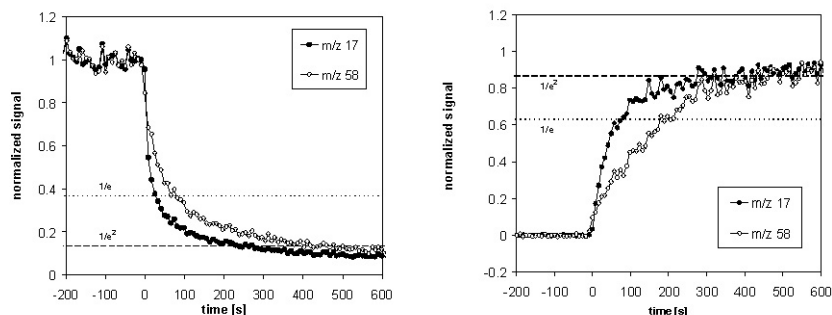


Figure 1: Switches between dry air containing ammonia / trimethylamine (approx. 80 ppbv) and zero air.

A new drift tube recently developed by IONICON [4] was installed and its performance with respect to ammonia is currently under investigation. First results indicate that the instrumental time response for ammonia has not been improved by the new drift tube design. However, a clearly different behavior was observed when switching from dry to humid air containing ammonia. Previously, the addition of humidity (resulting dew point $\sim 10^\circ\text{C}$) caused a short-time increase of the m/z 17 signal followed by a quasi-exponential decay. Subsequently, the m/z 17 signal resided higher than under dry conditions, which is interpreted as NH_3^+ production in the ion source because of back diffusion of H_2O (and N_2) from sample air. With the new drift tube (using the identical setup for generating the ammonia concentrations/humidity switches), this temporary increase of the m/z 17 could not be observed anymore (Figure 2).

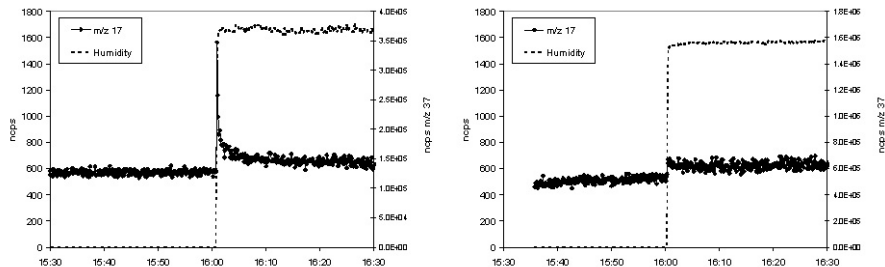


Figure 2: Switches between dry and humid air with the same NH_3 concentration – different behavior of instrument with conventional (left panel) and new drift tube (right panel).

We speculate that the previously observed peak was a consequence of the competition between H_2O and NH_3 molecules for surfaces suitable for adsorption. It appears that the new drift tube has better properties in terms of such adsorption effects. On the other hand, the overall still insufficient time response for ammonia measurements requires further investigations on the mechanisms of ammonia adsorptions in the drift tube or inlet. The time resolution for ammonia and the investigated amines achieved so far is still far away from the necessary performance for potential use in direct flux measurement methods.

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Development of Laser Desorption-Proton Transfer Reaction-Mass Spectrometry for Biomolecule Detection

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Abstract

We recently developed Laser Desorption Proton Transfer Reaction (LD-PTR) Mass Spectrometry (MS), a technique combining with Laser Desorption method (LD) and Proton Transfer Reaction Mass Spectrometry (PTRMS). The laser desorbed compounds, especially nonvolatile biomolecules, were collided with the reagent ions, e.g. $H+(H_2O)_n$ ($n=1-6$), and ionized via proton transfer reactions. Protonated intact molecules were dominant in the reaction of water cluster ions due to the reduced reaction exoergicity. Mass analysis was carried out by using a linear time-of-flight mass spectrometry. LD-PTR-TOF-MS has two features: examining the laser desorbed neutral compounds, and extending the application of PTRMS to nonvolatile compounds on surface. In this work, we give a clear demonstration of protonation of a biomolecule in gas phase. Development of this novel methodology and some preliminary results are presented.

Introduction

Over the last few decades, many advances have been made in the technique to obtain mass spectra of nonvolatile compounds, e.g. fast atom bombardment (FAB)¹, electrospray (ESI)² and MALDI^{3, 4}, which have contributed notably to researches in medical and biological chemistry. However, most of these methods are still subjected to some crucial problems, such as the fragmentation of hot parent ions and inefficient ionization. In other words, a softer and more efficient desorption/ionization method is still critically needed. Besides, owing to the interferences between components in condensed phases, the conventional methods are not suitable for quantitative analysis. Proton transfer reaction mass spectrometry (PTR-MS)^{5, 6} has been developed for more than ten years to detect volatile organic compounds (VOCs). In most cases, hydroxonium ions, H_3O^+ , were preferentially employed as the primary reagent ions, and they were utilized to ionize the VOCs via proton transfer reactions in a flow-drift-tube (FDT). This technique allows quantitative on-line monitoring of VOCs, so that it has been widely applied in the fields of environmental sciences⁷, food technology⁸ and medicine research⁹. In our laboratory, we coupled PTR-MS with a laser desorption (LD) neutral beam source for the first time to establish a laser desorption-proton transfer reaction time-of-flight mass spectrometry (LD-PTR-TOF-MS). The technique led to the sensitive detection of nonvolatile compounds on surfaces, especially for the biological molecules.

Experimental Methods

A home made hollow cathode¹⁰ ion source was utilized to generate the reagent ions. In this work, $\text{H}^+(\text{H}_2\text{O})_{1-6}$, were produced and were extracted into the source chamber (30 L) for consequent PTR, as is shown in Figure 1. The pressure of the source chamber was maintained at 1×10^{-3} torr by two turbo-molecular pumps (one with a pumping speed of 300 L/s (SEIKO SEIKI STP 400), and another one with 150 L/s (Oerlikon Leybold TMP151)). The gas pressure of PTR region (drift region), located right in front of the ion source exit, was estimated at roughly 0.1 torr. When the laser (355nm, third harmonic wavelength of Nd-YAG laser) irradiated the sample deposited on the sample plate near to the exit of ion source, laser-desorbed plume would blend into the buffer gas in the PTR region. The ratio of electric field-to-number density of buffer gas (E/N value) could be adjusted by the drift electrode, thus the drift velocity (v_d) of the ions could be optimized for ion-molecular reactions at low collision energy region.

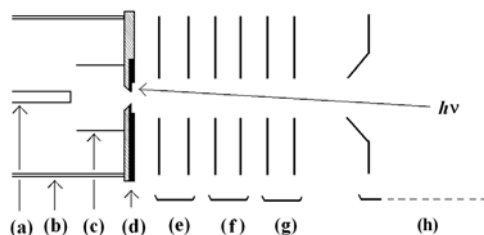


Figure 1: The hollow cathode ion source: (a) anode tube; (b) Pyrex tube; (c) hollow cathode; (d) the stainless steel sample mount and the replaceable sample plate; (e) drift and acceleration electrodes; (f) deceleration and pulsed extraction electrodes; (g) ion lens; (h) two stage differentially pumped TOF MS; hv: desorption laser.

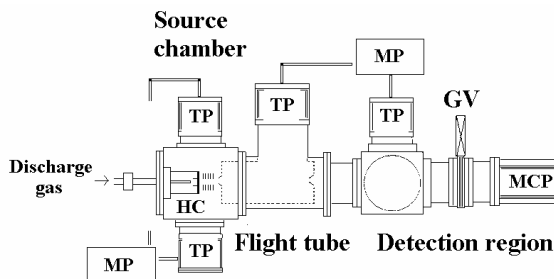


Figure 2: The whole view of the LD-PTR-TOF-MS instrument, which consists of three major parts: the source chamber, the flight tube, and the detection region. (TP: turbo pump; MP: mechanical pump; GV: gate valve; HC: hollow cathode; MCP: multichannel plate.)

In the end of the PTR region, ions were extracted by a static field toward an extraction region, where a pulsed voltage (positive 6-20 kV) was utilized to extract the ions into a two-stage differentially pumped linear time-of-flight mass spectrometry (TOFMS) as shown in Figure 2

(whole view). The TOF region behind a first skimmer (with an opening of $\phi 6$ mm I.D.) of the source chamber was evacuated to a pressure of 1×10^{-5} torr by a turbo-molecular pump (Turbovac 360, Leybold GmbH, Germany). The detection chamber located after the TOF region was differentially pumped through a second skimmer ($\phi 8$ mm I.D.) and was evacuated by another turbo-molecular pump (Turbovac 360, Leybold GmbH, Germany) to below 5×10^{-6} torr. A microchannel plate (MCP) (C-70125, Jordan TOF Products, Inc, USA) was installed at the end of the detection chamber to detect ions, and the signals was recorded by an oscilloscope (WaveRunner, Lecroy Corporation, Chestnut Ridge, USA). The total flight distance was 1.0 meter long.

Results

Angiotensin I (MW=1296) is one of the most extensively studied biomolecules by mass spectrometry¹¹ and was used in this work. To prepare the sample, 5 μ L of a 0.005 M angiotensin I solution (in 50% acetonitrile aqueous solution) and 15 μ L of a 0.05 M α -cyano-4-hydroxycinnamic acid (4-HCCA) solution (in 50% acetonitrile aqueous solution) were mixed thoroughly before sample deposition. After mixing, 1 μ L of the above mixture was deposited on the sample plate and was air-dried. 4-HCCA was used with the analyte because it absorbs laser energy efficiently and disintegrated into small fragments including molecules and ions, such reaction exerted mechanical force to push angiotensin I into the gas phase. The ions generated from matrix-assisted-laser-desorption/ionization (MALDI) mechanism or laser-desorption/ionization (LDI) could be distinguished from those of PTR by applying ion extraction pulse at various extraction delay. In comparison with MALDI and LDI at the same sample conditions, the PTR produced better signal intensities and exhibited negligible fragmentations. Figure 3 shows the LD-PTR mass spectra of angiotensin/4-HCCA (1:30) by using $H^+(H_2O)_{1-6}$ as the reagent ions and H_2O as buffer gas in the mass range from 0 to 2000 amu.

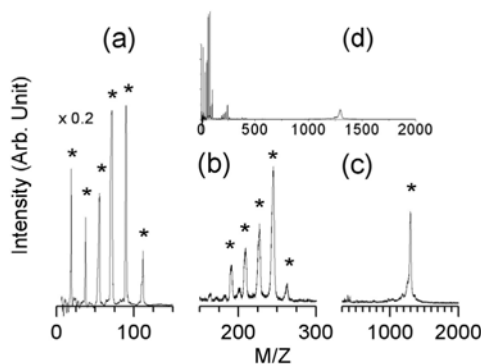
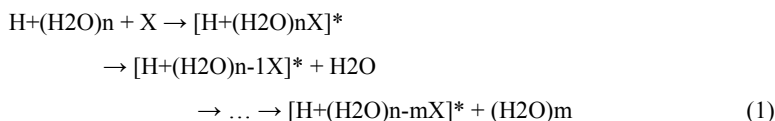


Figure 3: The LD-PTR mass spectrum of angiotensin/4-HCCA (1:30) by $H^+(H_2O)_n$ ($n=1-6$) as reagent ions, and H_2O as buffer gas, in the mass range of 0-2000 m/z , is shown and divided into three sub-panels in different m/z -scale. The *-labeled peaks in (a) panel are the reagent ions, $H^+(H_2O)_n$, where $n=1$ to $n=6$ from left to right. The intensity of signal in panel (a) is reduced by multiplying a factor of 0.2. In (b) panel, the *-labeled peaks are $[4\text{-HCCA}+H^+(H_2O)_n]$ ($n=1-4$) from left to right. In (c) panel, the signal from protonated angiotensin is *-labeled. In (d) panel, the original mass spectrum is shown.

A clear distribution of reagent ions, $H^+(H_2O)_{1-6}$, are shown in Figure 3(a), and it is peaking at $n=5$ and 4. these cluster sizes were reported previously as the most reactive species for protonation among water cluster ions ($n=2-6$) interacting with acetone and dimethylsulfoxide¹²⁻¹⁴. Figure 3(b) shows the PTR mass spectrum in the range of 125-300 m/z , wherein the adduct formation of $[4\text{-HCCA}+H^+(H_2O)_n]$ ($n=0-4$) were clearly seen and those with $n=3,2$ were most populated. This result may imply that the $H^+(H_2O)_n$ with $n=5,4$ were the major reagent ions and the reaction were through the following process with an evaporation of 1-2 water molecule(s) from the intermediate reaction complex:



where X denotes reactant. With a resolution of $m/\Delta m=130$, signal from protonated angiotensin is shown in Figure 3(c) without water-adduct formation, revealing that angiotensin I has higher proton affinity than water clusters so that the following direct proton transfer reaction can proceed.

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New Application of PTR-MS for OH Reactivity Measurements in the Atmosphere

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Abstract

Hydroxyl (OH) radicals are the most important atmospheric oxidant and enable the atmosphere to cleanse itself of various gaseous pollutants. Currently the overall OH atmospheric sink which is also called the total OH reactivity, is poorly quantified. Using a PTR-MS as a detector coupled to a turbulent flow glass reactor, a new analytical technique for direct online measurements of OH reactivity is presented. This technique employs the comparative reactivity kinetics approach. Pyrrole, which is not normally present in air, is passed through a glass reactor and its concentration is monitored with a PTR-MS. OH radicals are then introduced in the glass reactor at a constant rate to react with pyrrole, firstly in the presence of zero air and then in the presence of ambient air containing OH reactants. Comparing the amount of pyrrole exiting the reactor with and without the ambient air allows the air OH reactivity to be determined. The present dynamic range for ambient air reactivity is about 6 to 300 s⁻¹, with an overall maximum uncertainty of 20% above 8 s⁻¹ and up to 40 % between 6 - 8 s⁻¹. Results are presented from an urban environment. Field deployment as a more economical and portable alternative to the existing laser induced fluorescence based OH reactivity measurement systems are also discussed.

Introduction

Photochemical reactions initiated by the hydroxyl radical transform gaseous pollutants such as carbon monoxide (CO), sulphur dioxide (SO₂), nitrogen oxides (NO_x = NO and NO₂) and VOCs (Volatile Organic Compounds) into forms, which are more readily removed from the atmosphere by deposition or formation of aerosol [1]. In order to understand the hydroxyl radical (OH) budget in the atmosphere and constrain photochemical models an accurate measurement of the OH reactivity (sink term) is essential. Often, the overall sink term is estimated by calculating OH loss frequencies (product of concentration and rate coefficient) for all individually measured species and summing them. Thus, the OH reactivity) of a chemical is given by

$$\text{OH reactivity of reactant X (s}^{-1}\text{)} = k_{(X + OH)} [X] \quad (1)$$

where $k_{(X+OH)}$ is the rate coefficient for the reaction of X with OH.

However, it is not certain whether all relevant OH reactive species are measured by the suite of measurement techniques deployed in current field studies [2]. A direct measurement of the OH reactivity is therefore a very important tool to assess the missing OH sink when compared with the total sink due to measured VOCs and other OH reactants. Moreover it serves to accurately constrain the OH sink in photochemical models.

We measure the OH reactivity of the atmosphere directly, using an in situ kinetics approach called the comparative reactivity method [3], which employs a PTR-MS as a detector. This is a new atmospheric application of the PTR-MS instrument as previous PTR-MS atmospheric applications have been mainly focussed on measurements of VOCs and VOC fluxes [4].

Experimental Methods

Figure 1 illustrates how the experiment is conducted. Pyrrole (C_4H_5N) is introduced into a glass reactor and its concentration C_1 is monitored with a PTR-MS, in the air exiting the reactor. After some time when C_1 is well determined, synthetically generated OH radicals ($OH \ll [Pyrrole]$) are introduced into the reactor at a constant rate to react with pyrrole. This causes C_1 , the monitored concentration of pyrrole, to decrease to C_2 , as pyrrole reacts with the OH radicals. The decrease in the monitored concentration of pyrrole (from C_1 to C_2) also gives the initial concentration of the OH radicals, as all the OH is completely titrated by pyrrole. Next, an air sample containing reactive species is introduced into the glass reactor. The various species present in ambient air then compete with pyrrole for the available OH radicals, so that the concentration of pyrrole in the air exiting the reactor increases to C_3 . Comparing the amount of pyrrole exiting the reactor without (C_2) and with the ambient air (C_3) allows the introduced air sample's OH reactivity to be determined in a quantitative manner, provided the system is suitably calibrated. In principle any OH reactant can be employed in this method. However there are certain practical considerations that make pyrrole a very attractive choice. Firstly most ambient air VOCs that are detectable with a PTR-MS, land at odd masses after undergoing protonation (e.g. methanol; $m/z = 33$, acetone $m/z = 59$), while pyrrole contains a nitrogen atom and hence is detected after protonation within a PTR-MS at an even mass ($m/z = 68$), ruling out potential interferences. The rate coefficient of pyrrole with the hydroxyl radical is well known (1.2×10^{-10} molecules $cm^{-3} s^{-1}$), which is important for converting the measured signals into the measured OH reactivity as under:

$$R_{air} = \frac{(C_3 - C_2)}{(C_1 - C_3)} \cdot k_p C_1 \quad (1)$$

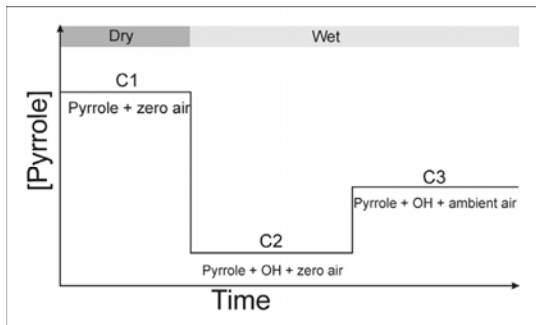


Figure 1: Schematic of Comparative Reactivity Method

Figure 2 shows a diagram of the glass reactor used along with its inlets and outlets labelled as arms A, B, C, D and E. Gas phase pyrrole is mixed with zero air and introduced through inlet A at a constant flow. Its concentration is monitored in the air exiting the reactor (outlet D) with a PTR-MS. Inlet B consists of a pen ray spectral mercury vapour lamp, over which humidified nitrogen / nitrogen is passed at a constant flow rate. The humidification is accomplished by bubbling gaseous nitrogen through water, which is maintained at room temperature (298 K). When the lamp is switched on, OH radicals are produced due to photolysis of the water vapour (at $\lambda = 184.9$ nm) present in the humidified nitrogen. This method of producing OH radicals has been used extensively in gas phase kinetic studies, including calibration of OH measurement instruments, and for more details the reader is referred to Heard and Pilling [5] and references therein. The tapered arm E is a Wood's horn which minimizes photochemical reactions along the length of the glass reactor. Outlet C is connected to an exhaust pump.

To sample ambient air for reactivity, the zero air is switched off and an equivalent amount ($130\text{--}150\text{ ml min}^{-1}$) of ambient air is pumped in, using a Teflon VOC sampling pump (Laboport N86-KN18; at arm A). This causes dilution of the ambient air within the reactor, and the dilution factor has to be taken into account when determining the total OH reactivity of the introduced ambient air. It is worth mentioning that the ambient air is not subject to any gas chromatography column, preconcentration step or laser excitation and its reactivity is directly converted into a modulation of the pyrrole signal so that any potential losses of VOCs and/or associated artefacts are minimised.

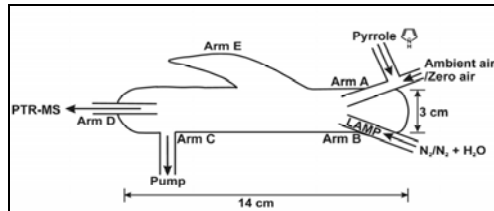


Figure 2: Design of the Glass Reactor

Results and Discussion

Figure 3 shows the diel OH reactivity profile for Mainz air, measured with the CRM technique from 18 - 20 August 2005. Ambient air was sampled outside our laboratory ($49^{\circ}59'N$, $8^{\circ}14'E$) at the Max Planck Institute for Chemistry in Mainz, circa 8 m above the ground. Just outside the laboratory there is an undergrowth of bushes and plants. The average value of the total OH reactivity of Mainz air was $\sim 10.4\text{ s}^{-1}$. OH reactivity was observed to be highest during the afternoon (13:00 L.T.), reaching peak values of $\sim 18 \pm 4\text{ s}^{-1}$, while lowest values ($\sim 6 \pm 3\text{ s}^{-1}$) were observed early in the morning between 2:00 to 4:00 L.T. The total OH reactivity measurements for Mainz air lie well within the range of total OH reactivity measurements reported in literature for urban air sites [3].

Applying the Comparative Reactivity Method (CRM) concept to the reagent and detector system of pyrrole and a PTR-MS, respectively, a new online measurement technique with a dynamic range of about 6 to 300 s^{-1} for ambient air and accuracy of $\pm 20\%$ has been developed. This makes it a method of choice for measurements of ambient air OH reactivity in regions of high OH reactivity such as tropical forests, where practical considerations have deterred deployment of LIF instruments. The system has also been tested for potential interferences such as NO and changing ambient humidity and with diverse air samples of known reactivity, with excellent results [3]. By combining the advantages of a PTR-MS such as its portability, cost (compared to laser based OH reactivity instruments), fast time response and soft chemical ionization for the reagent molecule pyrrole, which ensures detection without fragmentation of pyrrole, a promising new addition to atmospheric OH reactivity measurement instruments has been accomplished.

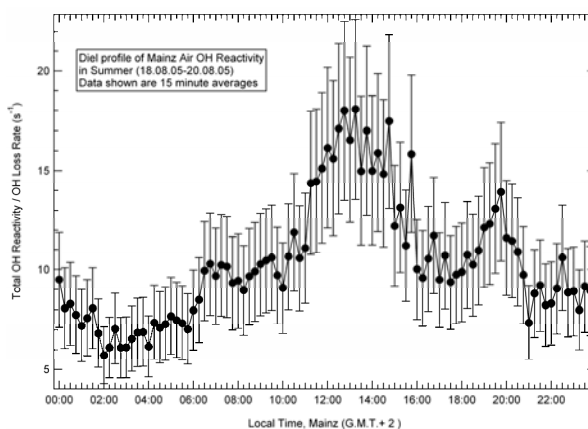


Figure 3: Diel mean profile (black circles) of the total OH Reactivity of Mainz (urban site) air measured during summer (August 2005) with the CRM instrument.

Acknowledgements

Special thanks to Sonja Jung for helping with her expertise in glass blowing and furnishing the requested myriad glassware and reactors. V. Sinha acknowledges the financial support rendered by the Max Planck Society. We thank all the members of the ORSUM group, in particular Thomas Kluepfel and Tom Custer who have facilitated the successful accomplishment of this work.

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Inter-comparison of formaldehyde measurement by PTRMS and the Hantzsch monitor over a wide range of humidity

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Abstract

PTR-MS detection of formaldehyde is hampered by the humidity dependence of instrument sensitivity. In this study we suggest a method to correct the PTR-MS signal according to concentration of water vapor in sampled air. The results of the correction are validated by inter-comparison of the PTR-MS and the Hantzsch monitor field measurements.

Introduction

Formaldehyde is an important atmospheric constituent which can be emitted directly or produced in-situ via oxidation of hydrocarbons. It is one of the most abundant volatile carbonyls in the boundary layer and HCHO measurements can provide useful information about photochemical activity in ambient air, given that it is formed via numerous oxidation processes.

Proton transfer mass spectrometry (PTR-MS) is an on-line technique that allows measurement of VOC at trace levels with a rapid time response. Hansel et al. [1] reported earlier a complication associated with formaldehyde detection by PTR-MS:



Due to a low proton affinity, the HCHO protonation R1 is reversed via R2 so that the instrument response becomes strongly dependent on water vapor concentration in the PTR-MS drift tube. Steinbacher et al. [2] reported a significant discrepancy between HCHO measurements by the Hantzsch monitor and PTR-MS. In a recent report Inomata et al. [3] suggested a method to correct the PTR-MS sensitivity with respect to sample air humidity. In this study we extend this approach and propose the correction which can be applied over a wider humidity range. The results are discussed by comparing the ambient formaldehyde measurements by PTR-MS and the Hantzsch monitor.

Experimental Methods

The PTR-MS used in this work was a commercial IONICON high sensitivity instrument. Typical conditions of operation were: drift tube pressure 2.2 mbar, water flow through ion source 6 sccm, reagent ion signal (m/z 21) 10^4 cps, inlet and drift tube temperature 45°C. The concentration of formaldehyde was calculated using signal at m/z 31. Background signal was measured by removing VOCs by passing sample air through charcoal cartridge or heated platinum wool. The

sensitivity factor was determined in two ways: by using a literature value of the proton-transfer reaction constant of R1 [1] and, experimentally, using a calibrated HCHO permeation source.

The concentration of formaldehyde in ambient air was also measured using a homemade instrument based on the fluorescence technique [4]. The measurement principle is similar to that of the Hantzsch monitor. The instrument was calibrated daily with liquid standards. Additionally, the sampling efficiency was tested using the permeation source.

Both instruments were deployed during field studies at Egbert, Canada (rural site) in May-June 2007 and at Whistler, Canada (mountain site) in May-June 2008.

Results

An excellent agreement between measurements by PTR-MS and the Hantzsch monitor was observed in the calibration laboratory experiment using the HCHO permeation source and synthetic air as carrier gas. Sample flow was very dry and one may expect the highest HCHO sensitivity of PTR-MS under such conditions. The sensitivity determined in this experiment was found to agree very well with the value calculated using a literature value of the proton-transfer reaction constant of R1.

The effect of water vapor on sensitivity can be calculated by solving the kinetics for the two reactions, R1 and R2. There is an analytical solution which can be simplified:

$$\text{sensitivity} = \text{sensitivity}(\text{dry}) \{1 - \exp(-k_{R2}[\text{H}_2\text{O}]t)\} / k_{R2}[\text{H}_2\text{O}]$$

where t is reaction time in the drift tube, $[\text{H}_2\text{O}]$ is water vapor concentration and k_{R2} is the R2 reaction constant.

The result of the correction is shown in Fig.1, where the regression slopes get closer to 1:1 lines as a result of humidity adjustment. Meteorological data were available during both field studies so that H_2O ambient concentration was calculated from relative humidity and air temperature.

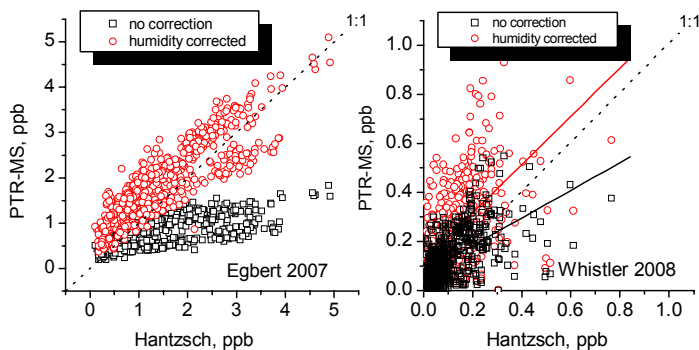


Figure 1: Intercomparison of formaldehyde detection by PTR-MS and Hantzsch monitor made for Egbert-2007 and Whistler-2008 field datasets.

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6. Contributed Papers (Posters)

Investigations on the influence of drift field and humidity on sesquiterpene product ion distributions in a PTR-MS instrument and implications for sesquiterpene detection sensitivity.

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Abstract

The effects of the ratio of the electric field strength to the buffer gas number density (E/N) in the drift tube of a commercial high sensitivity PTR-MS (Ionicon Analytik GmbH) on the product ion distributions of the sesquiterpenes β -caryophyllene, α -humulene, α -cedrene and longifolene have been investigated. Product ion distributions of α -cedrene and longifolene have also been determined at different water vapour pressures in the inlet line.

Chemical ionization of the sesquiterpenes resulted in important fragmentation of the nascent excited ion/molecule complex at the highest E/N values. The yield of the protonated molecule increased on average by a factor 1.6 by decreasing E/N from 140 to 80 Td. Taking into account the influence of E/N on the reaction time and on the reactant ion mobility, it is estimated that this decrease in E/N may lead to an overall increase in the PTR-MS detection sensitivity of sesquiterpenes (based on the ion signal at m/z 205) by a factor 3.5.

This increase in detection sensitivity was confirmed through branch enclosure PTR-MS BVOC flux measurements on a beech tree in natural conditions in the framework of the IMPECVOC project [1].

Introduction

Terrestrial vegetation is known to be an important source of non-methane volatile organic compounds (NMVOC, about 1150 Tg C yr⁻¹ worldwide). Among these biogenic VOCs isoprene (C₅H₈) and monoterpenes (C₁₀H₁₆) are generally found to have the highest emission rates and therefore they have received a lot of attention in the past. However, oxygenated BVOCs and sesquiterpenes (C₁₅H₂₄) are also known to be emitted directly by vegetation in non-negligible amounts. Quantification of sesquiterpene emissions is an experimentally difficult task because of the low vapor pressure of these compounds and their ability to react very rapidly with atmospheric oxidants (mainly O₃), which results in atmospheric lifetimes of only a few tens of seconds for some compounds. Because of their high potential to contribute to net oxidant and secondary organic aerosol (SOA) formation and in order to decrease the uncertainties on regional

and global sesquiterpene emissions, there is a growing need for more precise and reliable sesquiterpene flux data using standardized experimental protocols [2].

Important efforts have been carried out lately to develop and improve analytical techniques for sesquiterpene detection and quantification [3]. Proton Transfer Reaction Mass Spectrometry instrumentation (PTR-MS) has also been used recently to measure sesquiterpene concentrations, e.g. in smog chamber studies focusing on sesquiterpene ozonolysis [4] and in VOC emission studies from herbivore infested branches [5].

In order to optimize sesquiterpene quantification with a PTR-MS it is interesting to know how sesquiterpene product ion signals vary with changing instrumental and environmental parameters.

Experimental

The experiments were carried out with a hs-PTR-MS instrument (Ionicon Analytik GmbH). The drift tube pressure and temperature were kept at 2.2 mbar and 333 K respectively. The contribution of O_2^{+} impurity ions to the sum of reactant ions was limited to 2 %. Stable sesquiterpene flows were produced by sending high purity nitrogen (40 sccm) over the liquid sesquiterpene contained in a glass reservoir, which was completely immersed in a temperature controlled water bath [6]. The resulting sesquiterpene/ N_2 flow was further diluted in a large (1000 sccm) laboratory air flow, scrubbed from dust, ozone and VOCs and humidity controlled by means of an LI-610 dew point generator (LI-COR). Part of this diluted flow was introduced into the PTR-MS through a heated PEEK capillary (333 K). PTR-MS mass discrimination measurements were performed regularly by using a commercial dilute mixture of a set of aromatic compounds (RESTEK #34432-PI).

Branch enclosure measurements were carried out on a 85 years old *Fagus sylvatica* L. tree in the framework of the IMPECVOC project [1]. Ambient air, scrubbed from ozone, dust and VOCs was sent into the enclosure, in which it was enriched with biogenic VOCs. Part of the air leaving the enclosure was pumped towards the PTR-MS inlet through slightly heated insulated Teflon tubing. At the end of June 2008, when some branches were visually infected with aphids, sesquiterpene ion signals were continuously monitored at m/z 205 and m/z 149.

Results and discussion

Sesquiterpene product ion distributions were obtained at E/N values ranging from 80 to 140 Td, in steps of 10 Td. For all four sesquiterpenes studied, the major product ion is the protonated molecule with yields ranging from 30 to 65% at the highest E/N values. The nascent excited complex, that is formed upon reaction of the proton hydrates with the sesquiterpenes, partially decomposes into a large variety of product ions, the distribution of which is strongly dependent on the E/N value of the drift tube region. Unfortunately, these product ions are common to all sesquiterpenes and therefore cannot be used as sesquiterpene-specific fingerprints.

Interestingly, the distributions are not affected by changing relative humidities, and therefore the sensitivity of the PTR-MS for sesquiterpene detection based on specific fingerprint ions (e.g. at m/z 205) is not expected to be humidity-dependent either.

The product ion distributions also show that one should be careful when quantifying monoterpenes in the presence of sesquiterpenes, since the typical monoterpene product ion signals (at m/z 137 and 81) are also typical PTR-MS fragment ions of sesquiterpenes.

Detailed results of these laboratory studies will be presented in [7].

The laboratory results also show that the yields of the protonated sesquiterpenes on average increase by a factor 1.6 when decreasing the E/N value from 140 Td to 80 Td. By using this information and by taking into account the E/N dependence of the reactant ion residence time in the drift tube (including variations of the ion mobility), we estimated the overall PTR-MS sensitivity for sesquiterpene detection to increase by a factor 3.5 when decreasing E/N from 140 to 80 Td.

This estimation was confirmed in a recent dynamic branch enclosure field experiment (on a *Fagus sylvatica* L. tree), during which sesquiterpene emissions were clearly noticed (see Fig. 1), probably as a result of aphid infestation. A decrease of E/N in the drift region of the PTR-MS analyzer from 140 to 80 Td resulted in an increase of the sesquiterpene ion signal at m/z 205 by a factor 3.2.

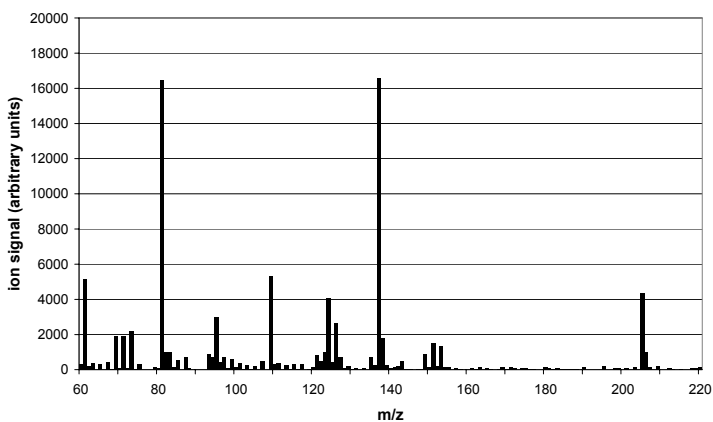


Figure 1: PTR-MS mass spectrum showing sesquiterpene emissions in a dynamic branch enclosure study on *Fagus sylvatica* L. (taking into account mass discrimination).

Acknowledgements

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PTR-MS evaluation of the effect of supercritical gas pasteurization on the volatile profile of a fresh apple juice

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Abstract

Supercritical pasteurization is receiving increasing attention as an alternative technology for foodstuff pasteurization, but often the possible effects on the perceptible quality are not sufficiently considered. PTR-MS was utilized to monitor the effect on the headspace of apple juice pasteurized with CO₂ and N₂O dense gases. The use of these gases allowed a satisfactory microbial inactivation but also brought to a reduction of volatile compounds that is proportional to the treatment time. Being volatiles directly related to odor and flavor of foodstuff their preservation must be taken into account into the optimization of pasteurization parameters and PTR-MS provides a rapid tool to control this important quality trait.

Introduction

The use of supercritical gases for foodstuff pasteurization is receiving increasing attention as an alternative technology to standard pasteurization based on thermal energy. While microbial reduction is carefully studied, often the possible effects on the perceptible quality are not sufficiently considered. A limited number of published studies focus on the sensory modifications induced by CO₂ treatment in food [1]. The effect of stabilization treatments on perceivable quality is, however, of outmost importance because it is the key factor for the consumer product acceptance. The very few data collected so far indicated that high pressure treated orange juice was almost indistinguishable from the untreated one [2]. The literature on N₂O appears even scarcer and less clear. To the best of our knowledge, no paper reports on the effect of N₂O on chemico-physical, nutritional or sensory features of foodstuff. Within a wider study to verify the impact of this pasteurization process on the chemical and sensory attributed of juices [3], we report in the present contribution the data related to volatile profile obtained by PTR-MS. Being volatile compounds responsible for odor and flavor of foodstuff, two attributes directly related to on quality traits linked to consumer perception and likeability, we monitored the effect of dense N₂O and CO₂ on the headspace of apple juice to obtain a preliminary evaluation of their sensory impact.

Experimental Methods

Juices

Freshly squeezed apple juice was produced at Macè Srl (Italy) using a blend of Golden Delicious and Granny Smith apples. The juices produced were sealed in plastic bags (1000 mL) and stored at -20°C . The day before any trial or further treatment the juice bags were thawed at 4°C (overnight). Before each experimental run, a certain quantity of the thawed juice was maintained at 4°C and not treated (Reference juice). After the treatment, reference and treated juices were stored again at -20°C until analysis.

Pasteurization

The trials were performed with the multi-batch pilot plant described in [4] utilizing CO_2 (RIVOIRA, purity 99.990%) or N_2O (RIVOIRA, purity 99.95%). All the experimental runs were carried out at constant temperature of 36°C and pressure of 100 bars since these values represent the optimal conditions to reach a satisfactory rate of inhibition in a short treatment time [4,5]. Two series of experiments were run: the first one, to evaluate the inactivation of the microorganisms initially present in the fresh juice corresponding as function of treatment time, the second one to evaluate how different treatment times (5, 10, 20, 40 min) modify the concentration of volatiles organic compounds.

Volatile monitoring

Volatile organic compounds were monitored with a commercial high sensitivity PTR-MS (Ionicon Analytik GmbH, Innsbruck, Austria). For each sample a 5 mL of juice was placed in a 120 mL silicon-septum closed glass bottle (Supelco, Bellefonte, USA) for 45 minutes in a water bath (36°C). The VOCs released into the headspace were then transferred through a heated (70°C) capillary line (uncoated deactivated fused silica tubing with an inner diameter of 0.25 mm; Supelco, Bellefonte, USA) directly into the drift tube of the PTR-MS at a rate of $10\text{ cm}^3/\text{s}$. The headspace was replaced by a flow of pure nitrogen gas (SOL s.p.a., Italy; purity: 99.999%). Details on this sampling method can be found in [6]. To avoid memory effects, the samples were measured in random order and nitrogen was flushed, for 5 min, between two consecutive samples.

Results and discussion

Figure 1 shows the effect on the total volatile of the pasteurization process operated at the optimal conditions for microbial inactivation (36°C ; 100 bar; 10 min). The averaged reduction was 38% and 31% for carbon dioxide and nitrous oxide respectively. Looking at single compounds, the highest reduction, more than 85% of the initial concentration, was observed for (E)-2-hexenal monitored at m/z 99 whose identity was ascertained by GC-MS (data not shown). In figure 2 is reported the evolution of total volatiles at different treatment time. The trend observed for the total volatiles is similar to the kinetic of microbial inactivation where depletion is function of time.

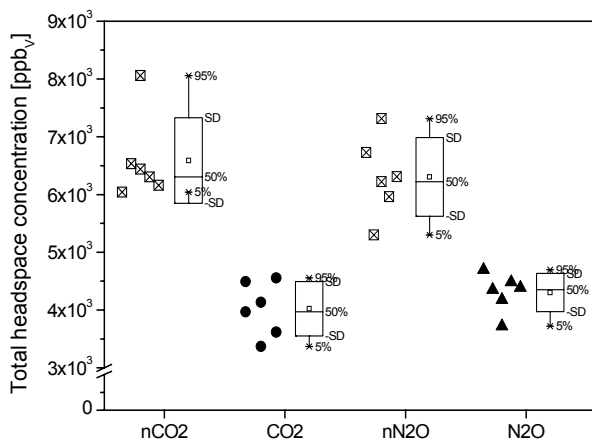


Figure 1: Effect of CO₂ and N₂O pasteurization (100 bar, 36 °C, 10 min) on head space composition. Cross centered square symbols indicate reference juices, triangles indicate N₂O treatment and circles indicate CO₂ treatment. Different batches of reference sample are reported separately (nCO₂ and N₂O).

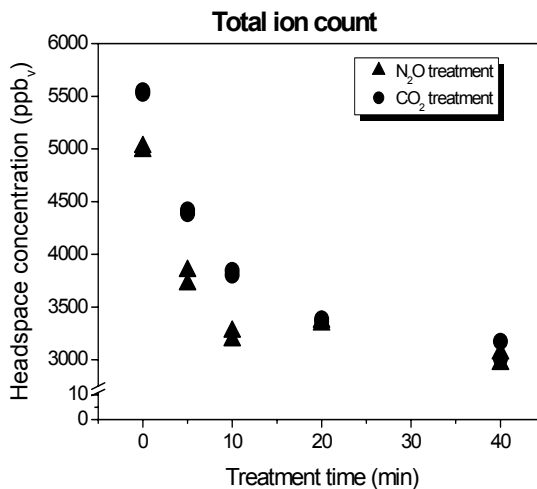


Figure 2: Effect of CO₂ and N₂O pasteurization (100 bar, 36 °C) on headspace total concentration as function of treatment time (2 replicates).

Conclusions

The pasteurization by dense phase CO₂ and N₂O can inactivate microorganisms naturally present in fresh apple juice but also brought, in the experimental conditions, to a general depletion of total volatile compounds present in the juices. This headspace modification must be taken into account during the optimization of pasteurization process to avoid sensory quality alteration that could compromise the acceptability of final products and to warrant the main advantage of high pressure gas treatment that should induce, due to the low temperature, lower impact on sensory profile of products. PTR-MS can be successfully used to monitor these modifications aiding the choice of optimal processing parameters and better supporting the scale up of laboratory plants.

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PTR-MS monitoring of benzene formation in beverages containing benzoate and ascorbic acid

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Abstract

The presence of benzene in food and in particular in soft drinks has been reported in several studies and should be considered in fundamental investigations on the formation of this carcinogen compound as well as in quality control. Proton Transfer Reaction Mass Spectrometry (PTR-MS) has been used here for rapid, direct quantification of benzene and to monitor its formation in model systems related to the use of benzoate in presence of ascorbic acid. Firstly, we show that PTR-MS allows a rapid determination of benzene that is in quantitative agreement with independent SPME/GC analysis. Secondly, as a case study, the effect of different sugars (sucrose, fructose and glucose) on benzene formation is investigated indicating that they inhibit its formation and that this effect is enhanced for reducing sugars. The observed inhibition effect of sugars on benzene formation depends on several parameters as the type and concentration of sugars and temperature: it can be more than 80% in situations that can be expected in the storage of commercial soft drinks. This is consistent with the reported observations of higher benzene concentrations in sugar-free soft drinks.

Introduction

It has been suggested that benzoate, a widespread, otherwise safe preservative, can induce, under certain circumstances, benzene formation in the presence of ascorbic acid [1] added as an antioxidant or being naturally present. Both substances are often present in commercial soft drinks and the worldwide beverage industry is aware of the potential risk of benzene containing products and of the necessity to minimize any potential formation, while still ensuring microbiological standards of the soft drink products [2]. During transport or storage, beverages are often exposed to direct sunlight and, especially during summer time, they can reach high temperatures; there are indications that these factors can favor benzene formation [2]. The fact that commercial sugar-free soft drinks contain more benzene than similar sugar-containing products [3,4] suggests a possible inhibition of benzene formation in presence of sugars but, as far as we know, neither direct experimental evidence of this effect has yet been published nor possible differences between different sugars have been considered. In this context, we propose proton transfer reaction-mass spectrometry as a possible new tool to address these issues [5]. In the present paper, as a prototypical case study, the formation of benzene in aqueous model solutions containing benzoate and ascorbic acid is discussed as well as the effect of adding different sugars at different concentrations. Results are also compared with GC data.

Experimental Methods

Reagents and model systems

The model systems used here consisted of aqueous solutions of sodium benzoate (SB, Carlo Erba/Sigma) at 400 mgL^{-1} plus ascorbic acid (AA, Carlo Erba) at 400 mgL^{-1} . The effect of sugars was studied adding to the model solutions sucrose (Suc, Carlo Erba, Italy) at three concentration levels (0.1, 0.25 and 0.5 M), glucose (Glu, Carlo Erba, Italy) at 0.1 and 0.5 M and fructose (Fru, Carlo Erba, Italy) at 0.5 M. The concentrations of SB, AA and sugars chosen for the model systems span the range expected for commercial products (soft drinks and juices).

The samples (300 mL) of the model solutions were filled into 500 mL glass bottles wrapped in aluminum foils to protect the solution from light.

Kinetic experiments: effect of temperature and sugars

The bottles containing solutions of SB plus AA were placed in a constant-temperature oven at 25 or 45 °C. At intervals corresponding to 5 min, 1 h, 3 h, 6 h, 8 h, 24 h, from the beginning of the experiment, HS was measured by PTR-MS. During the experiments at 45 °C aliquots of 15 mL were removed from the solutions for GC quantification at the same time intervals listed above. The effect of sugars was studied monitoring the benzene formation in the model solutions with sucrose at three concentrations (0.1, 0.25 and 0.5M). From the bottles containing the model solutions with sucrose at 0.5M three aliquots (15 mL each), at 3.5, 6 and 7 h, respectively, were sampled for GC quantification. To compare the effects of different sugars we repeated the experiments with fructose at 0.5M and glucose at 0.1 and 0.5 M.

Benzene quantification

PTR-MS was calibrated using a certified cylinder (Apel-Riemer Environmental Inc., Denver, CO, USA) containing benzene at the concentration of 1007 ppb, ($\pm 5\%$), which was dynamically diluted, using a system of 2 mass flow controllers, down to 1.0 ppb, in humidified VOC free air.

For GC measurements, samples were added with benzene-D6 as internal standard and headspace was pre-concentrate on a SPME fiber (carboxen/PDMS; Supelco, Milan, Italy) and desorbed in a GC-TurboMass Gold (PerkinElmer, Norwalk, CT).

Results and discussion

In Figure 1 PTR-MS quantification for benzene is plotted against GC-MS quantification showing a good accordance between the two determinations. After this consistency check we used only PTR-MS for quantification of benzene in the following experiments.

The behavior of benzene formation at two different temperatures is shown in figure 2. The benzene signal is roughly constant (less than $0.1 \text{ }\mu\text{g}\cdot\text{L}^{-1}$), close to the noise level, over the first 12 h when the solution is kept at 25 °C. Only after 24 h the signal starts to slightly increase reaching a concentration of $0.44 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ after 70 h. When the solution is kept at 45 °C a strong benzene formation sets in after 3 h reaching a maximum concentration ($118.5 \text{ }\mu\text{g}\cdot\text{L}^{-1}$) after about 24 h and remaining roughly constant ($\sim 125 \text{ }\mu\text{g}\cdot\text{L}^{-1}$) over the next 48 h. Our findings are in accordance with those of McNeal [6] who reports a concentration of about $300 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ in a similar experiment at 45 °C for 20 h.

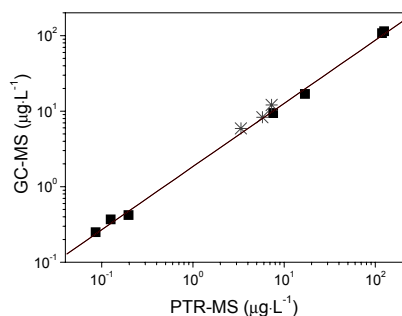


Figure 1: Inter comparison of benzene quantification by PTR-MS and SPME/GC-MS. Model systems at 45 °C with AA plus SB with (*) or without sucrose 0.5M (■).

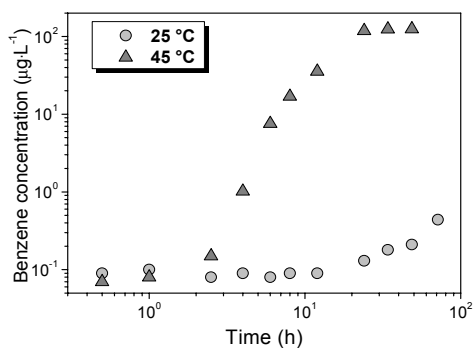


Figure 2: Example of time dependence of benzene formation at 2 temperatures.

When sugars are added, the benzene formation is reduced and this effect is proportional to sugar concentration as can be observed in figure 3 where the relative concentrations of benzene formed in the solutions exposed to 45 °C for 22 h are reported.

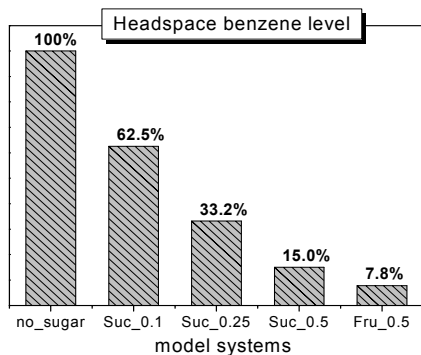


Figure 3: Effect of sugar concentration on the benzene formation in the models after 22 h at 45 °C. Data normalized to the solution with no sugar.

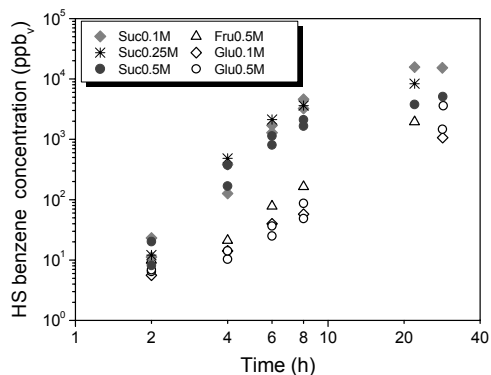


Figure 4: Effect of the reducing (fructose and glucose) and non reducing (sucrose) sugars on the benzene formation at 45 °C.

The different type of sugars seems to have different inhibition efficiency on benzene formation in our model systems. In figure 4 this effect related to the reducing nature of the sugars is manifest: the reducing sugars, fructose and glucose, have a higher protection effect than sucrose, a non reducing sugar.

Conclusions

Here we indicate the possibility to use the PTR-MS for direct quantification of benzene in beverages and investigated its formation in model systems containing ascorbic acid and benzoate that mimic the benzene formation in soft drinks that often contain both substances. The present study suggests the use of PTRMS as a new tool for a rapid control of the presence of this carcinogenic molecule in commercial beverages and for the monitoring of its formation in dependence of the storage conditions (temperature) and the sugars presence in the formulation. The results show that the benzene formation can occur at a temperature that can be reached during transport or storage, especially in summer time. Furthermore our findings indicate that fructose should be a better choice because its higher efficiency in sweetening (1.7 times than sucrose) and in inhibiting benzene formation.

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Measurements of biogenic VOCs over grassland in an Alpine valley

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Abstract

Since April 2008 continuous measurements of biogenic volatile organic compounds (BVOCs) were performed over a meadow near Neustift (Tyrol, Austria) for a growing season using a PTR-MS. Concentrations of several VOCs (such as Methanol, Acetone/Propanal etc.) were monitored and Ozone fluxes were estimated using the disjunct eddy covariance method.

Introduction

Volatile organic compounds (VOCs) enter the atmosphere from a variety of biogenic and anthropogenic sources with an estimated emission of 1300 Tg C yr⁻¹. Biogenic emissions are associated with processes such as growth, maintenance and decay of organic material [1]. 90% of the global VOC emissions are of natural origin [2].

Thus biogenic VOCs play an important role in tropospheric chemistry; VOCs are involved in the production of ozone and other secondary pollutants [3]. Special attention has been paid to the ability of biogenic VOCs to form secondary organic aerosol.

The magnitude of the biosphere-atmosphere exchange of organic trace gases however is poorly understood as quantitative measurements are hard to obtain.

Over grassland, which covers 40 percent of the Earth's surface, only very few studies have been conducted. Relatively high background emissions of Methanol [7] and other VOCs are expected over grass due to plant growth. As a consequence of plant stress like cutting grass [4, 5] or in response to environmental stress conditions (temperature, ozone etc.) [9]. VOCs are emitted. Therefore the recent long term campaign aims to study the amount of organic trace gases which are emitted and deposited over grassland in response to such processes over a whole vegetation period.

Experimental Methods

Monitoring Site

The field site is situated in Tyrol, Austria. Measurements are performed in the middle of a meadow at an altitude of 970 m above sea level close to the village Neustift (47°07' N, 11°19' E) in Stubai valley. The grassland there is usually cut two to three times a year.

Instruments

For continuous monitoring of the ambient VOC concentrations at a high time resolution a PTR-MS was located at the monitoring site. An Eddy covariance system consisting of an infra-red gas analyzer (IRGA) and a sonic anemometer is available. The system is completed by the installation of two commercially available ozone detectors.

The gas-inlet for the system is capped with particulate filter and located outside of the container at a height of 2.5 m above the ground. A heated Teflon line of 16 m length distributes the air from the inlet to the different instruments. An automatic weather station is operated nearby.

Results and Discussion

Figure 1 shows the time series of $m/z = 33$ measured with the PTR-MS during the period from 24th of April 2008 to 3rd of November 2008. The signal at $m/z = 33$ is attributed to Methanol following discussion in [6].

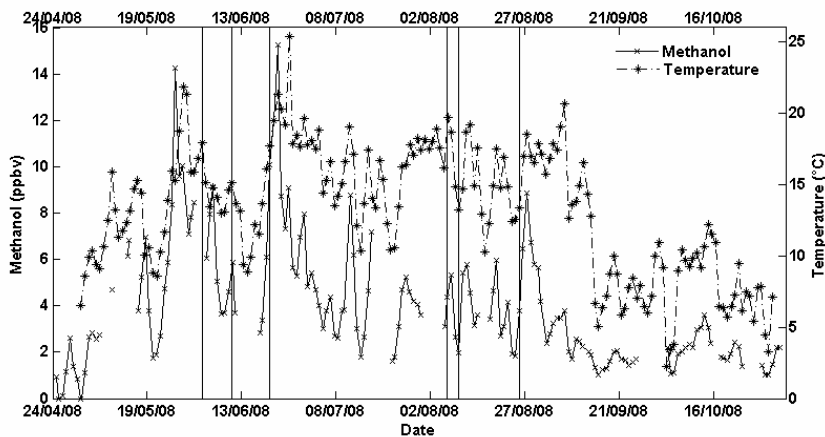


Figure 1: Daily means of Methanol and temperature during the period from 24.04.2008 to 03.11.2008. The grass was mowed on the days which are marked by a vertical black line. For the first two events the hay was removed on the day of the cut while for the other events the grass was dried several days before it was removed.

During the day the wind normally blows valley-up and therefore hayfields which are located valley outwards of the air inlet are the source area for the measured biogenic VOCs. Influences of the grassland which is situated valley inwards compared to the air inlet, however, are predominantly visible by night (down-valley winds).

The first cut of the fields which are located valley outwards compared to the monitoring site was on 2nd and 10th of June and the second on 6th and 9th of August. The neighboring meadows valley inwards were cut on 20th of June and 25th of August (events marked by the vertical black lines in Figure 1). The highest methanol concentrations are detected during spring. This could be explained by cell wall elongation during plant growth [8].

During the whole measurement period the concentration of m/z 59 showed a strong diurnal cycle (Figure 2). PTR-MS detects both protonated Acetone and Propanal at $m/z = 59$. However the contribution of Propanal in atmospheric samples is reported to be small (<10%) [6]. Therefore a possible explanation for the observed diurnal cycle of the m/z 59 signal is the advection of the long-lived compound Acetone. During the night there is weak down-valley wind which changes to up-valley wind in the early morning (Figure 2, lower panel). This makes an advection with the up-valley wind by day quite possible. However, it is not completely clear which process is driving the diurnal cycle of Acetone/Propanal in Stubai valley.

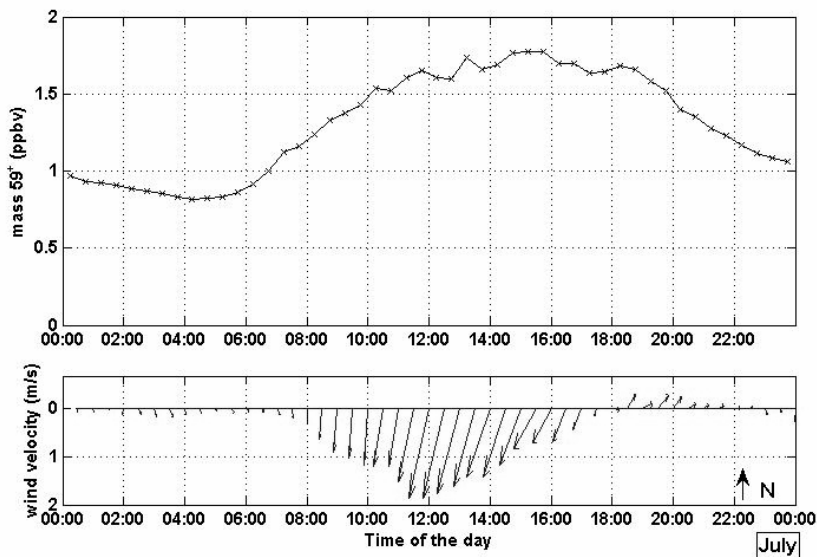


Figure 2: Diurnal cycle of Acetone/Propanal during July in the upper panel and the wind velocity vector in the lower panel averaged over the month of July 2008. During the night wind speeds are very low. In the early morning the wind picks up and blows predominantly South in inward direction of the valley.

Acknowledgements

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PTR-MS Characterisation of an Olfactometer

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Abstract

PTR-MS was used to characterise the performance of an olfactometer typically used in olfactory dysfunction assessments. The fast measurement capabilities of PTR-MS, with direct coupling to the olfactometer, enabled on-line evaluation of the rapidly-delivered aroma pulses. A variety of odorous compounds was used for testing, including hydrogen sulphide, 2,3-butanedione, ethyl butanoate and ethyl hexanoate. PTR-MS signals of the olfactometer pulses indicated that relative peak intensities were compound-dependent. Ethyl butanoate and ethyl hexanoate performed best, albeit with deviations from expected values. In contrast, hydrogen sulphide displayed little variation between different pulse intensity settings. Furthermore, relative intensity depended on pulse duration, with an increase in the latter producing a peak intensity increase. This could indicate compound losses in the delivery lines and/or instrumental performance problems. A temporal decrease in maximum intensity for identical pulses over an extended duration showed headspace concentration depletions, suggestive of Henry's law effects. This preliminary olfactometer performance assessment poses unanswered questions requiring further investigation.

Introduction

Olfactory dysfunction is a sensory disorder estimated to afflict more than 1 % of the population under 65 years of age, and more than 50 % of those older than 65 years [1]. Impairment of olfactory function can have several manifestations, including partial loss (hyposmia), complete loss (anosmia), or a distorted (dysosmia) sense of smell, and can arise for many reasons, e.g. after respiratory tract infection, sinonasal disease, head trauma, congenital defects, or aging, amongst others [2]. The consequences of olfactory dysfunction are not merely repressed appreciation of everyday smells, but also a hindrance in perceiving certain hazards (e.g. smoke, gas leaks, or inedible foods prior to consumption), as well as poor personal body odour assessment and affecting taste. Consequently, deterioration in quality of life and increased hazard exposure has been reported by subjects with olfactory loss [3,4].

To establish the cause and degree of olfactory impairment in affected persons, various clinical tests have been developed over the years. These commonly involve presenting the patient with an odorant stimulus and simultaneously monitoring perceived smells or reactions. One such procedure utilises an olfactometer, which is an instrument capable of providing odorants at short, defined pulses of known concentration [5,6]. Since the accuracy of an olfactometer is intrinsic in a patient's diagnosis, it is important that the set odour levels are reflected at the instrument's output. PTR-MS [7] was used to assess a commercially available olfactometer in terms of pulse intensity and duration. The aim of these experiments was to characterise the olfactometer with respect to these parameters to provide a first evaluation of its operating performance.

Experimental Methods

Instrumentation

A hs-PTR-MS (Ionimed Analytik), operated at E/N 120 Td, with a standard 1 m long, 1/16" OD, 0.04" ID Silcosteel™ inlet line, heated to 65 °C and with a flow of 250 ml min⁻¹, was used for the measurements. A model OM6b olfactometer (Burghart, Wedel, Germany) provided the aroma pulses. A brief outline of the operating principles of the olfactometer is as follows (for further details see [8]): Odorous compounds in the olfactometer are provided either from a gas cylinder (e.g. for hydrogen sulphide) or individually (liquid-phase) diluted in a water chamber, through which air is purged to establish a defined headspace concentration. This gas (from either cylinder or headspace) is supplied to the outlet port of the olfactometer, where it may be released into or diverted away from a constant carrier gas flow – made via precise pressure variations directly upstream of the output – and allow aroma pulses to be generated at the instrument's delivery line.

Compounds

A mixture of four odorous compounds was used in the assessment. Initial PTR-MS fragmentation pattern measurements of the pure compounds determined the respective predominant analyte ion: hydrogen sulphide (H₂S; m/z 35⁺), 2,3-butanedione (C₄H₆O₂; m/z 87⁺), ethyl butanoate (C₆H₁₂O₂; m/z 89⁺), and ethyl hexanoate (C₈H₁₆O₂; m/z 145⁺).

Assessments

For the tests described herein, the PTR-MS inlet was directly coupled to the olfactometer outlet port. The olfactometer aroma pulse intensities and durations for the compound test mixture were monitored via the respective PTR-MS signals. An initial series of measurements established the minimum suitable PTR-MS m/z dwell time to be 50 ms (data not shown), which was used for all subsequent tests. The relative PTR-MS signal maxima were measured for three pulse intensities (constant 200 ms pulse duration). Thereafter, pulse intensity was kept constant and four pulse durations (200, 500, 1000, and 5000 ms) were monitored. Finally, pulses of identical intensity and duration were monitored over a period of 50 min. Data represent mean values of three repetitions.

Results and Discussion

A linear increase in aroma pulse intensity (set to relative levels of 100, 50, and 25 %) was not reflected in the PTR-MS signal maxima (see fig. 1; values normalised to 100 % level). Ethyl butanoate displayed the best performance, with only slight deviation from expected values. 2,3-butanedione and ethyl hexanoate achieved correct tendencies, albeit with up to 20 % deviation

from set levels. Hydrogen sulphide, on the other hand, showed no linearity. Results indicate either a non-linear olfactometer performance or delivery problems to the PTR-MS of such brief pulses.

To establish the pulse duration dependence on the PTR-MS signal, the olfactometer set pulse period was varied (pulse intensity constant). The PTR-MS signal maxima increased with prolonged pulse duration (constant signal expected; see fig. 2). Ethyl butanoate again performed best, albeit with >20 % intensity variation over the four pulses. The other three compounds showed larger variations, with signal intensities increasing with pulse duration. Results indicate that shorter pulses were delivered less accurately to the PTR-MS, although it remains uncertain if this resulted from memory losses in the delivery line or from olfactometer performance problems.

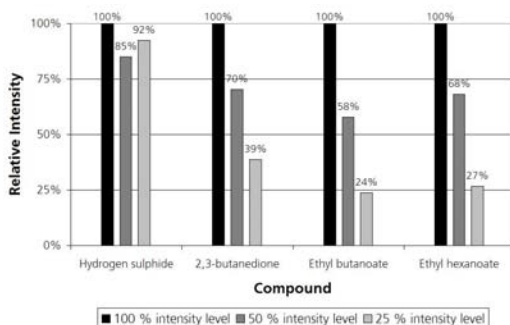


Figure 1: Mean individual compound pulse (200 ms) maxima for varying set intensity levels of a four-compound mixture. Values in legend represent set intensity level at olfactometer. Values in graphic indicate relative levels, as determined by PTR-MS.

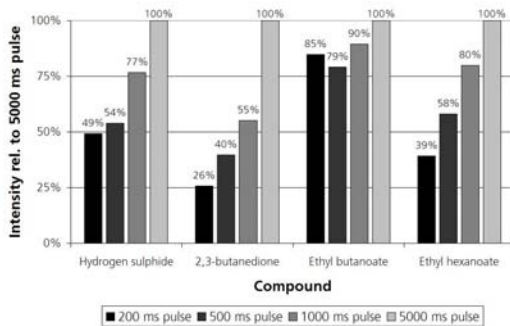


Figure 2: Mean individual compound pulse maxima for varying pulse duration (constant intensity) of a four-compound mixture. Values in graphic indicate relative levels, as determined by PTR-MS.

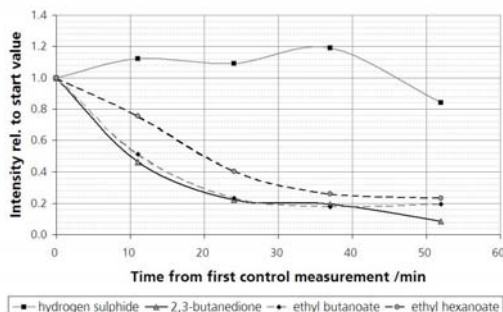


Figure 3: Mean individual compound pulse maxima for identical pulse duration and intensity of a four-compound mixture over a 50 min period.

A further assessment was made of the compound signal intensities for identical pulses over an extended duration of ~50 min. A significant fall-off in PTR-MS signal intensity (around 80 %) was observed over this period for 2,3-butanedione, ethyl butanoate and ethyl hexanoate (see fig. 3). Despite variations in the hydrogen sulphide signal, no systematic loss was seen. A possible explanation for this behaviour relates to the source of the compounds: the latter was delivered via a gas cylinder whereas the former compounds were from the headspace above their respective mixing chambers. This could indicate chamber headspace concentration depletion according to Henry's law, although may also relate to memory losses in the delivery lines. Further tests are necessary to confirm these observations.

Conclusions

The present experiments provided a first performance assessment of the olfactometer instrument in terms of pulse duration and intensity for a selection of compounds. The PTR-MS-olfactory coupling was compound-dependent. Pulses of varying intensity were found mostly to be non-linear, with only ethyl butanoate showing acceptable performance. Large deviations were observed for the remaining compounds, with hydrogen sulphide displaying no signal linearity. The intensity of the peak maxima also increased with pulse duration, suggesting that pulses of lesser duration suffer from losses and/or instrumental performance problems. Furthermore, signal decreases from mixing chamber compounds over an extended period indicated losses according to a Henry's law behaviour. Further experiments are planned to provide a more thorough assessment of the olfactometer for a wider range of aroma compounds to confirm present observations.

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Odour monitoring in composting plants by PTR-MS and PTR-TOF-MS

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Abstract

Volatile Organic Compounds (VOCs) monitoring in composting plants is a fundamental issue both because of the public concern on induced malodour and because it allows the control of the plant operation and the monitoring of the efficiency of biofilters used thereby providing a valuable support for waste management. This drives the research on methods for odour quantification that can be implemented on-site and that provide continuous and reliable odour quantification. In this context, olfactory methods are the reference because they provide a direct measurement of the human response to odour stimuli but they are slow, expensive, and can hardly be implemented on a continuous, on-site, basis. Other drawbacks are the necessity to enrol and train people and the fact that they cannot be used if the presence of dangerous substances is suspected. Classical methods of chemical analysis based on GC/MS and various concentration/pre-treatment procedures are highly developed but are slow and often not suited for on line monitoring. The use of electronic noses seems to open a new perspective for odour control but they still lack, in our opinion, in sensitivity and specificity. PTR-MS and in particular PTR-TOF-MS appears to fulfil most of the requirements asked for in composting plant odour quantification.

Many classes of compounds are potentially relevant in the composting process because they are present both in the initial waste and as final and intermediate products of biochemical metabolic pathways including short chain fatty acids, amines and ammonia, aromatic compounds, hydrogen sulphide, organic sulphides often oxidised to dimethyl sulphide and dimethyl disulphide. Many other compounds can, however, be present in addition and also contribute to the odour of composting plants and their possible presence and relative significance must be considered.

In previous studies we demonstrated the possibility to correlate PTR-MS data with olfactometric results on samples collected at different stages of the composting process. A possible drawback of PTR-MS is the unit mass resolution of the commercial instrument based on a quadrupole analyser that provides information only on the nominal mass of the investigated VOCs. In recent years several groups tried to solve this problem coupling the PTR-MS ion source with different analysers, mostly ion traps and time-of-flight mass analyzers, and now a commercial version of this latter methodology (PTR-TOF-MS) is available (IONICON Analytik, Innsbruck, Austria) and will be soon operative at FEM laboratories.

Here we show that it is indeed possible to provide a reliable estimation of the total odour concentration based on PTR-MS data and that a TOF analyser coupled with proton transfer ionisation provides a tool to identify, at least partly, compounds that are relevant for the formation of odour. In particular the TOF version of the PTR-MS allows the separate identification of nitrogen or sulphur compounds (usually with a very low odour threshold) from interfering, less relevant compounds present on the same nominal mass. Moreover we will show thanks to test measurements at Ionicon's site that the increased mass separation for these instruments is in addition accompanied by a better time resolution and a higher sensitivity.

In conclusion, PTR-MS is a promising tool for the real time monitoring of composting plants that provides an estimation of the odour concentration and the new TOF version allows, partly, to resolve the problems related to the identification of compounds that are expected to have an important impact on odour formation. We suggest that the information contained in PTR-MS-TOF spectra can be enough to face most practical problems related to waste management and composting plants.

QTL mapping of volatile compounds in ripe apples detected by proton transfer reaction-mass spectrometry

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Abstract

Volatile Organic Compounds (VOCs) play an important role in the research related to fruits and, more generally, to agroindustrial products because they are directly related to the perceived sensory quality. They also provide a non invasive and rapid tool for the monitoring of many metabolic and physiological processes induced, e.g., by ripening, storage and stress conditions including wounding or plant-insect interaction. Proton Transfer Reaction-Mass Spectrometry (PTR-MS) is a fast and direct tool for the on-line detection of a wide variety of VOCs. It is based on a soft chemical ionisation by proton transfer from protonated water and provides an absolute measure of concentration with a detection limit of a few part per trillion.

FEM-IASMA has a well-established experience in PTR-MS applications in food science and technology and in particular it has investigated the links between non-invasive PTR-MS fingerprinting of whole fruits with genetic traits. For instance it was possible to identify the cultivar of single whole strawberry fruits [1] and to relate PTR-MS data to gene expression [2]. Based on these latter results we decided to investigate if the PTR-MS fingerprinting of VOCs emitted by ripe apples can be used to identify putative QTL (quantitative trait loci) linked to distinct levels of particular VOCs.

In a first study presented at the previous PTR-MS conference, apples were studied from each of 57 genotypes of the progeny 'Fiesta' X 'Discovery' at Cadenazzo, Ticino (Switzerland). PTR-MS analyses were carried out on 2 apples collected from the same plant of each genotype. The measurement consisted in the direct sampling for 4 minutes of the volatile mixture formed in the head space of a whole apple closed in a glass vessel at room temperature. Spectra for a mass/charge ratio from 20 up to 240 were considered [3]. QTL analyses of the ANOVA estimates of the traits for each genotype were carried out for the intensity of 220 PTR-MS peaks using the software Map@QTL 4.0 to combine phenotypic and genetic marker data. This preliminary investigation showed that it is possible to find quantitative trait loci (QTLs) related to PTR-MS characterisation of the headspace composition of single whole apple fruits indicating the presence of a link between molecular characterisation and PTR-MS data. In particular, a qualitative comparison of overall PTR-MS spectra indicated good agreement between data of fruits of the same genotype and the presence of polymorphic effects. QTL analysis allowed the identification of ten genomic regions associated with seven spectral intensities ($m/z = 28, 43, 57, 61, 103, 115,$

145). The percentage of phenotypic variation explained by the QTLs ranges from 18% to 48%. Separate QTL analysis on the two replicates provided comparable results [3].

The encouraging results of this first experiment induced us to evaluate more apple fruit genotypes (105) of the same progeny described above but grown in three different locations in Switzerland (Conthey, Cadenazzo, Wädenswil). PTR-MS and QTL analysis has been carried out as in the previous experiment and, although data are still under analysis, we could confirm QTLs for 4 of the 10 genomic regions detected before. QTL mapping was consistently confirmed for all three different environments and in one location we identify the same QTLs in both years. QTLs for $m/z = 28$ and $m/z = 43, 57$ and 61 were significant in all the three environments only for genomic regions localized in two linkage groups (LG 2 and LG 15). Fragment masses 43, 57 and 61 masses are mapped in the same positions and we found, for heterozygous individuals, the linear relationship between fragments expected for esters (e.g. $m/z = 43$ and $m/z = 61$). The presence of a clear segregation for the phenotypic traits related to the fragments considered was evident and has been confirmed in all environments after one year. We thus can consider the markers in LG2 for $m/z = 43$ and 61 as markers for the presence of esters and, in particular, acetates. The other genomic regions ($m/z = 103, 115$ and 145) were confirmed only for the Cadenazzo progeny, the same evaluated in the previous experiment, indicating that the phenotypic effect of these QTLs were sensitive to the environment in which they were evaluated.

This work confirms the possibility to associate variability of VOCs emission directly measured by PTR-MS to the genome, resulting in fact in the identification of associated QTLs. A future step will involve assessment of their association with sensory and physiochemical traits aiming at the identification of quality traits measurable by a simple and fast method such as PTR-MS and at the identification of linked molecular markers allowing the prediction of fruit characteristics on young juvenile seedlings.

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PTR-MS study of volatile sulfur compounds in water and water/ethanol solutions: fragmentation patterns and partition coefficients

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Abstract

Volatile sulfur compounds (VSCs) as sulfides and mercaptanes, are produced in many biological and technological processes and are often characterized by very low odor thresholds and, even when present at a very low concentration, their contribution to malodor in ambient air or to odor and flavor of food is always relevant. Thus the detection and quantification of these compounds, as well as their monitoring, is of significance in different fields from food technology [1] to malodor control in waste management's plants [2], to medical applications as for oral hygiene control [3] or as indicators of adverse clinical conditions [4].

A particularly interesting case is the control of VSCs concentration in wine either to control possible off-flavors [5] or to identify peculiarities related to cultivars or terroirs [6] but, despite the important role played by VSCs, there is still a lack of information even on fundamental chemical parameters such as water/air partition coefficients (Henry's law constants) and even scarcer data on their partition coefficients for alcoholic solutions or wine.

Direct injection mass spectrometry and PTR-MS in particular, is a fast growing methodology that allows on line detection of volatile organic compounds with high sensitivity and high time resolution. The application of PTR-MS for direct analysis of wine has been proposed and applied suggesting that the problems related to high ethanol concentration (main H_3O^+ signal suppression, unstable reactions, detector saturation) can be partly overcome diluting the head space [7] or, on the opposite, saturating the headspace with ethanol thus modifying the standard proton transfer ionization from protonated water to proton transfer through protonated ethanol [8]. Also in the case of PTR-MS data, although the potential interest of sulfur compounds, there is still a lack of information on the proton transfer induced ionization of VSCs and, in particular, on the induced fragmentation patterns that is a major problem in the understanding of PTR-MS data in presence of ethanol.

An interesting application of direct injection mass spectrometry, proposed for PTR-MS by Karl et al [9], is the possibility to have a fast and accurate determination of partition coefficients that does not rely on absolute quantification of the concentration in the head space and that does not require the quantification of the concentration in the liquid phase. This method is particularly interesting for its simplicity and because it avoids several problems of static methods [9].

The work presented in this paper, as in previous work on esters [10], aims, on one side, at the determination of partition coefficients based on dynamic PTR-MS head space monitoring of a series of important VSCs both in water/air systems and in hydro alcoholic solution/air systems. On the other side, for the same compounds, aims at the investigation of the fragmentation patterns induced by proton transfer and of their dependence on experimental parameters. The final goal is the availability of chemical and technical information to allow a better monitoring of VOCs in food related issues and, more generally, whenever the monitoring of sulfur compounds is a concern. The data presented will be better exploited in the case of measurements with the recently introduced PTR-TOF-MS instrument [11] because sulfur compounds, in this case, doesn't interfere with the signals of other non-sulfur compounds.

In particular, we present here and compare with available literature and with theoretical data, the Henry's law constants of six VSCs and their partition coefficients in hydro-alcoholic solutions at different ethanol concentrations. For the same compounds the fragmentation patterns induced by proton transfer in standard PTR-MS operation and as a function of E/N are presented as well.

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Determining enzymatic activity and concentration by direct injection mass spectrometry

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Abstract

Direct injection mass spectrometry and, in particular PTR-MS, provides a sensitive and accurate tool for the monitoring of volatile organic compounds (VOCs) and therefore, it can address many technological and research issues involving their precise and rapid measurement. Here we propose, as a new application, the use of PTR-MS for the study of enzyme-catalysed reactions.

Enzymes are biomolecules that catalyze (i.e. increase the rates of) chemical reactions, by lowering their activation energy (E_a or ΔG^\ddagger). The molecules at the beginning of the process are called substrates, and the enzyme converts them into different molecules, the products. All enzyme assays measure either the decrease of substrates or the increase of products over time. Continuous assays, where the assay gives a continuous reading of activity, are the most convenient, as they directly provide the rate of the reaction investigated. This category includes spectrophotometric, fluorescence, chemiluminescence, calorimetric and light-scattering assays. We propose here to follow, on line, the concentration of the volatile compounds produced or consumed during the reaction by a mass spectrometric method using PTR-MS, by direct injection of the volatiles in the head space mixture over the liquid phase where the reaction takes place. As a case study we present the decomposition process of hydrogen peroxide (H_2O_2) into H_2O and O_2 catalysed by the enzyme catalase, one of the key-enzymes involved in cellular oxidative stress.

Firstly, we show that the enzymatic activity of a commercial preparation of bovine catalase can be determined accurately by PTR-MS and that this result is in good agreement with that obtained by the more traditional spectrophotometric method described by Aebi (Aebi, 1984). In this application, we use PTR-MS in a non-conventional way because we exploit the linear dependence of the signal at $m/Z=32$ (O_2^+) from the concentration of oxygen in the injected air. In other words, we are not using proton transfer reactions but a different kind of ionisation process that is usually minimised in standard PTR-MS procedure.

Secondly, as a case study of scientific relevance, we extend this approach to the investigation of catalase specific activity in some grape berry crude extracts, to verify the previously reported occurrence of an oxidative burst during fruit ripening (Pilati et al., 2007). These crude extracts are enriched in secondary metabolites that interfere with the H_2O_2 spectroscopic signal, thus making this approach prone to possible artifacts for catalase specific activity determination. The higher selectivity of mass spectrometry should overcome this problem by directly detecting the molecular oxygen generated by the catalase reaction even in complex matrices.

The outcomes of this study indicate that it is indeed possible to estimate an enzymatic activity, such as that of purified bovine catalase, or the enzyme concentration in a complex sample (i.e. its specific activity). The mass spectrometric method, as compared to the spectrophotometric assay, is characterized by a wider dynamic range and by a reduced influence of other products biological matrices complexity. Finally, it can monitor simultaneously a plethora of volatile compounds allowing the study of more than one reaction in the same media, or the intermediate and final products of reactions belonging to a metabolic pathway. Due to the sub-optimal operative conditions set for the case study, that is the monitoring of the non protonated product at $m/z=32$, which has a relatively high background signal, we could not fully exploit PTR-MS sensitivity. Thus, we envisage that much better results will be attained in the case of reactions involving volatile compounds that can be detected by standard PTR-MS operation.

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Analysis of fragmentation patterns of different monoterpene isomers using a Proton Transfer Reaction Linear Ion Trap MS and a Townsend Discharge Ionization Triple Quadrupole MS

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Abstract

Proton Transfer Reaction Mass Spectrometry (PTR-MS) allows for quantitative analysis of VOCs in real time at low concentrations [1], but cannot differentiate isomeric or isobaric molecules. The recent development of a high resolution PTR-Time-Of-Flight-MS [2] allows differentiation of most isobaric compounds, but is of course unable to identify isomers.

Here we present a comparison of two approaches to identify isomers:

- Collision induced dissociation (CID) in a Triple-Quadrupole-MS (QqQ-MS)

The QqQ-MS (a modified Varian 320-MS) has two quadrupole mass filters connected by a 180° curved collision cell. A Townsend discharge is used to produce primary H₃O⁺-Ions and associated water clusters. The ionisation of VOCs takes place by either direct proton transfer or switching processes. Both processes lead to protonated (m+1) quasimolecular ions with little fragmentation.

The first quadrupole is used for preselecting the ions with a nominal mass of interest. In the collision cell, the protonated molecules are fragmented. Argon at pressures up to 2.4 mTorr is used as the CID-gas. By varying the offset-voltage of the second quadrupole, collision energies of up to 50 eV in the laboratory frame are accessible. The resulting fragmentation pattern as a function of collision energy is recorded using the last quadrupole mass filter.

- CID with a PTR linear quadrupole ion trap (PTR-LIT)

The PTR-LIT instrument [3] couples a PTR ion source to a linear ion trap using helium as a trapping and CID gas. Protonated VOCs are accumulated in the trap up to a few seconds. After the filling, ions are isolated and manipulated using the dipolar excitation technique. With the PTR-LIT multiple MS (MSⁿ) is possible with almost unit trapping efficiency.

Therefore fragment ions can be isolated and fragmented further with almost no loss in sensitivity.

In a laboratory comparison experiment seven monoterpenes were analyzed with both instruments. Constant and defined volume mixing ratios of about 100 ppbv were supplied using liquid syringe injection of individual monoterpene species into a flow of zero air. This allowed quantitative analysis of CID patterns. Both instruments were simultaneously connected to the liquid injection-unit with a heated transfer line.

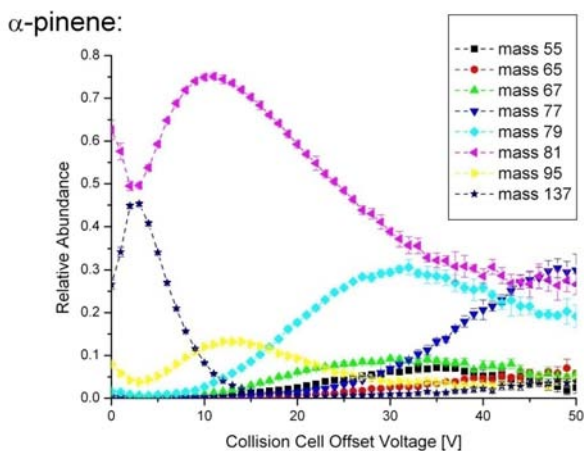


Figure 1: Fragmentation pattern of α -pinene as a function of collision energy (lab. frame) measured with the QqQ-MS

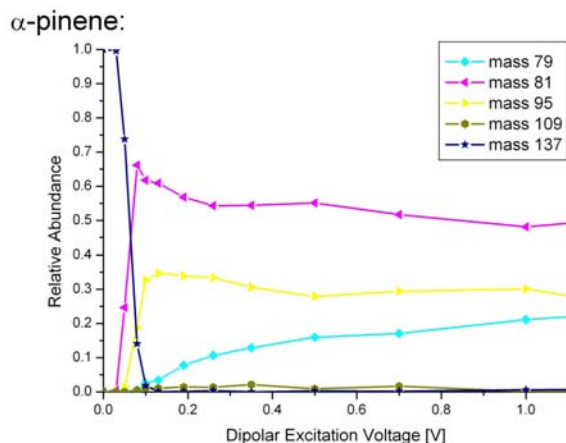


Figure 2: Fragmentation pattern of α -pinene as a function of dipolar excitation voltage measured with the PTR-LIT.

Both instruments were able to distinguish between different types of structures, i.e. the double ring structure of α -pinene was distinguished from the linear structure of ocimene. However, a quantitative analysis of mixtures of monoterpenes in ambient air seems not possible.

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Garlic breath sampling and monitoring by Proton Transfer Reaction - Mass Spectrometry

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Abstract

A method for sampling and monitoring garlic breath by Proton Transfer Reaction - Mass Spectrometry (PTR-MS) was developed. Breath composition and relevance of allyl methyl sulfide as impact compound in garlic breath was confirmed by combined Headspace Gas Chromatography - Mass Spectrometry (HSGC-MS) and Olfactometry. Breath concentrations of allyl methyl sulfide as measured by Proton PTR-MS showed the same elimination profile for three test persons. Allyl methyl sulfide concentration was found to be approximately twice as high after ingestion of 46 g as compared to 20 g of garlic in the same subject.

Introduction

Garlic represents one of the most popular spices and is appreciated as a seasoning for its pungent and pleasant flavour all over the world. Historically, it is used in many indigenous systems of medicine and has been attributed manifold health benefits. More recently, elucidation of garlic-related health effects has received much attention in literature and covers anti-oxidative, anti-inflammatory, hypocholesterolemic, hypotriglyceridemic, cardioprotective and neuroprotective functions as well as inhibition of carcinogenesis by interfering with the tumorigenic metabolism [1-3]. On the contrary, garlic-related breath malodours discourage consumers from garlic consumption. It is the objective of the this study to develop an effective method for monitoring garlic-related breath malodours and elimination profiles by PTR-MS.

Experimental Methods

Garlic Ingestion and breath sampling procedure

20 g or 46 g of raw garlic was cut into small pieces and ingested within five minutes after preparation. Panelists avoided garlic and other allium-type vegetables in their diet for 48 hours before the experiment. In order to avoid overlay of garlic odours from residues in the mouth, the sample was ingested without chewing. Before and after consumption of garlic panelists brushed their teeth with an aroma-free toothpaste according to [4]. Breath samples were collected before and at defined time intervals after consumption of garlic.

Consistent with Steeghs et al. [5], Tedlar bags were found to release substantial amounts of N,N-dimethylaminoacetaldehyde (m/z 88) and phenol (m/z 95) interfering with expected garlic breath

volatiles. Therefore, breath was collected in 3L Siltek[®] SilcoCan[®] canisters (Restek, Bellefonte, U.S.). The interior surface of these canisters is covered with a desactivating coating that eliminates losses of sulfur compounds due to reaction with metal surfaces. Vacuumised canisters were filled with breath samples via a specially designed Teflon mouthpiece which allows panellists a comfortable and leak-free breathing into the canister. Between sampling, canisters were cleaned by alternately vacuumising and flushing the canisters with pure nitrogen at least five times. Preliminary tests with acetone/air mixtures prove the suitability of the cleaning procedure. In order to avoid condensation effects of the breath samples on cold surfaces, canisters were stored at 40°C before filling as well as between measurements.

Headspace Gas Chromatography - Mass Spectrometry [HSGC-MS]

For identification and confirmation of breath volatiles, 10 mL of breath was automatically sampled from the SilcoCan[®] canister and analysed via HSGC-MS and combined Olfactometry. Analysis was performed on a Trace GC Ultra (Thermo Fisher Scientific Inc. Waltham, U.S.) equipped with a CombiPal Autosampler (CTC Analytics, Zwingen, Switzerland) and a Cold Trap 915 (Thermo Fisher Scientific Inc. Waltham, U.S.). Breath volatiles were trapped at -150°C and subsequently transferred to the GC-system by thermal desorption. Separation was performed on a DB5 capillary column (30 m x 0,25 mm x 0,25 µm) starting with a temperature of 0°C held for 2 min., subsequent heating at 6°C per min to 240°C held for 3 min. The column effluent was equally split for detection via a heated sniff port (240°C) a flame ionisation detector (240°C) and mass spectrometric detection (Saturn 2100T, Varian Inc., Palo Alto, U.S.) allowing for unequivocal identification of volatiles as well as their olfactory contribution to breath malodours.

Proton Transfer Reaction - Mass Spectrometry [PTR-MS]

The Siltek[®] SilcoCan[®] canister was connected to a High Sensitivity Proton Transfer Reaction - Mass Spectrometer (Ionicon Analytik Ges.mbH, Innsbruck, Austria) by piercing the heated inlet of the instrument through the septum of the canister. Preliminary tests with acetone/air mixtures prove a leak-free connection of the canister with the analytical instrument. Air was sampled from the canister via a continuous flow of 150 mL/min and analysed according to the method described by Lindinger et al. [6]. Applying the scan mode, samples were scanned for the full mass range from m/z 20-180 at a dwell time of 0.1 second per mass. For multiple ion detection, the following mass to charge ratios and individual dwell times were selected: primary ion: m/z 21 (0.1 s), breath acetone: m/z 59 (0.1 s), dimethyl sulfide: m/z 63 (5 s), allyl methyl sulfide: m/z 89 (0.1 s), dimethyl disulfide: m/z 95 (5 s), diallyl sulfide: m/z 115 (10 s), diallyl disulfide: m/z 147 (10 s). For comparability of results, signal intensities were normalised to a theoretical intensity of the primary ion H_3O^+ of 1×10^7 counts per second.

Results and Discussion

Compounds identified by HSGC-MS in 10 mL of breath after consumptions of garlic were allyl methyl sulfide and traces of diallyl disulfide. However, allyl methyl sulfide was the only compound detected at the sniff port indicating that other compounds have little or no relevance for the characteristic odour of garlic breath. Different from Ruiz et al. [7], who also identified p-cymene and d-limonene in garlic breath, these terpenes could not be identified in the present study. Other unidentified terpenes than p-cymene and limonene were found to be present in the

breath of the same subject before and after consumption of garlic and could therefore not be related to garlic consumption.

Consistent with Ruiz et al. [7] and Taucher et al. [8], allyl methyl sulfide was the most abundant sulfuric volatile and its concentration was found to reach its maximum after 4-5 hours after consumption of garlic for all three subjects (Figure 1). Additionally, the amount of allyl methyl sulfide formed was clearly dependent on the amount of garlic consumed. Allyl methyl sulfide concentration was found to be approximately twice as high after ingestion of 46 g as compared to 20 g of garlic in the same subject (Figure 2).

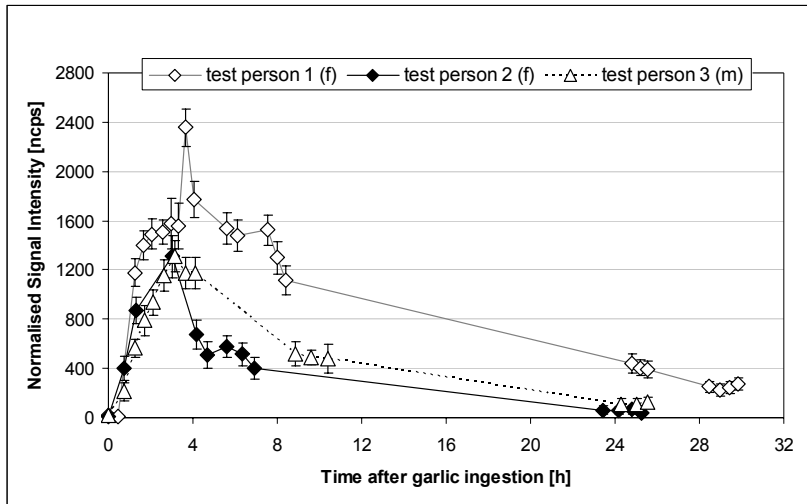


Figure 1: Elimination profiles of allyl methyl sulfide at m/z 89 in breath samples of three panelists after consumption of 20 g garlic as measured by PTR-MS

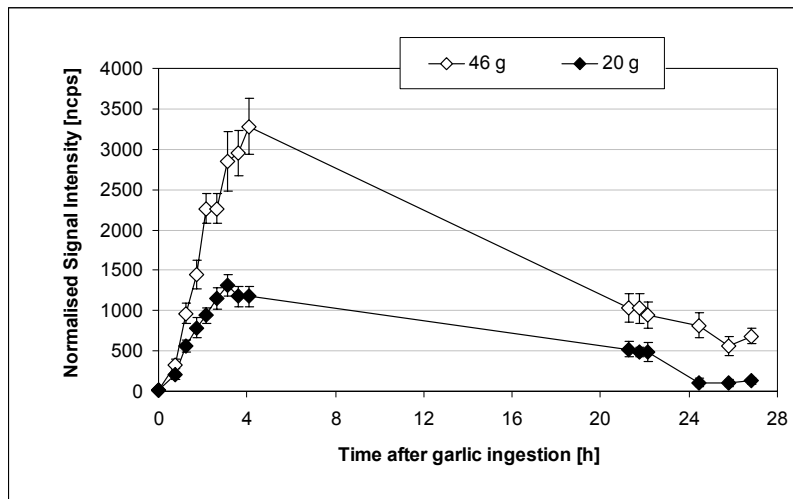


Figure 2: Signal intensity of allyl methyl sulfide at m/z 89 in breath samples of one panelist after consumption of 20 versus 46 g raw garlic as measured by PTR-MS

Different from the present study, Ruiz et al. [7] and Taucher et al. [8] additionally detected traces of diallyl sulfide, diallyl disulfide and diallyl trisulfide. However, concentration of these compounds reached an initial maximum right after consumption and showed a rapid decline in breath concentration within 1 hour after consumption. Ruiz et al. relates the rapid decrease to the elimination from oral and pharyngeal regions where they are present right after consumption of garlic. In the present study, oral hygiene measures were taken in order not to confuse volatile release from garlic residues in the mouth with other garlic-born metabolites. Consistently, sulfuric volatiles related to garlic residues in the mouth were not detected in the present study. Furthermore, elimination profiles of allyl methyl sulfide displayed the same profile as Ruiz et al. found when oral contamination with garlic residues was avoided due to consumption of enteric coated garlic tablets [7].

Conclusion

An effective method for sampling and monitoring garlic breath by Proton Transfer Reaction - Mass Spectrometry while avoiding the formation of artifacts, was successfully developed and implemented.

Acknowledgement

The authors wish to thank Dr. Alfons Jordan, Ionicon Analytik Ges.mbH, Innsbruck Austria for helpful advice and discussion.

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PTR-MS in pomology: study of the development of VOCs during post-harvest ripening (shelf-life).

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Abstract

The development of volatile organic compounds (VOCs) in apple during shelf-life is an important aspect to evaluate fruit quality which is tightly correlated with the post-harvest storage technique utilized. Proton transfer reaction mass spectrometry (PTR-MS) measurements were performed on apples conserved under four different storage conditions: ULO (ultra low oxygen), DCA (dynamic controlled atmosphere), ILOS+ (initial low oxygen stress) and 1-MCP (1-methylcyclopropene) + ULO. The most important family of molecules, which are the major contributors to the characteristic apple-like aroma, were identified and the essential differences during shelf-life regarding these family were evaluate.

Introduction

Post-storage ripening of apples or ‘shelf-life’ depends on a great number of factors, such as growth, harvesting operations or storage conditions [1]. Optimisation of storage conditions is possible by limiting the production of ethylene using low temperature and an atmosphere with high carbon dioxide (CO₂) and low oxygen (O₂) concentration. This constitutes the basis of the so-called “controlled atmosphere” (CA) storage [2]. The state-of-the-art CA technique is “Ultra-Low O₂” (ULO) storage [3]. A modification of the ULO method is “Initial Low O₂ Stress” (ILOS) [4]. The main advantage of this technique is that it controls to a certain extent the physiological disorder scald which causes commercial losses.

An improvement of the CA-ULO technique consists in the “Dynamic Controlled Atmosphere” (DCA) which represents a dynamic adjustment of CA conditions to the physiological state of apples, without the need of post-harvest chemical treatment of fruit [5]. Online fluorescence measurements allow to detect the stress level of the fruits caused by low O₂ and to adapt the storage atmosphere to the physiological demands of apples to improve the storage process.

Another innovative fruit storage method is the use of specific molecules that are able to bind to ethylene receptors thus delaying the fruit’s maturation processes. 1-MCP (1-methylcyclopropene C₄H₆) formulated as SmartFresh® is largely used for this purpose [6].

The choice of the storage method influences the parameters which determine the quality of the final product. It is necessary, therefore, to understand how the storage method affects the evolution of principal aromas in apples in order to obtain a high quality product.

Proton transfer reaction mass spectrometry (PTR-MS) represents a good alternative for VOC analysis (instead of common gas chromatography techniques) and allows fast and real-time quantification of trace components with concentrations as low as a few pptv without sample preparation [7].

Experimental Methods

Storage conditions

Apple fruit (*Malus domestica* Borkh.) cv 'Red Delicious', picked in the optimal harvest window for long-term CA storage (year 2007) in the Venosta Valley (~600 m above sea-level) of South-Tyrol (Italy), were stored for 7 months with four different long-term storage techniques. i) ULO: 1.0 kPa O₂ plus 1.0 kPa CO₂ ii) DCA: 0.4 kPa O₂ iii) ILOS+ 0.7-0.8 kPa O₂ plus 0.9 kPa CO₂ iv) treatment with 1-methylcyclopropene (0.625 µl l⁻¹ 1-MCP; AgroFresh Inc./Rohm & Haas Company, Philadelphia, PA, USA), formulated as SmartFresh[®], at 2.5°C for 24 h in the above mentioned gas-tight container with ventilation and monitoring of O₂ and CO₂, after six days of pre-refrigeration at 2.5°C in normal air. The 1-MCP-treated fruits were subsequently stored in ULO-CA. Temperature was kept at 1.3°C and relative humidity at ~98 % for all storage methods.

Shelf-life conditions

Apples stored for 26 days at constant temperature (20°C) and relative humidity of 60-70% were analysed at seven-day intervals in order to evaluate the development of aromas in this period and to detect differences between the four storage methods.

PTR-MS setup

PTR-MS operating conditions were as follows: drift tube voltage, 600 V; drift tube pressure, 2.00 ± 0.05 mbar; drift tube temperature, 70°C; O₂⁺/ H₃O⁺ ratio ≤ 0.1 %; inlet temperature, 80°C.

Results and Discussion

Dimick and Hoskin [8] reported that nearly 300 volatile organic compounds have been isolated from apple. Of these, the alkylic esters (from C₃ to C₁₈), which are produced especially in apples' peel, are considered major contributors to the characteristic apple-like aroma and flavour in most cultivars and especially in "Delicious" apples [9]. Moreover Fellman et al. [10] consider three acetic esters (butyl acetate *m/z* 116, 2-methylbutyl acetate *m/z* 130 and hexyl acetate *m/z* 144) the most important and characteristic molecules of apples' flavour. The masses of the three acetic esters are clearly present in the PTR-MS mass spectra and can be discriminated from the background. In fact, even if chemical ionisation, which takes place in the drift tube, is a soft ionisation, numerous peaks related to the characteristic fragmentations of acetic esters [11] were noticed in all apple mass spectra. Considering the importance of acetic esters in apple aroma the development of mass *m/z* 43 and 61 can be chosen as an analytical index for the quantification of the total amount of aromatic molecules. Thanks to the potentialities of PTR-MS instrument it is possible to evaluate the aromatic profile (VOCs) of one apple in few minutes without using a destructive technique. The development of all esters during shelf-life is similar: they are almost constant for the first eight days and then drastically increase until day 26. However, apples treated with 1-MCP and then stored under ULO conditions produce the smallest quantity of esters. This

is in agreement with the fact that inhibition of ethylene receptors by 1-MCP significantly blocks the maturation process of apples.

Another important family of molecules which plays an important role in apple aroma is the family of terpenes [12]. This mixture of molecules are biosynthesized from isoprene ($C_5H_9 = m/z$ 69 $M+H^+$) through known head-tail reactions. Only monoterpenes ($C_{10}H_{17} = m/z$ 137 $M+H^+$) and sesquiterpenes ($C_{15}H_{25} = m/z$ 205 $M+H^+$) made up by two and three isoprene units are volatile enough to be revealed by PTR-MS.

All the other masses correlated with m/z 137 and 205 can be predominantly assigned to fragmentation characteristic of these molecules [13].

Monoterpenes and sesquiterpenes for apples stored under ULO, ILOS+ and DCA conditions increased significantly in the first eight days and then decreased until day 26.

Apples stored with DCA reach the highest concentration of these molecules, followed by ULO and ILOS+.

The main differences in aromatic profile have been found between apples treated with 1-MCP and untreated ones, stored in different controlled atmospheres. This behaviour could be due to the inhibition of the ripening process by 1-MCP even during shelf-life conditions, despite the fact that ambient temperature and regular air composition accelerate ripening and senescence processes of apples well preserved in cool storage at very low O_2 and high CO_2 concentrations. For these reasons apples treated with 1-MCP developed the smallest quantity of VOCs during shelf-life.

Due to online real-time measurements and the fast data acquisition by means of the PTR-MS technique it has been possible to analyse the dynamics of aroma development in apple. These results could represent a further step to make PTR-MS a standard technique for apple aroma analysis combined with the traditional sensory tasting panels.

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On-Line PTR-MS Monitoring of Volatile Metabolites in the Dynamic Headspace of Microbial Cultures

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Abstract

A method for real-time analysis of volatile organic compounds (VOCs) from microbial cultures was established using proton-transfer-reaction mass spectrometry (PTR-MS). A newly developed sampling system was coupled to a PTR-MS instrument to allow for on-line monitoring of VOCs in the dynamic headspace of microbial cultures. The novel PTR-MS method was evaluated for four reference organisms: *Escherichia coli*, *Shigella flexneri*, *Salmonella enterica*, and *Candida tropicalis*. Headspace VOCs in sampling bottles containing actively growing cultures and uninoculated culture media controls were sequentially analyzed by PTR-MS. Characteristic marker ions were found for certain microbial cultures: *C. tropicalis* could be identified by several unique markers compared with the other three organisms, and *E. coli* and *S. enterica* were distinguishable from each other and from *S. flexneri* by specific marker ions, demonstrating the potential of this method to differentiate between even closely related microorganisms. Although the temporal profiles of some VOCs were similar to the growth dynamics of the microbial cultures, most VOCs showed a different temporal profile characterized by constant or decreasing VOC levels, or single or multiple peaks over 24 hours of incubation. These findings strongly indicate that the temporal evolution of VOC emissions during growth must be considered if characterization or differentiation based on microbial VOC emissions is attempted. Our study may help to establish the analysis of VOCs by on-line PTR-MS as a routine method in microbiology and as a tool for monitoring environmental and biotechnological processes.

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Traffic and vegetation contributions to methanol, acetaldehyde and acetone concentrations in an Alpine valley: a study involving long-term PTR-MS measurements

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Abstract

Continuous PTR-MS measurements of volatile organic compounds (VOCs) were carried out over a 15-month period beside a motorway in the Alpine Inn valley. NO, NO₂, CO and PM₁₀, as well as several meteorological parameters, traffic numbers and pollen abundances of selected plant species, were additionally monitored. Diurnal changes in VOC levels reflected meteorological conditions of the valley atmosphere. Seasonal variations of biogenic VOCs (BVOCs) were also observed, with lowest concentrations in winter and highest concentrations in late spring, including a long phase with high levels in summer. This indicates a strong correlation between BVOC emissions, plant growth, and plant decay driven by temperature and sunlight. This study was the first continuous long-term on-line measurement under real outdoor conditions of a large spectrum of biogenic and anthropogenic VOCs such as methanol, acetaldehyde, acetone, isoprene, monoterpenes, and aromatic compounds. The complete dataset allows the VOC concentrations to be separated according to biogenic and traffic-related contributions; this abstract focuses on methanol, acetone and acetaldehyde.

Observations of fragmentation of simple organic compounds in proton transfer mass spectrometry measurements

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Abstract

The mass spectra and branching ratios of the product ions resulting from reaction of hydronium ions with 23 volatile organic compounds have been determined at 6 values of E/N. This work is undertaken to clarify what compounds the proton transfer reaction mass spectrometer (PTR-MS) is responding to when it gives a spectrum of counts at a range of m/z values. Currently there are a number of compounds (that occur for instance in the atmosphere) where the branching ratios of product ions have not been measured or have been measured at only one of the possible PTR-MS operating conditions (E/N). In some cases, the product fragment ions may be unmeasurable by PTR-MS.

Examples are presented of the branching ratios of product ions of hydroxyacetone, ethyl formate and methyl isobutyl ketone, and, for comparison ethanol which is already known.

The goal of this work is to measure the branching ratios of product ions for simple organic compounds present in the atmosphere that have not already been measured.

Introduction

A significant proportion of the gaseous organic compounds emitted to the atmosphere are simple C1 – C7 volatile organic compounds (VOCs) some of which are harmful to human health (e.g. benzene), and all are oxidized in the atmosphere and are therefore involved in the formation of secondary pollutants including ozone and secondary organic aerosols [1].

In PTR-MS, proton transfer reactions with hydronium ions, H_3O^+ , are used to ionize VOCs in air. The PTR-MS detects the product ions via a quadrupole mass spectrometer usually as the molecular mass of the species (amu) plus one, due to proton. The limitation of this alone, is that isobars, compounds of the same molecular mass, that undergo proton addition are indistinguishable to the PTR-MS. With some compounds, other product ions are formed including protonated fragments of the original molecule, and protonated products with added water molecules. The extent of this clustering and fragmentation is determined, in part, by E/N (unit Townsend, Td) where E is the electric field strength (V cm⁻¹ and N is the buffer gas density (molecules cm⁻³). 1 Townsend = 1017 V cm² molecule⁻¹. Increasing E increases the ion kinetic energy between reagent ions. At high values of E/N cluster ion formation is limited however fragmentation of product ions is more frequent.

The PTR-MS mass spectra of over one hundred compounds have been determined including a range of alcohols [3,4,5], aldehydes and ketones [2,3], carboxylic acids [6] esters [2,7], aromatics [3,8] and monoterpenes [9]. Only in some cases have mass spectra been determined over a range of PTR-MS operating conditions [8,9]. Many atmospheric measurements by PTR-MS have been conducted with simultaneous independent VOC measurement techniques to assist with the qualitative and quantitative analysis of the masses detected [10].

For reliable stand alone measurements of complex air matrices by PTR-MS, a database of branching ratios of the product ions resulting from reaction of hydronium ions with atmospherically relevant VOCs across the range of operating conditions would be useful in order to optimize PTR-MS operation to detect species of interest and to accurately interpret the mass spectra.

Experimental Methods

The CSIRO PTR-MS is a High Sensitivity PTR-MS (Ionicon Analytik GmbH) that operates with the aid of auxiliary equipment that regulates the flow of air in the sample inlet and controls whether the PTR-MS is sampling ambient or zero air or calibration gas. Zero air is generated by passing air through Pt wool at 350°C to oxidize all detectable VOCs in sample air. The PTR-MS inlet and drift tube temperature was maintained at 75°C.

Table 1: 23 Volatile organic compounds for which fragmentation patterns have been determined over a range of values of E/N.

Alcohols	Acids	Esters	Other OVOCs
Methanol (CH ₃ OH)	Formic Acid (HCOOH)	Methyl formate (HCOOCH ₃)	Formaldehyde (HCHO)
Ethanol (C ₂ H ₅ OH)	Acetic Acid (CH ₃ COOH)	Ethyl formate (HCOOC ₂ H ₅)	Acetonitrile (C ₂ H ₃ N)
1-propanol (C ₃ H ₇ OH)	Propanoic acid (C ₂ H ₅ COOH)	Propyl formate (HCOOC ₃ H ₇)	Acetaldehyde (C ₂ H ₄ O)
2-propanol (C ₃ H ₇ OH)	Oxalic Acid (C ₂ H ₄ O ₄)	Butyl formate (HCOOC ₄ H ₉)	Acetone (C ₃ H ₆ O)
1-butanol (C ₄ H ₉ OH)		Methyl acetate (CH ₃ COOCH ₃)	Hydroxyacetone (C ₃ H ₆ O ₂)
2-butanol (C ₄ H ₉ OH)		Ethyl acetate (C ₂ H ₅ COOCH ₃)	MEK (C ₄ H ₈ O)
Tert. Butyl alcohol (C ₄ H ₉ OH)			MIBK (C ₆ H ₁₂ O)

23 VOC standards (Table 1) were prepared by injecting a small amount of liquid standard (>98% purity) into Tedlar gas sample bags 8-10 L volume filled with zero air from the PTR-MS system. Mass spectra were obtained at 5 or 6 values of E/N (90, 110, 130, 140, 150, 170 Td). The primary ion signal was reduced to < 106 cps to avoid saturating the SEM. To avoid measurements in the non-linear range of the PTR-MS the primary ion signal was not depleted by more than 10 - 30% during sampling of the standards.

The tedlar bag containing the standard mixture was attached directly to the PTR-MS inlet. Data were recorded in PTR-MS scan mode from m/z 21 – 250 with a dwell time of 0.5s, and were averaged over 5 cycles for each value of E/N. A separate bag filled only with zero air was also prepared to determine any interference from the bags.

Results and Discussion

The mass spectra and branching ratios of the product ions resulting from reaction of hydronium ions with ethanol, ethyl formate, methyl isobutyl ketone (MIBK) and hydroxyacetone are presented for a range of E/N values in Figure 1.

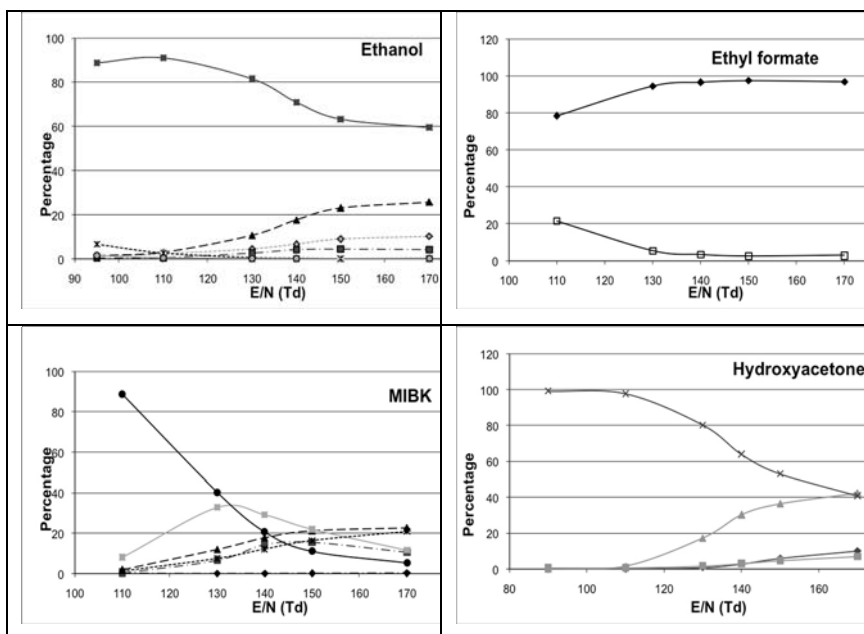


Figure 1: The mass spectra and branching ratios of the product ions resulting from reaction of hydronium ions with ethanol, ethyl formate, methyl isobutyl ketone (MIBK) and hydroxyacetone (Acetol) for a range of E/N values. Branching ratios are presented as a percentage of the total ion signal.

Among the 23 VOCs studied, compounds with the same molecular mass such as butanol, propanoic acid, hydroxyacetone and ethyl formate were distinguishable from one another by their different fragmentation patterns.

The C2-C4 alcohols underwent hydrolysis (loss of a H₂O molecule), at high values of E/N (>130Td). The signal for ethanol and methanol had only minor contributions (<1%) from hydrated cluster ions and dimers. Maleknia et al. in a study of the headspace of ethanol liquid [11] observed a dominant peak at mass 93 for (E/N of ~134 Td) likely the ethanol dimer. At lower concentrations the dimer will be less evident. Low PTR-MS sensitivity to ethanol, previously reported by Warneke et al (2003), was observed in this study, with the total ion signal decreasing from 4.5×10⁵cps at 95Td to 9000 cps at 170Td, likely due to fragmentation to an unmeasurable ion, perhaps mass 19 or 37.

The protonated parent ion was the dominant ion observed in the branching ratios of acetonitrile, acetaldehyde, acetone and MEK. The mass spectra of hydroxyacetone and MIBK are dominated by the protonated parent molecule and one fragment ion at low E/N values (<130Td). This is similar to the branching ratios previously reported for MIBK [12] and hydroxyacetone [13]. Smaller fragments were increasingly observed at higher E/N values for MIBK and hydroxyacetone.

Fragmentation, typically by hydrolysis, was frequently observed in the C3 – C4 acids and esters. Small contributions from hydrated clusters were observed only for formic- and acetic acid. The ethyl formate signal is dominated by a fragment ion at mass 47 and a small contribution from the protonated parent ion.

The PTR-MS had no response to oxalic acid. The proton affinity of oxalic acid is unknown, and may be too low for proton transfer from hydronium ions. However, it is possible oxalic acid protonates and then fragments to an unmeasurable ion. Ervasti et al [14] observed dissociation of self-protonated oxalic acid to H₃O⁺ + CO + CO₂.

More work is to be done to extend the range of compounds studied and to interpret fragmentation patterns in terms of chemical theory.

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¹³CO₂ feeding experiment of four common European boreal tree species: ¹³C incorporation into monoterpenes

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Abstract

Monoterpenes emission from tree species with large storage pools such Scots pine (*Pinus sylvestris*), European Larch (*Larix decidua*), Norway spruce (*Picea abies*) are traditionally calculated using exponentially temperature dependent algorithm. The basic principle lies in the fact that monoterpenes are largely stored into specific pools (such as resin ducts), and their emission rate are strongly dependent on temperature ⁽¹⁾. On the other hand, the monoterpenes emission from non-specific storage pools trees, (e.g. Holm Oak -*Quercus ilex*-, Silver birch -*Betula pendula*-) are calculated using light and temperature dependent algorithm, similar to the algorithm used for isoprene emission ⁽²⁾. This algorithm is based on the light dependence of the electron transport and temperature dependency of the enzymes activity.

It is likely that the monoterpene emissions from tree species with large storage pools are at least part of the year originating partly from specialized storages and partly directly from photosynthesis. Recently there is the development on dynamic process of isoprene emission but there is also the need for similar model development for the monoterpene emissions.

In order to constrain this emission model we need information on the fraction of the emission originating directly from photosynthesis and from the storage pools.

The aim of this study is therefore to quantify this fraction.

For the experiments four common tree species characterizing the European Boreal forest were used: *Pinus sylvestris*, *Larix decidua*, *Picea abies* and *Betula pendula*. In addition, *Quercus ilex* was measured and compared to the *Betula pendula*. Two dynamic cuvettes were run in parallel to measure on-line the gas exchange (Walz GFS-3000, Germany) and the emission of Volatile Organic Compounds (Proton Transfer Reaction Mass Spectrometer, Ionicon, Austria), in particular isoprene and monoterpenes. The label was applied with ¹³CO₂ mixed with synthetic air at 380 ppm (purchase from AirLiquide, France) and cartridges were collected at the steady state for the identification of the VOCs by GC-MS analysis.

The data clearly show a slow incorporation of ¹³C into monoterpenes for the Pinus, Larix and Picea and very fast for Betula and Quercus ilex. This slow incorporation reflects the large monoterpenes storage pools and the slow turn-over. In contrast, the non specific storage trees species (i.e. Betula and Quercus) show a very fast incorporation of ¹³C.

The *de novo* biosynthesis of conifer trees significantly contributes to the total monoterpenes emission. Since the biosynthesis of monoterpenes and their storage in conifer trees might take place during specific stage of plant development or at certain time during the year (e.g. spring), it is likely that the fraction of monoterpenes coming directly from *de novo* biosynthesis changes as well.

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Dynamic Approaches to Measure Partition Coefficients of VOCs

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Abstract

To determine water-air partition coefficients (Henry's law constant, HLC), a series of dynamic approaches have been developed and step-wise improved, towards increased accuracy, extended temperature range and volatility range. These allow the rapid and reproducible determination of HLCs even for molecules with very low volatility, without the need of calibration. The various configurations have been applied to determine the HLCs of volatile organic compounds (VOCs) of different volatility, over the temperature range 20 to 90°C.

Introduction

Partition coefficients play a significant role in describing daily processes like aroma release from food products (e.g. coffee), in environmental chemistry like cloud formation, or in medicine giving insight in human metabolism. The equilibrium of a gas dissolved in a liquid, namely water, can be described via partition coefficients, obeying, in the case of ideal dilute solutions, Henry's Law: $K = c_{aq}/p_{gas}$ [(mol/l)/atm]. In food and flavor sciences, it is usually expressed via the volatility: $K_{PC} = c_{gas}/c_{aq}$ [(mg/l)/(mg/l)], which can be converted into Henry's law constant: $HLC = H = K = 1/(10^{-2} R T K_{PC})$ with the temperature T [K] and the molar gas constant R [J/(K mol)].

Experimental Methods and Results

Based on work by Leroi *et al.* [1] and Mackay *et al.* [2], we developed a stripping cell coupled to a proton-transfer-reaction mass-spectrometer (PTR-MS) to measure dynamically and on-line partition coefficients of VOCs in air/water. Here, an inert gas bubbles through the water solution of a VOC of interest and takes up part of the dissolved gas reaching the equilibrium state corresponding to Henry's law. The time-concentration profile of the compound in the inert gas measured via PTR-MS allows one to determine its partition coefficient. The respective experimental setups for measuring compounds with different degree of volatility are shown in the following. We designed a single stripping cell coupled to a PTR-MS [3],[4] for measuring the partition coefficients of highly volatile compounds, as well as a double cell coupled to the PTR-MS for medium to low volatile compounds [4]. For higher reproducibility and accuracy, we improved the experimental setup by (i) placing the whole setup inside a temperature controlled oven and (ii) adding a cell filled with doubly distilled water in front of the stripping

configuration so as to control the humidity and temperature of the inert gas passing through the setup [5]. In the following, the respective setups are described in detail together with some experimental results obtained with these.

Single stripping cell configuration

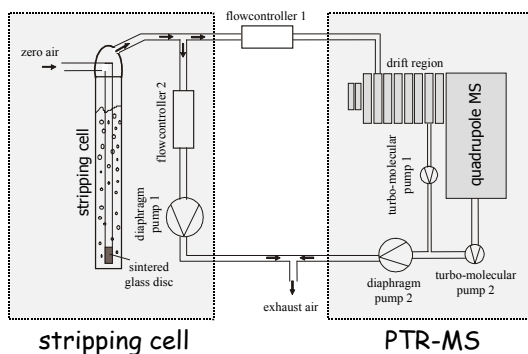


Figure 1: Single stripping cell configuration [3], [4].

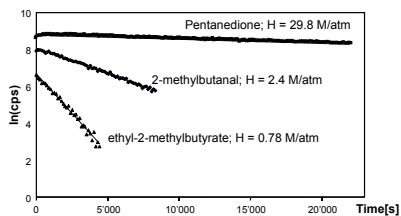


Figure 2: Determination of HLC of the compounds pentanedione (square, low volatile), 2-methylbutanal (rhombus, medium volatile) and ethyl-2-methylbutyrate (triangle, high volatile) at 22°C (flow: 100 ml/min, $V = 100$ ml). The straight lines correspond to a fit according to the equation 2. [3]

Clean air (zero air) flows through a stripping cell filled with doubly distilled water and a small amount of a volatile organic compound (VOC). Passing a sintered glass disc, small gas bubbles are generated taking up dissolved VOC until equilibrium according to Henry's law is reached. The decrease of VOC in water with time is described by equation 1 and can be monitored by plotting the counts per second of the protonated VOC as a function of time according to the equation 2, as the concentration of the VOC is proportional to the counts per second of the protonated VOC ([3] and references therein). There is no need of calibration. For high volatile compounds, the HLC can be determined within minutes. For compounds of lower volatility, measurements of partitions coefficients can take several hours, requiring a very high stability of the setup. To circumvent this problem we have developed a modified setup described in the following section – the double stripping cell configuration.

$$-V \frac{dc}{dt} = \frac{pF}{RT} = \frac{FC}{HRT} \quad \text{eq. 1}$$

$$\ln(cps(t)) = -\frac{F}{HVRT}t + \ln(cps(0)) \quad \text{eq. 2}$$

(V : liquid volume, c : liquid concentration, t : time, p : partial pressure of the compound, F : air flow (STP) through the cell, R : molar gas constant, T : temperature, H : Henry's constant, $cps(t)$: counts per second at time t , $cps(0)$: counts per second at time $t = 0$)

Double stripping cell configuration

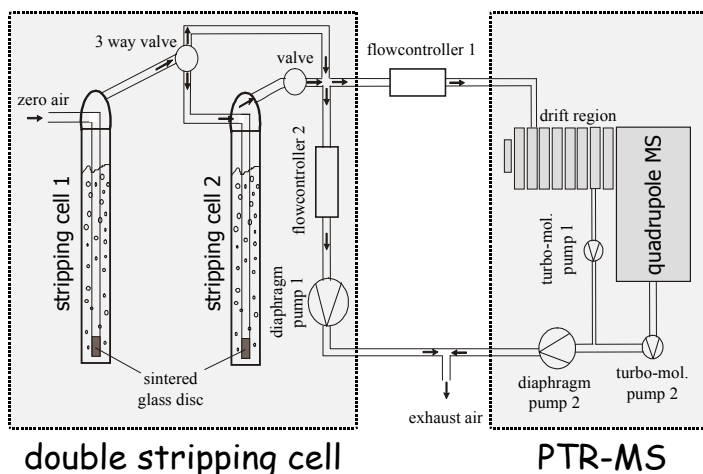


Figure 3: Double stripping cell configuration. [4]

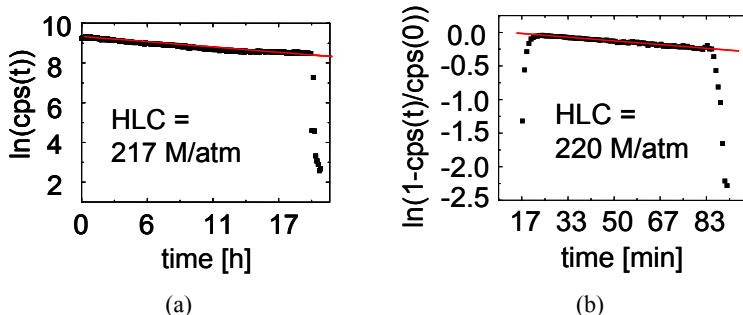


Figure 4: Determination of the HLC of Methanol at 22°C with (a) single cell setup (open circles: measured data, straight line: fit to the equation 2) and (b) double cell setup (open circles: measured data, straight line: fit to the equation 3). Please note the different time scales of (a) and (b). 100ml/min [4]

First, the inert gas bubbles through the stripping cell 1 filled with a highly concentrated aqueous solution of the VOC of interest. Passing through stripping cell 2, the doubly distilled water therein is enriched with the VOC. After the water in stripping cell 2 is saturated, the VOC concentration measured with PTR-MS increases dramatically. The HLC can be determined by plotting the concentration-time profile according to the equation 3.

$$\ln \left(1 - \frac{p(t)}{p_0} \right) = - \frac{HF}{VRT} t \quad \text{eq. 3}$$

Improved stripping cell configuration

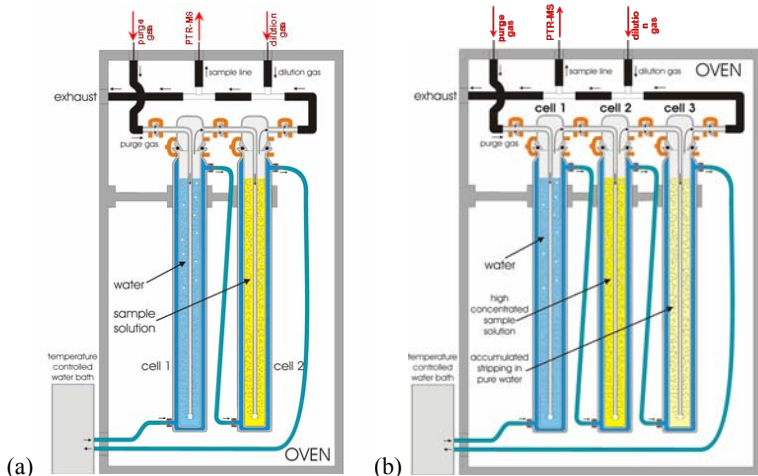


Figure 5: Improved setup of (a) single and (b) double cell configuration. [5]

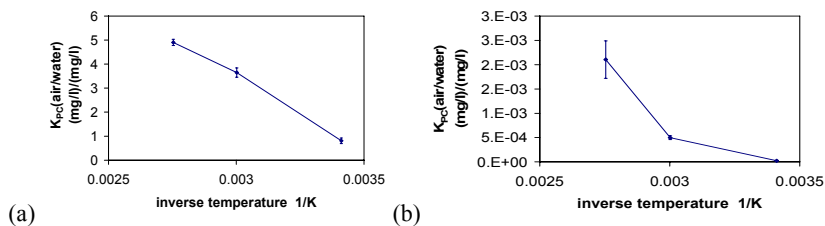


Figure 6: Temperature dependence of the air-water partition coefficient $K_{PC}(\text{air/water})$ of (a) limonene ($HLC = 0.1$), for 20°C, 60°C and 90°C, measured with single cell setup and (b) 2,3,5-trimethylpyrazine ($HLC = 3652$) measured with double cell setup (rhombus: measured data, straight line: fit to the equation 4) [5]

For higher reproducibility and accuracy, and in order to measure partition coefficients over an extended temperature range, the setup was improved in two critical aspects – figure 5. First, the whole setup was placed inside a temperature controlled oven, allowing to work at well defined temperatures (precision better than $\pm 1^\circ\text{C}$). Second, a stripping cell was added in series in front of the setups shown in figures 1 and 3, inside the oven, which was filled with doubly distilled water, so as to tightly control the humidity and temperature of the inert gas passing through the setup [5]. Such improved configurations allow measuring partition coefficients with very high accuracy.

Figures 6 (a) and (b) show the temperature dependence of the air-water partition coefficient $K_{\text{PC}}(\text{air/water})$ of limonene and 2,3,5-trimethylpyrazine, respectively, according to equation 4. These graphs show the importance of measuring the partition coefficient at a precise temperature.

$$\ln\left(\frac{H}{H_0}\right) = \frac{\Delta H_E}{R} \left(\frac{1}{T_0} - \frac{1}{T}\right) \quad \text{eq. 4}$$

(H : HLC at temperature T , H_0 : HLC at T_0 , R : molar gas constant, ΔH_E : enthalpy of solution)

Conclusion

We presented several configurations to measure air/water partition coefficients dynamically via stripping cells coupled to a PTR-MS which allow a highly reproducible, fast and simple determination of Henry's constants. The single cell setup is designed for high volatile organic compounds ($\text{HLC} < 1$), whereas the double cell configuration allows a rapid determination of partition coefficients even for medium or low volatile organic compounds ($\text{HLC} > 1$). Placing the setup in a temperature controlled oven and letting the inert gas flow through a tempered cell filled with doubly distilled water before passing the solution containing stripping cell allows measurements under controlled temperature, gas flow and humidity conditions. We measured a series of air/water partition coefficients, with high accuracy and under tightly defined conditions as was shown for (i) pentanedione, 2-methylbutanal and ethyl-2-methylbutyrate measured with the single cell setup, (ii) methanol measured with both configurations and (iii) the temperature dependence of the partition coefficients of the highly volatile compound limonene as well as the low volatile compound 2,3,5-trimethylpyrazine.

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Volatile Organic Compound Production and Consumption from Litter Samples

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Abstract

Biogenic volatile organic compounds can have important effects on atmospheric chemistry, soil ecology and terrestrial biogeochemistry. Microorganisms produce a wide range of such VOCs and the emissions of such microbial VOCs (mVOCs) from decomposing litter can be substantial, but knowledge gaps remain. Specifically, we do not know what percentage of measured VOCs emitted from litter are produced via abiotic processes. We have limited understanding of how VOC fluxes change over the course of litter decomposition and how the types and quantities of VOCs produced differ across a range of litter types. The final knowledge gap to be examined is how the addition of nitrogen might affect VOC production or consumption. The experiment is scheduled for the winter of 2008 and will incorporate 12 litter types including a mix of grasses, conifers and deciduous tree species.

Introduction

Biogenic volatile organic compounds (VOCs) are reactive gases of low molecular weight produced by biological activity in a wide range of natural systems. Biogenic VOCs can have important effects on atmospheric chemistry, reducing hydroxyl radical (OH) concentrations, increasing tropospheric ozone and producing organic nitrates in the atmosphere^{1,2}. Although the emissions of VOCs from plants have received the vast majority of the attention from atmospheric scientists and ecosystem ecologists, plants are not the only biological source of VOCs in terrestrial ecosystems³. Microorganisms produce a wide range of VOCs and the emissions of such microbial VOCs (mVOCs) from decomposing litter can be substantial⁴⁻⁶.

These mVOCs emitted from litter have not been well-studied. Besides their impact on atmospheric chemistry, there is a growing awareness that they can have important effects on soil fertility and microbial interactions within soil. Several studies have shown that certain VOCs can alter microbial rates of nitrification, denitrification and nitrogen mineralization^{7, 8}. Microbial interactions within the soil may be regulated by mVOCs inhibiting or stimulating growth⁹. In short, mVOC production and consumption during litter decomposition may be an important component of terrestrial ecosystem function and biosphere-atmosphere interactions, but key knowledge gaps remain.

Specifically, we do not know what percentage of measured VOCs emitted from litter are produced via abiotic processes⁶. VOC fluxes correlate well with CO_2 suggesting that abiotic

process play a small role but there no research verifying this assumption. Likewise, we have limited understanding of how VOC fluxes change over the course of litter decomposition and how the types and quantities of VOCs produced differ across a range of litter types. An additional knowledge gap is how the addition of nitrogen might affect VOC production or consumption. Preliminary evidence suggests that nitrogen additions can dramatically influence VOC fluxes from litter (N. Fierer, unpublished). If this phenomenon is found to be widespread, it could be important given the potential role of these VOCs in ecosystems and the well-documented increase in N inputs to terrestrial ecosystems from anthropogenic sources.

Question 1: What percentage of the VOCs produced during litter decomposition are derived from abiotic vs. biotic (i.e. microbial) sources? *H1: Abiotic production of VOCs will be measurable, but short-lived with rates that are far lower than rates of microbial VOC production.*

Question 2: Does VOC production and consumption vary between different litter types during litter decomposition? *H2: Each litter type will have distinctly different VOC production and consumption profiles during decomposition because distinct litter types have unique litter chemistries and are likely to be decomposed by unique microbial communities.*

Question 3: Does the addition of nitrogen change VOC production and consumption during litter decomposition? *H3: The addition of nitrogen will alter rates of VOC production and consumption, particularly in low-nutrient litter types, due to changes in decomposer community composition*

Experimental Methods

Question 1: Twelve litter types will be separated into individual containers, sterilized, and maintained under aseptic conditions. Litter types that will be used will include 2-3 pines, 2-3 grasses and 6-8 deciduous trees. All containers will be autoclaved in order to sterilize the litter. The non-sterile replicates will be re-inoculated using a small amount of soil to introduce the microbial decomposers. Containers without litter will be used for experimental controls (“blanks”) to measure background VOC concentrations. Headspace VOC and CO₂ measurements will then be taken on regular intervals using a Proton Transfer Reaction – Mass Spectrometer (PTR-MS) and an infrared gas analyzer respectively. Measurements can be taken until abiotic VOCs taper off. Use of a PTR-MS will allow for frequent measurements of samples for an extended duration. However, because the PTR-MS cannot distinguish between different compounds with the same mass, measurements of the headspace will also be analyzed using a GC-MS housed at National Oceanic and Atmospheric Administration.

Question 2: Following the above experiment, the same setup can be used with the exception of the need to sterilize the setup. I will track VOC production and consumption across a 60-d period in each of the 12 litter types, with 4 replicate containers of each litter type.

Question 3: At the same time as the above experiment, a replicate of the above setup can be used with an addition of nitrogen (NH₄NO₃) at the beginning of the experiment to each container. For this question the data from question 2 will act as a control.

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Influence of respiratory manoeuvres on the 'on-line' detection of volatile organic compounds (VOCs) in exhaled by hs-PTR-MS

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Abstract

Background: Modern gas analysis techniques now permit real time and on-line quantification of multiple VOCs within a single exhalation. However, the influence of various respiratory manoeuvres affecting exhalation flows, pressures and the kinetics of metabolite release to the gas phase are largely unknown. **Methods:** We examined the variation in the concentrations of selected VOCs over a range of expiratory flows (50; 100; 250ml/s), as well as after 30s periods of breathhold and paced hyperventilation. On-line and real time measurement of breath samples from healthy volunteers was performed by hs-PTR-MS (n=10). Integration of the hs-PTR instrument with the LR2500 analyser allowed for on-line monitoring of CO₂, NO and expiratory pressures. **Results:** The concentration of ethanol was inversely correlated ($r=-0.87$) with expiratory flow rate and measured differences were statistically significant between flows of 50 and 250ml/s (410 vs 314ppb respectively, $p=0.02$). In contrast, acetone concentrations increased with higher flows ($r=0.95$; 805, 838 and 868 for flows of 50, 100 and 250ml/s; $p<0.05$). Exhaled methanol (206 vs 179; $p=0.001$), acetaldehyde (26 vs 22; $p=0.001$), and ethanol (410 vs 208; $p=0.001$) were also significantly reduced following hyperventilation, whilst methanol (206 vs 217; $p=0.01$), acetone (805 vs 869; $p=0.001$) and isoprene (348 vs 390; $p=0.01$) levels were all increased after the same duration of breathhold. **Conclusions:** These findings demonstrate that respiratory manoeuvres influence significantly the measured concentration of a number of human breath metabolites that are of potential importance within the clinical setting. Similarly to exhaled NO, our results underscore the requirement for the adoption of standardised practices for monitoring of exhaled VOCs by on-line mass spectrometry methods.

Introduction

Analysis of volatile organic compounds (VOCs) within exhaled breath is a promising field of medical research which offers an opportunity for the development of novel non-invasive diagnostic approaches. Recent technological advances within the field of exhaled breath gas analysis, characterised principally by the development of instruments such as hs-PTR-MS and SIFT-MS, now permit the real time and on-line quantification of multiple VOCs within a single exhalation. Currently however there remains a limited resource of experimental evidence defining

the characteristic effects and potential significance of confounding factors which are predicted to influence the quantification of many of these compounds within the breath. In particular measurement-specific sources of variability, which are perhaps the most amenable to regulation within the clinical setting, have received little attention. We have therefore investigated the effect of a range of respiratory manoeuvres, including expiratory flow rate, on the absolute concentrations of trace gases detected within the exhaled breath of healthy volunteers using PTR-MS.

Experimental Methods

Local ethics committee approval was obtained for the current study and prior to enrolment all subjects provided informed verbal consent.

Sampling manifold

The on-line and real time monitoring of multiple selected VOCs within the exhaled breath of human subjects in addition to respiratory pressures and flows was achieved by integration of two independent gas analysis systems, hs-PTR-MS (IONIMED Analytik GmbH, Innsbruck, Austria) and the LR2500 multiple-gas analyser (Logan Research Ltd. Rochester, UK) [Figure. 1].

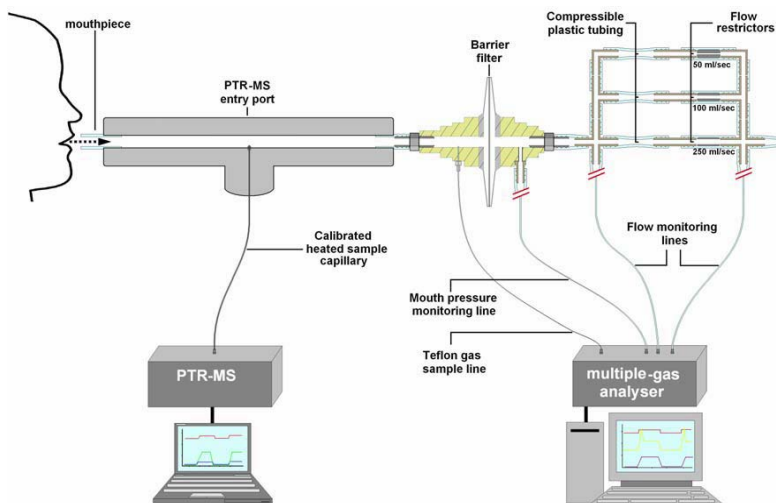


Figure 1: Schematic representation of *hs*-PTR-MS manifold.

Respiratory manoeuvres:

The current experiment was divided into two phases. Initially subjects were asked to exhale into the experimental manifold over a range of expiratory flows (50; 100; 250ml/s). At each flow rate subjects were instructed to exhale into the manifold for approximately 20s whilst maintaining a mouth pressure of $>5\text{cmH}_2\text{O}$. Subjects were then asked to perform two further manoeuvres; breathhold (30s) and paced hyperventilation (30s), after completion of which they were then

instructed to exhale into the manifold as already described. For exhalations performed following breathhold and hyperventilation a flow rate of 50ml/s was set as standard. For each of the five manoeuvres examined subjects performed three separate repeat measurements. VOC concentrations in ppb, were calculated using the results of previously completed calibrations experiments. Statistical analysis was performed using SPSS 14.0 software package (SPSS Inc., Chicago, USA.). Median values and standard deviation were computed for all exhaled compound concentrations. Comparisons between breath manoeuvres were performed using the Sign test. The level of statistical significance was assigned to one-sided p-values less than or equal to 0.05.

Results

Human subjects: The sample group of subjects consisted of 10 healthy, non-smoking volunteers (6 male; 4 female) of age 29 ± 7.3 yrs (21-45), and BMI 24.6 ± 4.6 (19-35). All subjects had been fasted for a minimum of 1hr prior to breath sampling.

Flow rate: It was observed that the concentration of NO within exhaled breath was inversely correlating ($R = -0.97$) with expiratory flow rate. Like NO, the concentration of ethanol within exhaled breath was also observed to be inversely correlated with expiratory flow rate, although a significant difference was only observed between measurements made at 50 and 250 ml/s ($R = -0.87$; $410\text{ppb} \pm 301$ vs 314 ± 197 ; $p = 0.02$). In contrast, acetone concentrations increased at higher flows ($R = 0.95$; 805 ± 489 , 838 ± 554 and 868 ± 568 ppb for flows of 50, 100 and 250ml/s, $p < 0.05$) [Figure. 2].

Hyperventilation: Following hyperventilation the concentration of CO_2 was significantly reduced ($4.5 \pm 0.4\%$; $p = 0.001$) in comparison to its level after normal breathing (5.5 ± 0.4 ; value recorded for standard exhalation at 50ml/s). In addition the concentrations of methanol (206 ± 80 vs 179 ± 73 ; $p = 0.001$) [Figure. 2], acetaldehyde (26 ± 13 vs 22 ± 4 ; $p = 0.001$), and ethanol (410 ± 301 vs 208 ± 101 ; $p = 0.001$) were also significantly reduced following hyperventilation.

Breathhold: After a 30s end inspiratory breathhold, the median concentrations of CO_2 (5.5 ± 0.4 vs 5.9 ± 0.4 ; $p = 0.05$), methanol (206 ± 80 vs 217 ± 83 ; $p = 0.01$), acetone (805 ± 489 vs 869 ± 506 ; $p = 0.001$) and isoprene (348 ± 204 vs 390 ± 215 ; $p = 0.01$) [Figure. 2] within exhaled breath samples were observed to be significantly increased above levels recorded after normal breathing.

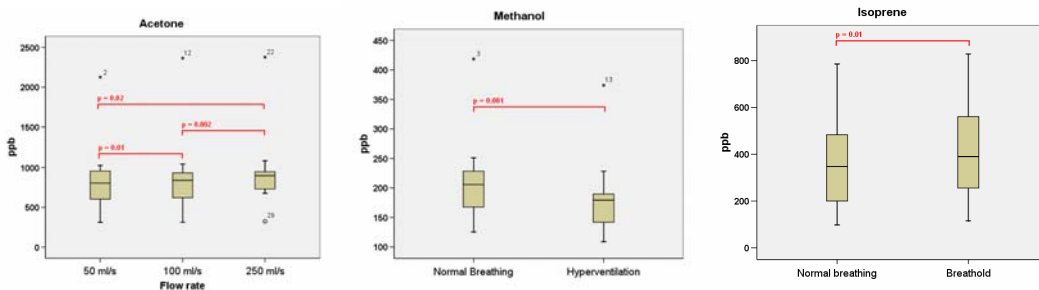


Figure 2: Influence of respiratory manoeuvres on the concentration of selected exhaled VOCs

Discussion

In accordance with the findings of Silkoff *et al.*, [1] and numerous other authors, we observed a significant inverse relationship between expiratory flow rate and NO concentration. In the present study we also identified that ethanol exhibited a similar degree of flow dependency with levels decreasing at higher expiratory flows. We predict that an increase in ethanol concentration at higher expiratory flow rates can be explained by a longer contact time between inhaled and subsequently exhaled air within the alveolo-capillary unit, thus allowing for increased evaporation of ethanol from the bloodstream into the gas phase. In contrast, the concentration of acetone within expiratory air increased significantly at higher flow rates. As suggested by other authors higher exhalation flows may result in shorter contact time between exhaled alveolar air and the airway wall, and thus a small re-uptake of VOCs such as acetone [2]. This however directly contrast with our explanation for the observed flow dependency of ethanol and would be in turn hard to confirm.

The finding of significantly decrease concentrations of CO₂ after 30s hyperventilation, importantly verified our protocol for this manoeuvre. VOCs whose levels were observed to decrease following hyperventilation included methanol, acetaldehyde and ethanol. Although unconfirmed it is likely that hyperventilation decreases the levels of selected exhaled trace gas in much the same way as it does CO₂. As a result of increasing minute ventilation, there is a greater rate of exchange between alveolar and ambient air, the effect of which being that for CO₂ and certain other VOCs the corresponding rate at which they are evaporated from the bloodstream and excreted from the body is increased. Eventually this process leads to depletion of circulating and hence expiratory levels of these compounds.

As expected we observed a significant increase in CO₂ concentration within exhaled breath samples for subjects following breathold. Also observed was a significant increase in the concentration of isoprene within breath samples analysed following breathold, a result which confirmed the recently published findings of Lärstad *et al.* [2]. Other VOCs appearing at higher concentrations follow breathold included acetone and methanol. One possible explanation for these findings is that end inspiratory breathold prolongs the time permitted for evaporation of certain VOCs from the blood into the alveolar air space. Should this prove to be correct one could also assume that diminished minute ventilation would have a similar, albeit relative effect. Although we have attempted such experiments, we have thus far been unable to satisfactorily induce hypoventilation in conscious volunteers.

Finally it is important to report that the concentrations of three VOCs, formaldehyde, propanol and hexanal, were not significantly affected by variation of either expiratory flow or minute ventilation.

Conclusions:

We conclude that variation of expiratory parameters has a significant effect on the concentrations of certain exhaled VOCs. We can tentatively suggest possible explanations for this variability, based on current knowledge; however future work should now focus on explaining the precise pulmonary dynamics of these compounds. We would propose that until such time as this work has been performed, investigators should adopt the current ATS/ERS recommendations for the measurement of exhaled NO [3], when performing on-line analysis of exhaled VOCs.

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Quantification of 2-Methyl-3-buten-2-ol Emissions above a Ponderosa Pine Forest in the Western U.S.

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Abstract

2-Methyl-3-buten-2-ol (232MBO) is the dominating volatile organic compound (VOC) emitted from coniferous ecosystems in the Western United States. Here we investigate the potential of the PTR-MS technique to quantify 232MBO in laboratory and field experiments. Fragmentation analysis of 232MBO and its isomers is presented as a function of the E/N-ratio in the PTR-MS drift tube. Ion fragmentation patterns obtained from pure standards are compared with ion distributions observed ambient air. The influence of potentially interfering VOCs (e.g. isoprene) is examined. The obtained data suggest that PTR-MS can provide an alternative approach for quantifying 232MBO emissions from vegetation.

Introduction

Volatile organic compounds (VOCs) critically influence the composition of the Earth's atmosphere by fueling tropospheric chemistry [1]. While isoprene dominates global biogenic VOC emissions, the mix of biogenic VOCs can be regionally quite distinct. For example, Goldan et al. [2] characterized the VOC composition of ambient air at a remote mountain site (3050 m elevation) in Colorado in June 1991 and found that a C-5 alcohol, identified as 2-methyl-3-buten-2-ol (hereinafter referred to as 232MBO), was the dominant organic trace compound, with concentrations 4 to 7 times those of isoprene. Based on the fact that diurnal changes in ambient 232MBO concentrations were very similar to those of isoprene, with known biogenic sources, and on the fact that 232MBO concentrations did not correlate well with those of benzene, an indicator of anthropogenic sources, they concluded that there was likely to be a large, unidentified local biogenic source of 232MBO. The oxygenated compound 232MBO may be the predominant reactive VOC above pine forests in the western U.S.. Quantification of 232MBO is challenging, because it is known to dehydrate in gas-chromatography-desorption systems [3]. Here we evaluate the PTR-MS technique for analysis of 232MBO in laboratory and field experiments. The field measurements were conducted at the BEACHON research site in Colorado (39° 6'2.59"N, 105° 6'11.75"W; approx. 2350 m a.s.l.) between August 10th and December 10th 2008. The site is dominated by ponderosa pine (*Pinus Ponderosae*).

Experimental Methods

Two high sensitivity PTR-MS instruments were used in this study. A modified high sensitivity PTR-MS system was deployed for field measurements. This instrument was operated at 2.3 mbar and 105 Td. Periodic calibrations using multi VOC component standards were used to characterize sensitivity changes over time. A second high sensitivity PTR-MS was used to quantify the relative abundance of the major MBO product ions m/z 87⁺, 69⁺, and 41⁺ in systematic laboratory experiments. Product ion distributions were investigated at 2 mbar drift pressure with E/N-values ranging from 99 to 132 Td.

Results and Discussion

Product Ion Distribution of 232MBO

Figure 1 shows the fragmentation pattern of 232MBO as a function of the E/N-ratio (E denotes the electric field strength, N the gas density in the drift tube, 1 Td = 10⁻¹⁷ cm² V molecule⁻¹). Three major product ions (m/z 41⁺, m/z 69⁺ and m/z 87⁺) are characteristic for this particular isomer. The major fragment ion is m/z 69⁺. The parent ion (m/z 87⁺) accounts for 16 to 19% of the total ion abundance. This fraction does not change drastically within the range of E/N values investigated here. High values of E/N mainly influence the partitioning between m/z 69⁺ and m/z 41⁺. Product ion distributions of 331MBO, 231MBO and 321MBO were also investigated. They produce different relative product ion distributions. At 119 Td the following relative abundances (m/z 87⁺ : m/z 69⁺ : m/z 41⁺) were observed for 331MBO, 231MBO and 321MBO respectively: 3.5:81.6:14.9, 11.4:65.5:23.1 and 0.4:77.4:22.2.

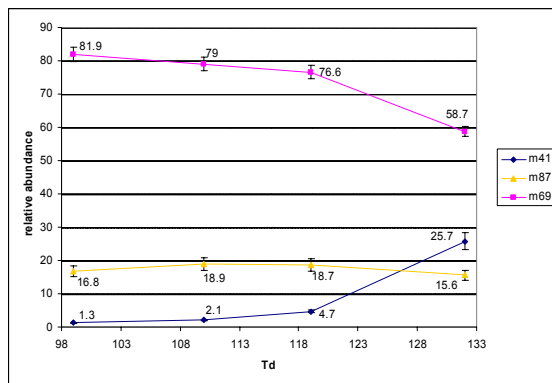


Figure 1: Fragmentation pattern of 232MBO as a function of E/N (in Td)

Fragmentation of MBO isomers forms product ions, which interfere with the detection of other important biogenic VOCs (e.g. m/z 69⁺ - isoprene). Examination of the relative product ratios can be useful to distinguish MBO isomers and isoprene. Considering the predominance of 232MBO emission in natural ecosystems of the Western U.S. the ratio between m/z 69⁺ and m/z 87⁺ can be used to investigate the residual fraction of isoprene contributing to m/z 69⁺.

Field Measurements

The relative fraction m/z 69⁺/(m/z 69⁺+ m/z 87⁺) observed during daytime at the BEACHON research site is shown in Figure 2. Ratios are plotted as a function of height (y-axis). The forest canopy extended to approx. 17m above ground. The ratio obtained from above canopy eddy

covariance (EC) measurements agrees well with the top of the canopy ratio derived from an inverse Lagrangian transport (ILT) model. Both lie within less than 1% of the ratio expected from pure 232MBO emissions. Mixing ratio data (blue circles) suggest a slightly lower abundance of $m/z\ 69^+$ (on average 2%) than expected if they were solely due to 232MBO emissions. This implies a small contribution on $m/z\ 87^+$ from other VOCs advected to the site. A slightly higher abundance of the emission signal on $m/z\ 69^+$ in the middle of the canopy inferred from the ILT model indicate a contribution from isoprene (or from other biogenic VOCs) Assuming that this increase was due to isoprene emissions we can estimate the fraction relative to 232MBO (2.8%). It would relate to an isoprene emission rate of approx. $0.4\ \text{mg}/\text{m}^3/\text{h}$. In conclusion, the ion-distribution observed at the BEACHON research site suggests that 232MBO is the major contributor (e.g. >95%) to $m/z\ 69^+$ and $m/z\ 87^+$.

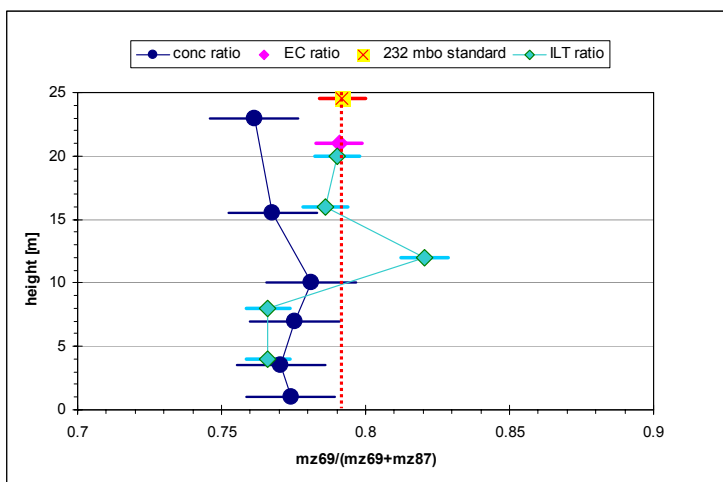


Figure 2: $m/z\ 69^+/(m/z\ 69^+ + m/z\ 87^+)$ observed in ambient air and calculated by two flux measurement methods (ILT, EC). For reference the ratio obtained from a 232MBO standard is also shown, indicated by the red dotted line.

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Acknowledgements

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A Commercial High-Resolution, High-Sensitivity (HRS) PTR-TOF-MS Instrument

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Abstract

Most PTR-MS instruments employed so far, use quadrupole filters to analyse product ions generated in the reaction drift tube. The low mass resolution of the quadrupoles used limited the possibility to identify the trace gases under study.

Here we report the product launch of a new version of PTR-MS using a time-of-flight mass spectrometer, which is capable of measuring VOCs at ultra low concentrations (as low as a few pptv) under high mass resolution (as high as 7000 in the V-mode) with a mass range of beyond 50.000 amu.

Instrument Description

The present HRS PTR-TOF-MS is an instrument based on the design of the commercially available High Sensitivity PTR-quadrupole-MS instrument from IONICON [1]. The instrument was constructed by interfacing the well characterized IONICON hollow cathode ion source and drift tube section with an orthogonal acceleration reflectron time-of-flight mass spectrometer. An instrument schematic of the HRS PTR-TOF-MS is given in Fig. 1.

A first exploratory prototype version with a detection limit of 20 pptv for 1 min integration time has been constructed and tested in the Institut für Ionenphysik by Hansel and co-workers [2] in collaboration with IONICON.

The IONICON High-Resolution, High-Sensitivity (HRS) PTR-TOF-MS based on this prototype [2] has recently been launched as a serial product and achieves a detection limit of less than 10 pptv for 1 min integration time. It is mounted in a single mobile rack, where the mass spectrometer, the ion source and drift tube system, the vacuum system (2 split-flow turbo pumps and 1 foreline backing pump) and the whole electronics including the pulse generator are integrated. The rack dimensions are 56 x 130 x 78 cm (22 x 51,2 x 30,7 in., WxHxD) and the whole instrument weighs approximately 170kg (375 lb). The instrument is featuring a touch screen display serving as status indicator for: pressures, voltages, pressure and flow controller values, temperatures and turbo pumps. The data acquisition and analysis software is situated in an external desk top computer and display system.

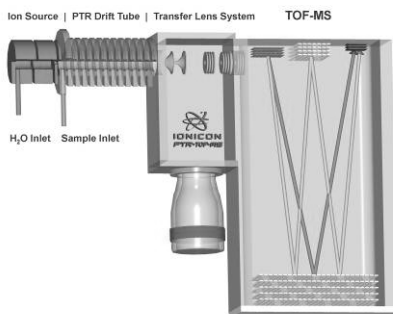


Figure 1: Schematic drawing of the HRS PTR-TOF-MS instruments, consisting of the hollow cathode discharge ion source, the drift tube reaction chamber, the transfer lens system and the reflectron time-of-flight mass spectrometer.

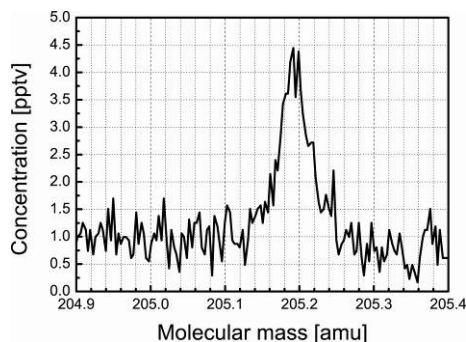


Figure 2: PTR-TOF-MS spectrum of sesquiterpene demonstrating the rather high mass resolution and low background for an extremely small signal from an urban air sample. The signal has been obtained by averaging 10 measuring cycles each running for a 4 min integration time

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Performance Evaluation

Several facts are responsible for the fast response time (smaller than 100ms), high sensitivity (up to several tens of cps/ppbv) and low detection limit (down to a few pptv, see Fig.2) achieved in the present instrument as compared to the previously reported TOF set-ups [3-5]. Most importantly, the extremely low detection threshold is due to the interplay of the higher duty cycle of the unique TOF-MS layout, the specially designed transfer lens system, the dimensions of the pulse extraction region, and the low background noise of the multichannel-plate (see Fig.2). Moreover, especially rewarding is the fact that in contrast to quadrupole mass filter detection, where the transmission of ions is strongly deteriorating with increasing mass (usually a factor of 3 when going from mass 100 to 180), in the present case sensitivity (transmission) is increasing

when going from 21 to larger masses (e.g. transmission factor is seen to increase by about a factor of 5-7 when going from 21 to 181). The certified mass range accessible for the present instrument is in excess of 50 000 amu. From extended measurements with calibrated gas samples we can confirm linearity of response over a concentration range from about 10 pptv up to 10 ppmv. This is similar to the performance of the commercially available IONICON High Sensitivity PTR-quadrupole-MS instruments.

Experimental Methods

In order to demonstrate the performance and the some of the specific advantages of the presently available HRS PTR-TOF-MS, i.e. high mass resolution, high sensitivity, ultra-low detection limit, we have carried out measurement of the urban air just outside of our laboratory in Innsbruck. One of the major traffic connections into the city is passing just 100 m in front of the laboratory. Besides VOCs from the airport Greenland (nearby) and the city in general, substantial VOC pollutants are expected in this environment from the adjacent road depending on the specific traffic situation. Urban air is sampled over a number of days via a heated PEEK sampling capillary (inner diameter of 1mm and the length of about 3m) from just outside the laboratory building (appr. 5 m above ground) directly into the HRS PTR-TOF-MS instrument.

Results

Figure 3 shows an example of mass spectra in their expanded view, recorded during daytime within a 40 min period (average of ten 4 minute scans). The mass resolutions of about 5000 (operating the instrument in V-mode) given in Fig. 3, immediately reveal that each nominal mass consists of several clearly resolved peaks indicating the presence of multiply species at a single nominal mass.

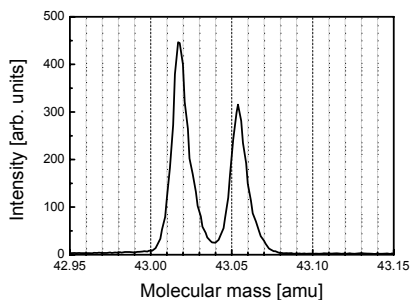


Figure 3a: Expanded view of nominal mass 43; protonated ketene at 43.0184 and protonated propene at 43.0548.

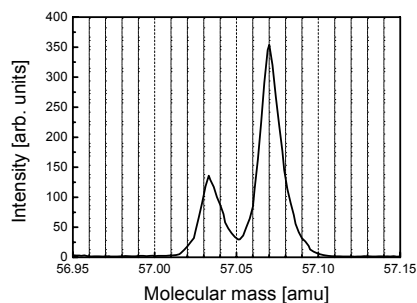


Figure 3b: Expanded view of nominal mass 57; protonated methylketene at 57.034 and protonated butene at 57.0704.

Figure 4 presents the results of absolute concentration measurements yielding the temporal variations of some major VOCs (traffic and biogenic compounds) present in the urban air. It is particularly interesting that in some cases the temporal behaviour of two compounds having the same nominal mass is quite different, in this case mass separation as achieved in the present case is of importance. A vivid example of this can be seen in Fig. 4a where methylketene at mass 57.034 takes a quite different behaviour over the three day period than butene at mass 57.0704, in contrast furan at mass 69.034 shows a rather similar dependence as isoprene at 69.0704 except for a strong deviation on Monday morning showing a strong increase for isoprene. In summer when isoprene emissions are much higher this may be different. Similar large differences at least in magnitude are present for other isobaric pairs, e.g. see in Fig. 4b (the two species located at mass 75.081 and 75.0446). Given these results, the differing behaviour observed and documented here for the first time highlights and demonstrates possible applications of PTR-TOF-MS in emission studies under complex primary source conditions.

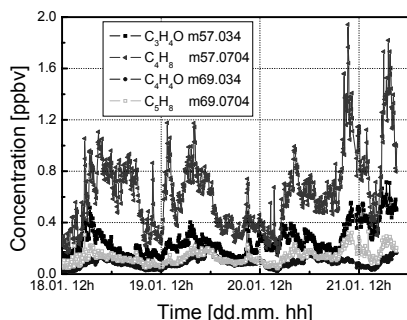


Figure 4a: Methylketene at 57.034, butene at 57.0704, furan at 69.034 and isoprene at 69.0704

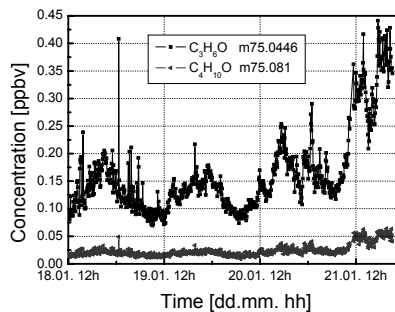


Figure 4b: C₃H₆O₂ likely acetic acid methyl ester and formic acid ethyl ester at 75.0446 and C₄H₁₀O likely dimethylethanol at 75.081

Conclusion

The present study clearly demonstrates the great potential of the newly developed PTR-Time of Flight-MS. In contrast to commercially available quadrupole PTR-MS instruments, the HRS PTR-TOF-MS described here can generate entire mass spectra (snapshots) of complex trace gas mixtures on short response times (smaller 100 ms) with high mass resolution (in V-mode typically around 5000 and higher) and with virtually no upper mass limit (confirmed range of over 50 000 amu). In addition the present instrument is highly sensitive (even for large masses yielding several tens of cps/ppbv) and features an extremely low detection limit (a few pptv). Together these unique features make this instrument a useful and valuable tool for trace gas analysis in all kind of fields, including atmospheric and environmental science, food and flavour science, medical applications and industrial monitoring.

Acknowledgements

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Better Response-Times with a New High-Temperature PTR Drift Tube

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Introduction

We present the development of a new drift tube design featuring a much faster response time compared to the versions used so far in commercially available PTR-MS instruments. Especially for fast dynamic systems, like flavour analysis or atmospheric flux measurements [1, 2], improvements in the time resolution are directly connected to the accuracy of measurements. The new drift tube features both a higher reaction gas exchange frequency and a new heating concept allowing for constant temperatures of up to 200°C.

Moreover, the better performance of the new drift tube is especially important when analyzing very “sticky” compounds where the response time can dramatically be improved by using higher temperatures (e.g. 200°C).

Experimental

Measurements were carried out with two independent PTR-MS systems, one equipped with a standard drift tube and one equipped with the new high resolution drift tube. The inlet capillaries of these two PTR-MS systems were connected to one mouth piece. With this set-up we carried out breath gas analysis (with high breathing frequency, i.e. one breath cycle approx. 2s) on test persons to investigate corresponding differences in the response time. The inlet flow on both instruments was set to 500sccm. The inlet temperatures as well as the drift tube temperature were maintained at 80°C during the experiments. The dwell time on both instruments for the measurement were set to 10ms. Approximately 1cm of the inlet tube at the mouth-piece-end was not heated in order to avoid artifacts by reactions from the test persons to the high temperature.

Results

Figures 1-4 below exhibit the results of measurements carried out with the experimental set-up described above thus allowing us to compare the performance of the two different drift tube designs. From these measurements clear differences in the observed response times can be deduced. We take the decrease of the acetone concentration (at the trailing edge of a breathing cycle) from 90% to 10% of the signal as a convenient measure for the response time of a system. For the new drift tube this time for acetone is about 40-50ms as shown in Fig. 2 whereas for the old drift this time is 80-90ms. Acetone is not very sticky and the difference in the decomposition

time is just due to the higher gas exchange frequency possible by the new drift tube layout. Thus the gas exchange frequency of the new drift tube is by a factor of 2.5 higher.

For very sticky compounds the new drift tube is, however, showing much larger differences for this decrease times (see the examples given in Fig.3 and 4). This is caused partly by the higher gas exchange frequency and partly by the different construction materials which are used in the new drift tube.

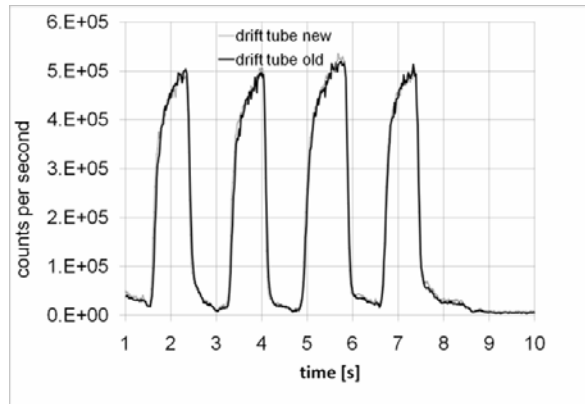


Figure 1: Comparison between old and new drift tube by measuring acetone (as an example of a “non-sticky” compound) in a breath gas sample (4 breathing cycles).

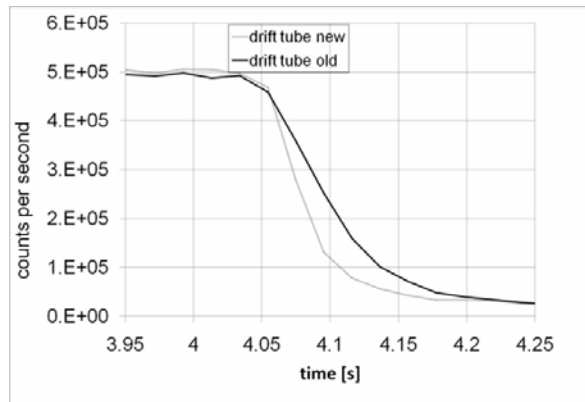


Figure 2: Comparison between old and new drift tube by measuring acetone (as an example of a “non-sticky” compound) in a breath gas sample with higher time resolution than in Figure 1 clearly demonstrating the faster response time in case of the novel design.

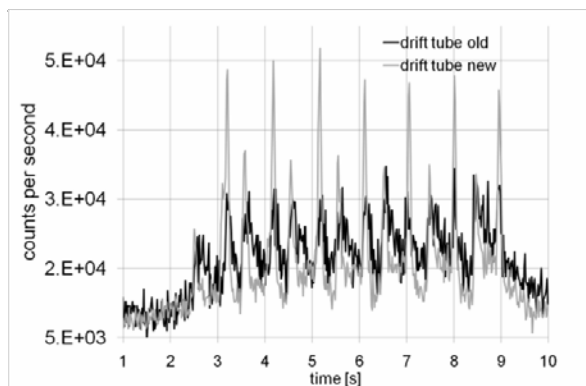


Figure 3: Comparison between old and new drift tube by measuring acetic acid (as an example for a sticky compound) in a breath gas sample (7 breathing cycles).

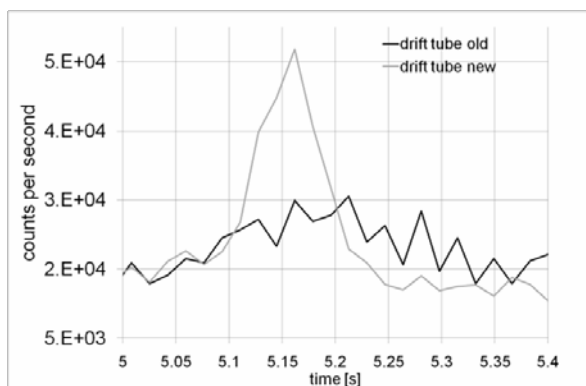


Figure 4: Comparison between old and new drift tube by measuring acetic acid (as an example for a sticky compound) in a breath gas sample with higher time resolution than in Figure 3 clearly demonstrating the superior performance of the novel design.

Discussion

The new drift tube design with its higher reaction gas exchange frequency has a much faster response time especially for “sticky” compounds, even at the same moderate temperatures as those drift tubes available, so far used to work with.

Great potential can be expected in terms of further improvements related to measurements of flavours and other difficult compounds by the possibility to heat the PTR drift tube up to 200°C compared to the previously used maximum of 120°C.

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Automated chamber measurements of VOC emission in boreal forest

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Abstract

We have used automated chambers with PTR-MS to measure branch scale emissions of several VOCs from Scots pine. Measurements showed Scots pine to emit methanol, acetaldehyde, acetone and monoterpenes.

Introduction

VOCs contribute to the formation and growth of atmospheric particles which are an important factor in the climate system. In the ecosystem scale boreal forest is known to emit oxygenated volatile organic compounds (OVOC) but the emissions dynamics of individual trees is rather poorly known.

Experimental Methods

Measurements were carried out at the SMEAR II measurement station [1] (station for measuring ecosystem-atmosphere relations) in Hyytiälä Southern Finland (61° N, 24° E, 180 m a.s.l.) in summers 2007 and 2008. The forest around the measurement station is dominated by Scots pine with some Norway spruce, aspen and birch. The canopy height of the forest is about 16 m.

PTR-MS measurements were divided to three cycles each of them lasting an hour. The first hour was allocated to the ambient air volume mixing ratio measurements [2], the second to ecosystem scale flux measurements [3] and the third to chamber measurements.

In our automated chamber system branches of Scots pine in the upper part of the canopy were enclosed into chambers. The chambers remained open most of the time and were closed periodically four times per hour for less than two minute periods. Sample air was drawn from the chamber to the PTR-MS via about 30 m long Teflon tubing (see Fig. 1 in [2]). During the summer 2007 we used one chamber and during the summer 2008 two chambers.

Results and Discussion

As an example one of week measurements from June 2007 are presented in Fig 1. Emissions of three OVOCs methanol, acetaldehyde and acetone as well as monoterpenes have a clear diurnal cycle. The emission of OVOCs corresponds to over 80% of the total emission of these four

compounds. Previously published results reported that in Hyytiälä the ecosystem scale monoterpene emissions correspond to about 50 % of the total emission of these compounds [4].

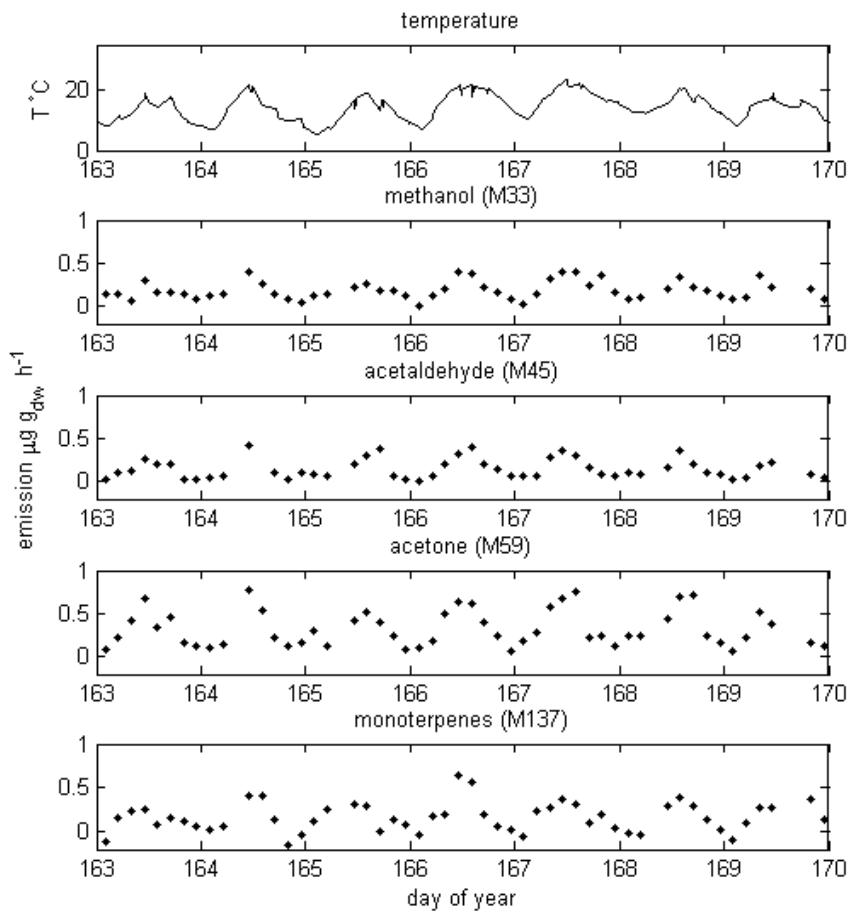


Figure 1: Hourly mean temperature and hourly mean emissions of methanol, acetone and monoterpenes during one selected week in summer 2007.

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Proton-transfer-reaction mass spectrometry online analysis of volatile organic compounds in the exhaled breath: kidney transplant rejection diagnosis

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Abstract

On-line breath gas analysis was performed on 110 kidney transplant patients. We used high sensitive Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) for trace gas analysis of volatile organic compounds in the exhaled breath. For its very fast response time single breath manoeuvres could be resolved into their different exhalation phases. Measured data points of end tidal air, which is representative for alveolar air, were selected mathematically for further analysis. In total 704 breath tests comprising at least 48 different components were conducted. Whenever the tight schedule of transplantation preparation permitted, the patient performed one breath test before and several tests following transplantation. During this period the transplanted kidney took up its function and started to clear the body from uremic solutes and toxins. Six patients underwent a rejection crisis during which they carried out 21 breath tests.

For data analysis we used the Interrelation Miner Methodology, which compasses multivariate statistical data analysis and outcome prediction. Both breath data and clinical parameters were used for the data mining process as predictors. Very high quality in the prediction with AUC values between 0.8 and 0.9 were received by taking into account the time development of the variables of single patients by using breath data relative to the first breath test. It has to be confirmed whether our prediction model based on a small number of six rejection cases is capable of describing additional new cases.

There is not a single breath parameter that can be used for the diagnosis of a rejection crisis, but only the use of a group of breath compounds results in a valuable prediction. Our results show that breath tests of single patients are similar and that changes in the breath pattern over time are an important quality of this data set.

Aroma Release with Atmospheric Pressure Chemical Ionization (APCI-) and Proton Transfer Reaction (PTR-) Mass Spectrometry: Competition and Quantitative Aspects

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Abstract

Atmospheric Pressure Chemical Ionization and Proton Transfer Reaction have been studied as mass spectrometry ionization techniques for the analyses of headspace above mixtures of aroma compounds at various concentrations in water. Heptan-2-one, ethyl hexanoate, heptan-2-ol and diacetyl have been chosen because they belong to different chemical classes and they are frequently used for food flavoring. The objectives were to compare sensitivity and quantification capabilities of both techniques and to evaluate if competitions between molecules, already noticed with APCI-MS, were specific or not of this ionization technique. Quantitative aspects have been specifically addressed when a given volatile compound is present alone or in successive mixtures containing increasing amounts of other volatile compounds. First results seemed to establish that the same competition phenomena occur with both ionization methods, raising quantification issues for the real-time headspace analyses of mixtures of aroma compounds with APCI- or PTR-mass spectrometry.

Introduction

Real time aroma release measurements (*in vivo* and *in vitro*) can be achieved with mass spectrometry using either Atmospheric Pressure Chemical Ionization (APCI) or Proton Transfer Reaction (PTR) as soft ionization techniques [1;2].

An APCI source and interface was designed by our group for connection to an ion trap mass spectrometer [3]. During breath-by-breath data acquisition using this equipment, it clearly appeared that competition between volatiles occurred within the chemical ionization process, preventing true quantitative data to be obtained. This point was specifically addressed studying the headspace above mixtures of heptan-2-one, ethyl hexanoate and heptan-2-ol varying in concentrations. The comparison of their APCI responses with pure compounds responses clearly highlighted that the APCI-MS signal for heptan-2-ol decreased when ethyl hexanoate concentration increased in the mixture [4]. On the contrary, the responses of GC-FID detector for heptan-2-ol were not affected in the presence of increasing amounts of ethyl hexanoate, as

expected [5]. It was also found that ethyl hexanoate APCI signal was overestimated in the presence of ketones and alcohols. In parallel, the APCI signals of ketones and especially of alcohols were underestimated in the presence of ethyl hexanoate. As the mixture composition had very little influence on the air/product partition in the concentration range used in the study [6], results were tentatively explained by the respective estimated proton affinities. However, no clear explanation could be obtained. These findings demonstrated a clear drawback of the APCI technique if quantitative determinations are expected.

Contrarily to APCI, Proton Transfer Reaction mass spectrometry is given for a quantitative technique for headspace applications [7]. The objective of the present work was to compare APCI-MS and PTR-MS for the headspace analyses of mixtures of 4 aroma compounds in various concentrations, keeping in mind quantitative issues.

Experimental Methods

Aroma compounds

Heptan-2-one, ethyl hexanoate, heptan-2-ol and diacetyl (Sigma-Aldrich, St Quentin Fallavier, France) were chosen for their belonging to different chemical classes and their frequent use for food flavoring. 21 solutions with different compositions and concentrations were studied: 12 solutions contained only 1 aroma compound at 3 different concentrations (low, medium, high), 1 solution was a mixture of the 4 molecules at a fixed medium-concentration and 8 solutions were mixtures in which the concentration of 3 aroma compounds were fixed to a medium value while the last molecule one varied between low and high levels. Concentrations were chosen within the linearity ranges that were previously determined.

Materials

The first mass spectrometer used was an ion trap mass spectrometer (Bruker Esquire LC, Bremen, Germany) operated in the full scan mode (20 to 200 a.m.u. in 0.1 s). A low dead-volume APCI source has been specially constructed, with the corona needle facing the ion entrance capillary [3]. Using the implemented auxiliary gas, a Venturi effect was created to allow introduction of vapors in the source via a fused silica capillary tubing (0.53 mm i.d.) inserted into a heated transfer line maintained at 150°C to avoid condensation of water. The fused silica tubing was inserted near the Venturi region in a capillary adjustment device that allows the inlet flow rate to be precisely adjusted from 0 to 100 mL/min. The vapor inlet flow rate was optimized for signal-to-noise ratio and set to 30 mL/min.

The second mass spectrometer used was a high sensitivity PTR-MS system (Ionicon, Innsbruck, Austria) operated in the standard real-time VOC detection mode. The PTR-MS instrument drift tube was thermally controlled (60°C) and operated at 2.0 mbar with a voltage set to 600 V. Measurements were performed on the Multiple Ion Detection mode with a dwell time per mass of 0.1 s. The inlet of the PTR-MS instrument was connected to the headspace sampling device by a 1/16" PEEK tube kept at 60°C. The headspace mixture was continuously extracted with a constant air flow.

Methods

Measurements were performed on the headspace generated from 250 mL aqueous solutions of volatiles contained in 20 L Teflon® bags inflated with 17 L of nitrogen. The advantage of this

Teflon® bags method is a constant headspace concentration delivery for several minutes. The bags, maintained at room temperature for thermodynamic equilibrium establishment for at least 12 hours, were then connected to the APCI source or to the PTR-MS via heated capillary transfer lines. Mass spectra were acquired and averaged for ca. 1 minute after stabilization of the signal and maximum intensities of the pseudo-molecular ion or specific fragment-ion profiles recorded. Ion intensities resulting of a fixed medium-concentration of ethyl hexanoate, heptan-2-one, heptan-2-ol and diacetyl each alone in solution were compared to ion intensities resulting of the same molecules at the same concentrations but present in different mixtures containing three concentrations (low, medium, high), chosen within the linearity ranges previously determined, of the other volatiles [for instance : 0.3 mg/L ethyl hexanoate compared to 0.3 mg/L ethyl hexanoate in one mixture also containing 0.16 mg/L heptan-2-ol (low), 0.25 mg/L heptan-2-one (medium) and 22 mg/L diacetyl (medium)]. Results obtained with both ionization techniques were compared.

Results and Discussion

Using the APCI- and PTR- MS instruments, the headspace responses of pure compounds have been compared to the headspace of the same compounds found in mixtures of various composition and concentration. As shown in Figure 1, the mass spectrometry responses of heptan-2-ol were considerably affected in both cases (APCI and PTR) by the presence of increasing amounts of ethyl hexanoate in the mixtures. This result confirmed previous ones obtained with APCI-MS [4]. Even though the phenomenon seemed less important with PTR-MS than with APCI-MS (but still clearly significant), this raised questions concerning the quantitative determination of aroma compounds present in mixtures in the tested concentration ranges using PTR-MS.

The other main result was a decrease of the heptan-2-one signal with increasing amounts of ethyl hexanoate whatever the technique. This decrease also confirmed previous results obtained with APCI-MS [4], but is also significant with PTR-MS.

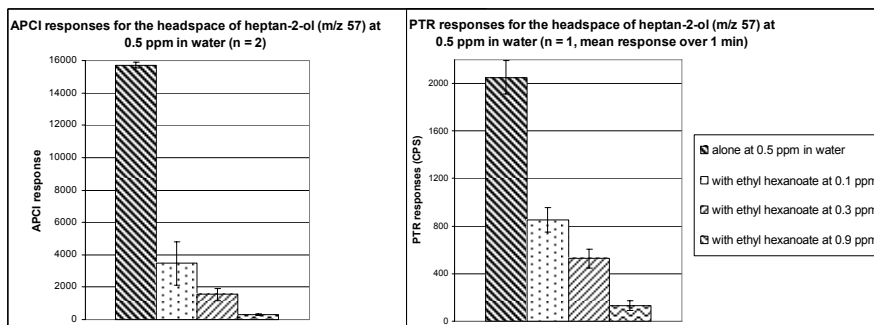


Figure 1: Headspace responses of APCI and PTR for heptan-2-ol at 0.5 ppm in water, alone or with increasing amounts of ethyl hexanoate (0.1, 0.3 and 0.9 ppm).

As already stated, the composition of the mixtures has very little influence, if any, on the air/product partition at the concentrations used in this study [6]. Thus, the results clearly highlight the existence of interactions between molecules when they are present in mixtures, whatever the ionization technique used (PTR or APCI), and no clear explanation could be obtained by comparing the respective estimated proton affinities. Data are still under treatment to evaluate the influence of such a phenomenon on quantification and further experiments are necessary to understand it.

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Application of Proton Transfer Reaction Mass Spectrometry to TATP Detection

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Abstract

The mass spectra were measured for explosive TATP with a homemade proton transfer reaction mass spectrometry (PTR-MS). The main product ions were observed at $m/z=43, 74, 75$ and 91 . When the reduced-field E/N was decreased, the characteristic protonated TATP ions occurred at $m/z=223$. The results indicate that PTR-MS may detect explosive TATP through changing the electric fields in the drift tube.

Introduction

Triacetone triperoxide (TATP) is a dangerous explosive with the power comparable to TNT [1]. Due to its sensitivity to heat, impact and friction, TATP is unstable and has no application in civil engineering or in military. However, TATP can be synthesized with available chemicals such as acetone, hydrogen peroxide and acid catalysts, thus it has become an improvised explosive material used by the terrorists including the July 2005 London transport bombings [2].

Since TATP is thermally labile and contains no nitro groups, the conventional explosives analytical devices are not suitable for its detection. In the past, many of analytical techniques have been strongly studied for TATP detection [3,4]. For instance, infrared spectroscopy [5,6], ion mobility spectrometry [7,8], and LC or GC coupled with MS [9-12] have been employed to detect TATP. Recently, selected ion flow tube mass spectrometry (SIFT-MS) [13] and desorption electrospray ionization (DESI) mass spectrometry [14-17] permit TATP to be detected with the advantages of rapid response time and minimum or no sample preparation.

Like SIFT-MS [13,18], proton transfer reaction mass spectrometry (PTR-MS) also is online monitoring technique, which has been widely used for the real-time detection of trace volatile organic compounds (VOCs) in environmental monitoring, food inspection and medical diagnosis [19, 20]. In order to simplify mass spectra, the PTR-MS instrument applies an electric field along the ion reaction tube to induce the ions collision with the kinetic energy just beyond the ion-molecular bond energies within the cluster ions. Thus the product ions, formed in the proton transfer reaction of reagent ions H_3O^+ with most analyte M , are expected to appear only in the form of protonated ions MH^+ . The ion energy in the drift tube is related to the reduced-field E/N , where E is the electric field and N is the number density of gas in the drift tube. Under the normal operation condition, E/N is set to a value typically in the range of $120Td\sim 160Td$ ($1Td = 10^{-17} V cm^2$) to produce single protonated ion for each compound. However, for volatile organic

compound TATP containing weak bonds; the protonation will probably be unavailable due to the collision induced dissociation. But, it is possible that under a low reduced field the collision induced dissociation can be controlled so that the characteristic protonated TATP ions may still occur. Here we report the mass spectra of explosive TATP with headspace sampling on a proton transfer reaction mass spectrometer in the case of the high and low reduced-field.

Experimental Methods

The TATP measurements were accomplished on a homemade proton transfer reaction mass spectrometer and a detailed description about the PTR-MS apparatus can be found elsewhere [19-21]. The reagent ions H_3O^+ were generated by glow discharge through water vapor. Small amount of TATP was synthesized according to the method described in the literature. And the TATP vapor was introduced into the drift tube by flowing laboratory air over the headspace of solid TATP sample at room temperature. The ions leaked into the spectrometer vacuum chamber were detected by a quadrupole mass spectrometer equipped with the pulse count detection system. In the experiments, the proton transfer reaction mass spectrometer was regularly run by adding benzene or acetone sample gas to the inlet carrier gas so as to conform its normal operation conditions. To show the ions signal arisen from TATP, the mass spectra shown below are the results after the background subtraction of the laboratory air sampling.

Results

Figure 1 (a) shows the TATP mass spectrum obtained at the normal reduced-field $E/N=141$ Td. As expected, the protonated TATP ions have not obviously been detected; instead, the several ion peaks are observed at $m/z=43$, 74, 75 and 91 which are the product ions of TATP proton transfer reaction. The $m/z=43$ ions is generally attributed to CH_3CO^+ . The $m/z=74$ and 91 ions are probably $[(\text{CH}_3)_2\text{C}(\text{O})\text{O}]^+$ and $[(\text{CH}_3)_2\text{C}(\text{O})\text{OOH}]^+$ respectively.

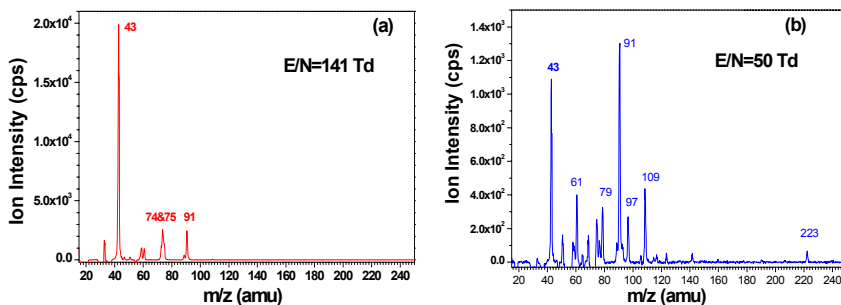


Figure 1: Mass spectra of TATP at $E/N=141$ and 50 Td after the air background subtraction.

To attain the characteristic protonated TATP ions, the reduced-field E/N must be decreased to prevent the protonated ions from collision induced dissociation. Fig.1 (b) gives the mass spectrum at an optimized $E/N=50$ Td according to ion intensities dependence on the values of E/N . Operating in such a low reduced-field, the protonated TATP ions occur at $m/z=223$, meanwhile, the water clusters $\text{H}_3\text{O}^+(\text{H}_2\text{O})_{n=1,2,3}$ at $m/z=37$, 55 and 73 are prominent. The ions at $m/z=43$, 91

still exist as the product ions of TATP reaction analogous to the SIFT-MS measurement where there is no electric field in the ion reaction region [13]. The ionic at $m/z=91$ mainly involves ions $[(\text{CH}_3)_2\text{C}(\text{O})\text{OOH}]^+$ with minor contribution of $\text{H}_3\text{O}^+(\text{H}_2\text{O})_4$ based on the $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ cluster ions relative abundance. The newly presenting ions at $m/z=61, 79$ and 97 , with 18 amu mass interval, maybe are the cluster ions containing $m/z=43$ ion attached by different number of water molecules. The $m/z=109$ ions are also tentatively assigned as clustering ions of $m/z=91$ with water molecule. The ion peaks at $m/z=43, 74, 75, 91$ and 223 are important characteristic in identifying TATP.

The results show that proton transfer reaction mass spectrometry can be applied to detect explosive TATP. TATP identification can be carried out according to the mass spectra changes at $m/z=223, 43$ and 91 under the low and high reduced-field conditions. The proton transfer reaction mass spectrometer used in the present experiment has a detection limit around 10ppb in the VOCs measurement. Actually, the proton transfer reaction mass spectrometer can reach a lower limit of detection down to 10pptv [19, 20]. It is possible that, if using a high sensitivity PTR-MS instrument to detect TATP, the mass spectra characteristic reported in this experiment will be more remarkable, and a better limit of detection can be achieved.

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Development of a high-temperature drift tube for the PTR-MS instrument

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Abstract

Currently, the PTR-MS reaction chamber is not designed for continuous high-temperature operation. The insulating spacers of the drift tube consist of Teflon PTFE limiting the maximum continuous operating temperature to approximately 120°C. In principle, Teflon PTFE withstands temperatures up to 260°C. However, creeping and deformation of the fluoropolymer was observed when operating the drift tube at temperatures > 120°C for extended periods. This resulted in leaking, short-circuiting and ultimately in instrument failure. Here we present the development of a high-temperature PTR-MS drift tube that can be continuously operated at temperatures up to 220°C. A resistive glass drift tube was used to circumvent the problem with the Teflon PTFE spacers. The ion source, the ion injection region and the ion extraction region into the mass spectrometer were redesigned and built out of high-temperature resistive materials. The instrument performance (sensitivity, detection limit, source stability) was characterized at room temperature and at 220°C using a VOC standard. Tests with low-volatility compounds are currently being performed. Preliminary results indicate that the new high temperature PTR-MS drift tube can be used for detection of organic compounds with low vapour pressure.

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Analysis of High Mass Resolution PTR-TOFMS spectra from 1,3,5-trimethylbenzene (TMB) Smog Chamber Experiments

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Abstract

Despite extensive efforts over recent years, significant uncertainties in our understanding of the photo-oxidation of aromatic compounds remain. The reactions of aromatics are highly complex with numerous reaction pathways and a large number of products, most of which are photochemically active. Low concentrations of a large number of highly oxygenated compounds with widely varying properties and stabilities, makes detection by conventional analytical methods difficult.

Proton-transfer-reaction mass spectrometry (PTR-MS) has emerged as a useful tool to study the atmospheric chemistry of oxygenated volatile organic compounds (oVOC) [1]. However, qualitative characterization of complex mixtures by conventional quadrupole PTR-MS instruments is usually difficult and sometimes impossible. Therefore, the High Resolution Proton Transfer Reaction Time-of-Flight Mass Spectrometer (PTR-TOF, [2]) has been developed. The PTR-TOF couples the soft ionization of a PTR source with the advantages of a state of the art time of flight mass spectrometer, i.e. the high mass resolution and the high duty cycle. With a mass resolution of about 5000 (FWHM) and a mass accuracy between 5 and 10 ppm the PTR-TOF allows to determine the empirical formulas of the analyte ions. This will allow to distinguish many compounds of similar nominal mass and thus help in the identification and quantification of compounds,

A series of photo-oxidation experiments was performed in the 27 m³ Paul Scherrer Institute environmental chamber [3]. The photo-oxidation of 1,3,5-trimethylbenzene (TMB) was studied with initial mixing ratios of 150-600 ppb under various NO_x conditions. Due to the well defined conditions under which smog chamber experiments can be performed, they are highly valuable for the development and the evaluation of chemical mechanisms and to get a better understanding of processes leading to secondary organic aerosol (SOA) formation.

Using the PTR-TOF, ~ 300 product ion peaks were identified during TMB photo-oxidation experiments and corresponding time traces were recorded. About one third of the observed peaks was equivalent to a concentration > 0.5 ppbv. Empirical formulas C_nH_mN_nO_o were determined and ions were separated and grouped according to their C, O and N numbers. This allowed to determine photo-oxidation grade dependent values such as the O:C ratio and time traces of mono-

and multi-oxygenated compounds. The results are compared with theoretical results predicted by the Master Chemical Mechanism (MCM).

Acknowledgement

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In vitro selection of nepetalactone-rich genotypes of *Nepeta rtanjensis* by using HPLC and PTR-MS

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Abstract

The accumulation of nepetalactone in shoots of *Nepeta rtanjensis* Diklić & Milojević, grown *in vitro*, was determined by HPLC, and its concentration in the atmosphere of glass jars was determined by PTR-MS. The obtained results varied with the carbohydrate type present in the culture medium. Furthermore, the variability in nepetalactone accumulation was significantly different between different genotypes.

Introduction

The essential oils are usually accumulated in glandular trichoms on the leaf surface, from where they can be released like volatile organic compounds (VOCs) into external media. Plants release VOCs into the atmosphere in response to various biotic and abiotic stresses [2]. Since 1998, PTR-MS has been used in a large number of laboratory and field studies of the biogenic VOCs released from vegetation to the atmosphere [3, 4 and 5]. PTR-MS offers the possibility of sensitive VOCs detection without sample preparation or chromatography [6], and is therefore a suitable tool for following the dynamics of VOCs emission.

The pharmacological effects of *Nepeta* species are ascribed to nepetalactones, which are usually the main components of their essential oils. After GC-MS analysis of *N. rtanjensis* essential oil, 4 $\alpha\alpha$, 7 α , 7 $\alpha\beta$ - nepetalactone (79,89 %) and 4 $\alpha\alpha$, 7 α , 7 $\alpha\alpha$ - nepetalactone (6.3%) were described as the main components, while α -copaene, α -pinene, β -pinene and 2-metoxy-p-cresol were present in less than 5% [1].

The objective of this study was to determine whether HPLC and PTR-MS could be used as indirect screening tools for the selection of nepetalactone-rich genotypes of *N. rtanjensis*. Shoot cultures were grown on various carbohydrate sources in order to optimize the composition of culture media for the *in vitro* growth, as well as for the nepetalactone accumulation.

Experimental Methods

In vitro culture

Shoot cultures of *N. rtanjensis* were grown in 350-mL glass jars closed with transparent polycarbonate caps, with 60 ml culture medium in each. Half-strength MS media [7] was supplemented with 100 mg l⁻¹ myo-inositol, 6x10⁻²M sucrose, glucose or fructose and 7 g l⁻¹ agar.

Cultures were grown in a growth chamber under long day conditions (16/8 h light/dark cycle) at a temperature of 25 ± 2 °C, and a relative humidity of 60-70%. Light was provided by 60 W white fluorescent tubes with photon flux density $50 \mu\text{mol m}^{-2}\text{s}^{-1}$ ("Tesla" Pančevo, Serbia).

HPLC analysis

Nepetalactone content in methanol extracts was determined using a Hewlett Packard HPLC system, model 1100 with DAD. The column used for the nepetalactone analysis was Hiperasil BDS-C18, (5μ), 125 x 2 mm I.D. The mobile phase consisted of purified water (MilliQ Water System Corporation, France) and acetonitrile (CHCN; HPLC grade, Acros Organics, Geel, Belgium). Water (A) and acetonitrile (B) were applied in the following gradient elution: 70% A (0.00 min); 60% A (15.00 min); 0%A (20.00 min); 0% A (25.00 min). The flow rate was set to $0.400 \text{ ml min}^{-1}$ and the detection wavelength to 225 nm, which is the UV maximum of nepetalactone. All analyses were performed at 40 °C.

PTR-MS analysis

Standard Proton Transfer Reaction Mass Spectrometer PTR-MS (Ionicon Analytik, Innsbruck, Austria) was used for the measurements of nepetalactone concentrations in the atmosphere of glass jars. Average instrument parameters during the measurements are shown in Table 1. Each measurement was performed within 20 cycles with a sample time of 5000 ms/mass.

Table 1: Average PTR-MS parameters during the measurements

Parameter	Units	Value
Press controller	[mbar]	365
Flow controller	[sccm]	7
USO	[V]	99
US	[V]	60
UQL	[V]	49
UNC	[V]	6
Source current	[mA]	7
Inlet temperature	[°C]	60
Udrift	[V]	597
Pdrift	[mbar]	2.18
Tdrift	[°C]	60
Detection pressure	[mbar]	4E-05
SEM Voltage	[V]	2400

Results and discussion

Some of the compounds previously detected in the essential oil of *Nepeta rtanjensis* by GC-MS analysis, were also detected by the PTR-MS in the atmosphere of glass jars (Figure 1). After their accumulation in the glandular trichomes of *in vitro* grown plants, VOCs are released and they spread over the glass jars, in which the plants are cultivated.

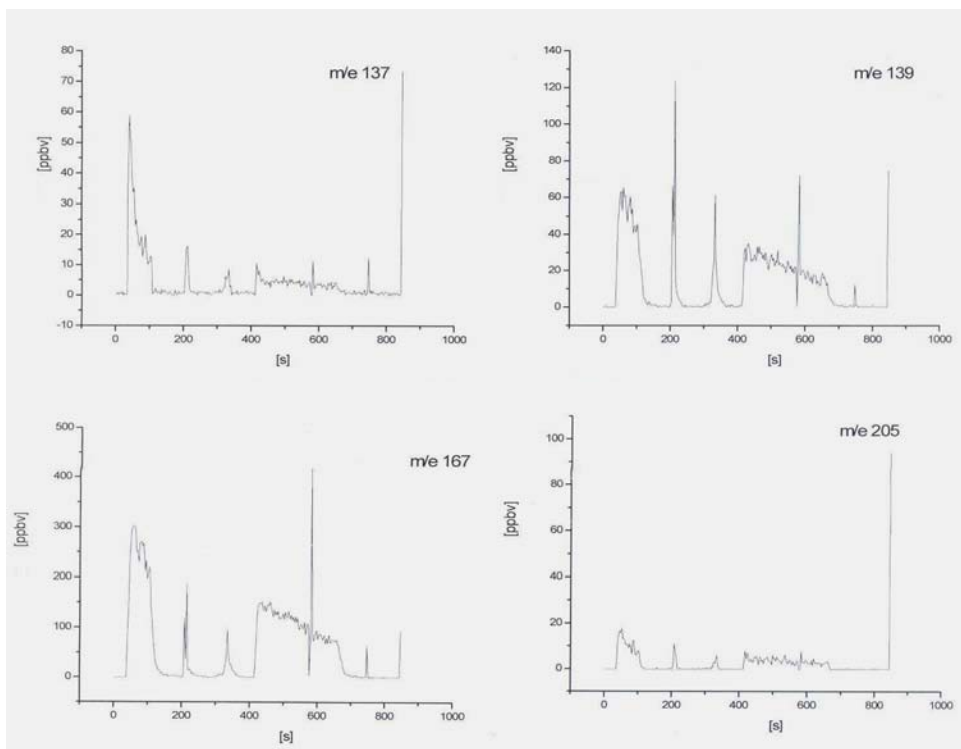


Figure 1: PTR-MS identification of VOCs in the atmosphere of glass jars, in which *N. ratanjensis* shoots were grown: m/e 137-pinene, m/e 139-2-metoxi-p-cresol, m/e 167-nepetalactone m/e 205-copaene

The dominant compound of *N. ratanjensis* essential oil, 4 α , 7 α , 7 β -nepetalactone, which shows various biological activities, was the compound of interest for our investigations. In order to select nepetalactone-rich genotypes, we analyzed the content of this iridoid monoterpene in shoots of three different genotypes. We also performed PTR-MS analyses of nepetalactone concentration in glass jars. The presence of nepetalactone in the atmosphere of culture jars is the consequence of its relief in the surrounding area from the leaf surface.

In words of nepetalactone accumulation and its relief from the leaf surface, significant difference between genotypes was determined. Regardless the genotype, the highest nepetalactone concentration in shoots, as well as in the jars atmosphere were detected when plants were grown on media containing sucrose (Figure 2A and 2B). It is well known that photosynthetic activity of tissues *in vitro* is reduced, mainly due to low light intensity, limited gas exchange, and high relative humidity in tightly closed vessels. Therefore, for *in vitro* growth and development, a

continuous supply of carbohydrates is essential. The best results obtained with sucrose, may have been caused by more efficient uptake and utilization of this sugar by plant tissues, what further stimulates the production and accumulation of nepetalactones.

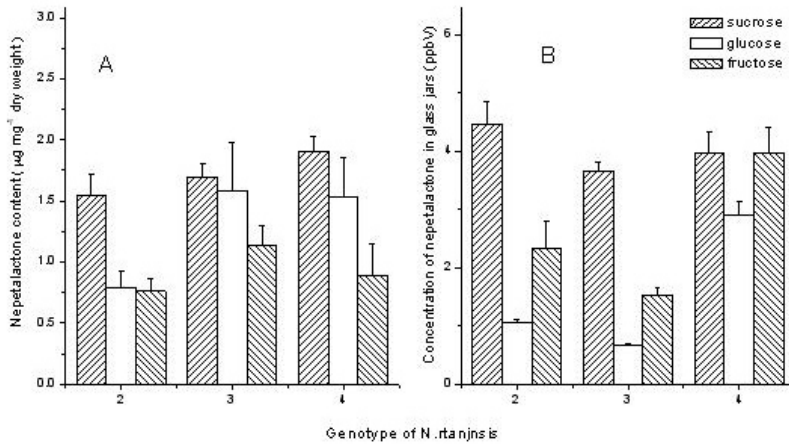


Figure 2: A) Nepetalactone concentration in the atmosphere of glass jars, in which shoots of three genotypes were grown on media containing different carbohydrates-as detected by PTR-MS; B) The concentration of nepetalactone in shoots of three *N. rtanjinis* genotypes cultured under various carbohydrate sources.

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PTR-MS breath samples analysis applied to the detection of exposure to ionizing radiation

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Abstract

The aim of this study is a non-invasive, high-throughput approach to detect human exposure to ionizing radiation, by means of proton transfer reaction mass spectrometry (PTR-MS) analysis of breath gas samples followed by a multidimensional statistical analysis of the collected data.

The presented results consist in a new and effective data analysis methodology, valuable insight on the volatile organic compounds (VOCs) involved in group differentiation, and therefore a promising test blueprint for the general PTR-MS breath analysis framework.

Introduction

PTR-MS of breath gas samples [1] is a non-invasive, high-throughput detection system, ideally suited to large scale emergency diagnoses, like the measurement of exposure to ionizing radiation of a population involved in a meltdown accident or terrorist "dirty bomb" attack [2].

In order to develop such a test, collected PTR-MS data from radiotherapy patients and unaffected people are investigated using a multidimensional statistical analysis.

Experimental Methods

The collected samples consist of 149 pairs of measurements, a breath air sample and a room air sample at a time, collected in the same conditions by mean of 3-litres Teflon bags (see [3] for details on the sample acquisition and measurement protocol).

Sample type	Sample size	Lung irradiation	Full body irradiation	Room air samples
Controls	84			73
Patients before irradiation	15	2	13	
Total not irradiated	99			
Irradiated patients	50	26	24	

Figure 1: table of patients' samples. "Controls" are healthy donors. One room air sample can be associated to many breath gas samples – e.g. when many donors are in the same room

Ninety-nine samples belong to non-irradiated people, the other 50 to patients already subjected at least to one session of a multi-session radiotherapy (see fig. 1). Some patients were subjected to full body irradiation and received between 4 and 12 Gray of cumulative exposition during a complete radiotherapy cycle, while others received only lung irradiation for cumulative organ doses ranged between 12 and 75 Gray. The samples are analyzed by a standard PTR-MS device producing a scan from 20 to 200 AMUs.

Breath PTR-MS data are not only rather noisy, but also high dimensional. The noise aspect means that no single mass signal appears to be a reliable test for irradiation exposition, i.e. there is no single “radiation marker”. These high dimensionality and noisiness make standard strategies like principal component analysis (PCA) or linear discriminant analysis (LDA) computationally demanding and numerically unstable.

Our preconditioning strategy consists in taking logarithms and performing room air normalisation of the breath samples. We also add a small constant β to all breath and room air sample counts before normalisation:

$$K_{\text{preconditioned}} = \ln \frac{K_{\text{breath}} + \beta}{K_{\text{roomair}} + \beta}$$

This approach uses the correlation between room air and breath air (see fig. 2) in order to reduce the noisy effects of the environment conditions and the PTR-MS device, and produces roughly gaussian data (see fig. 3).

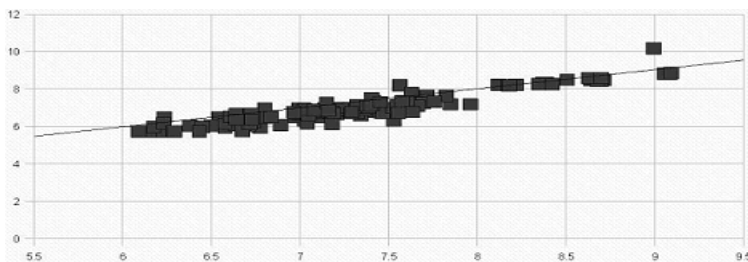


Figure 2: linear correlation of air samples for mass 45. On the horizontal axis the log-concentrations of the breath samples, on the vertical one for the room air samples.

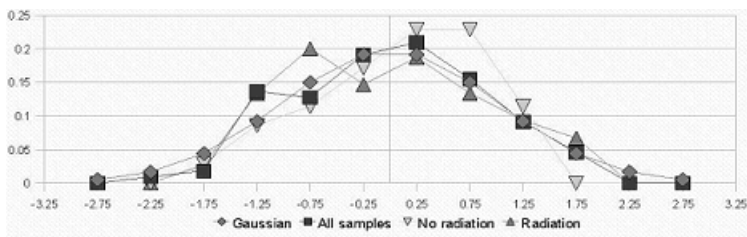


Figure 3: gaussianity of the log-concentrations for mass 45. On the horizontal axis the distance to the mean, normalized with the standard deviation. On the vertical axis the probability/relative frequency.

The sample data is then partitioned into two non-overlapping subsets, a training subset and a validation subset. A test will be constructed on the basis of the information conveyed by the training set, while the score of the test will be measured on the validation set.

A separation score for each mass signal is computed. The assumptions of gaussianity for log-signals and of equal variance between the two populations suggest the distance between the means, normalized with the standard deviation, as a good separator indicator.

Let N be a small integer, i.e. 7-20. The N mass signals with the highest separation scores are selected, and the N -dimensional problem is studied with known strategies like the Linear Discriminant Analysis (LDA) or a simplified version of the LDA which we call *weighted scalar product* (WSP) in which the empirical correlation matrix (that is, the matrix consisting of the statistical correlations of the selected normalized mass signals) has been set to zero with the exception of the diagonal entries.

Certain mass signals are masked out because of being known markers for other unrelated phenomena (see for instance [4]):

- mass 42 is typically associated to acetonitrile, a marker for smokers which correlates strongly to people developing lung cancer and being consequently subjected to radiotherapy;
- masses 69, 72 and 41 are typically associated to Isoprene, apparently correlated to increased heart rate (exercise, activity);
- masses 22 to 28 appear to be background signal;
- masses 88, 89 and 95 are significantly contaminated by bag impurities.

Results

The aforementioned strategy produces a test using only the training sample subset as learning data. The test is then applied to the validation sample subset, and the Receiver Operating Characteristic (ROC) curve are computed to gauge its performance. The test is a function of the parameter β and N .

This is a standard procedure in statistics, and offers a measure of the performance of a test constructed from given samples when applied to new, unknown ones. The ROC curve gives a visual measure of the efficacy of the test in separating two populations. The ideal test has perfect sensitivity (1) and perfect specificity (1), while the goodness of a less-than-ideal test can be gauged by the distance (many mathematical definition of a “good” distance can be given, some are more traditional, no-one is theoretically superior) to the top-left corner of the diagram (which corresponds to the mentioned ideal condition).

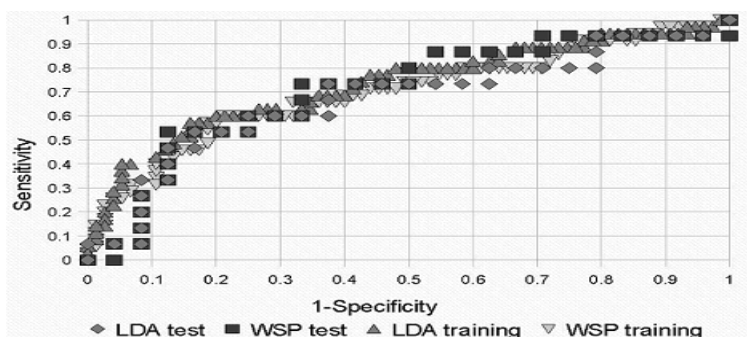


Figure 4: ROC curve for $\beta = 30$, $N = 7$.

After observing the behavior of ROC curves as we change the parameters and consider different randomly selected partitions between training subset and validation subset, we made the following observations:

- one good parameter choice is $\beta = 30$, $N = 7$ (see fig. 4);
- masses 45, 46, 59, 60, 63 pop up for most of the partitions (the first four typically associated to acetaldehyde and acetone respectively, while 63 is tentatively associated to dimethyl sulfide) suggesting that the chemical equilibrium between these signals is related to radiation exposure;
- LDA and its simplified version WSP do not seem to perform too differently: this might be due to the greater computational complexity of the LDA, and henceforth to a greater susceptibility to noise which compensates its theoretically better performance when applied with the above parameter choice.

Discussion

A multidimensional statistical analysis of breath gas PRT-MS data is presented, with the introduction of a data preconditioning based on room air normalization and gaussianity, and a dimensionality reduction approach.

This is then applied to the problem of detecting human exposure to ionizing radiation, offering a non-invasive, low-cost, high-throughput test, which yields promising ROC curves. The test is although not yet good enough for medical screening because of the less-than-ideal performance, and because of the relatively inhomogeneous sample set used so far (eating condition, age, body mass index etc. were not considered).

Acetaldehyde, acetone and dimethyl sulfide appear to be related to radiation exposure. A study of the possible biochemical reasons is of high interest. In particular, since most of the irradiated people are suffering from cancer, it may be that cancer markers instead of radiation markers are showing up: acetone was identified as a marker for lung carcinoma, diabetes or hunger, while dimethyl sulfide with liver disease [5].

Further development foci are a new sample collection protocols in order to reduce noise, and a refinement of the model assumptions, like signal normality and decorrelation.

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VOC concentration measurements in North European urban site

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Abstract

VOC concentrations were measured at high latitude urban measurement station SMEAR III in Helsinki. In this study we were interested biogenic and anthropogenic sources of VOC.

Introduction

Volatile organic compounds were measured online for four and half months continuously in SMEAR III station which is a high latitude urban measurement station in Helsinki, Finland. The aim of this study was to measure atmospheric concentrations of VOCs, such as methanol and carbonyl compounds. Behaviour of the concentrations of these compounds in high latitude urban areas is not well known. We were especially interested in relative contributions of biogenic and anthropogenic sources of VOCs.

Experimental Methods

VOCs were measured with PTR-MS during 19.12.2005-3.5.2006 at SMEAR III in Helsinki. SMEAR III is an urban measurement site where trace gases, aerosol particles and urban micrometeorology is studied. It is located 5 kilometres northeast from the downtown of Helsinki. In the vicinity of measurement station there are both vegetated areas and major roads [1].

Ambient air was measured with PTR-MS at the fourth floor of a four storey building. Sample air was pulled through two meters Teflon tubing in to the PTR-MS. In these measurements PTR-MS was calibrated using a gas standard once a week. The calibration procedure and volume mixing ratio calculations are presented in detail by Taipale et al. [2]. In total 51 masses were measured.

Results and discussion

In urban area winter-time methanol concentration is higher than in rural site [3]. In spring methanol concentration has a diurnal cycle indicating biogenic influence (Fig.1). Benzene has also higher concentration and more pronounced diurnal cycle than in rural site. In spring monoterpene concentrations show a diurnal cycle with highest concentrations during daytime. This behaviour is opposite to that observed in rural coniferous forest site [3], [4].

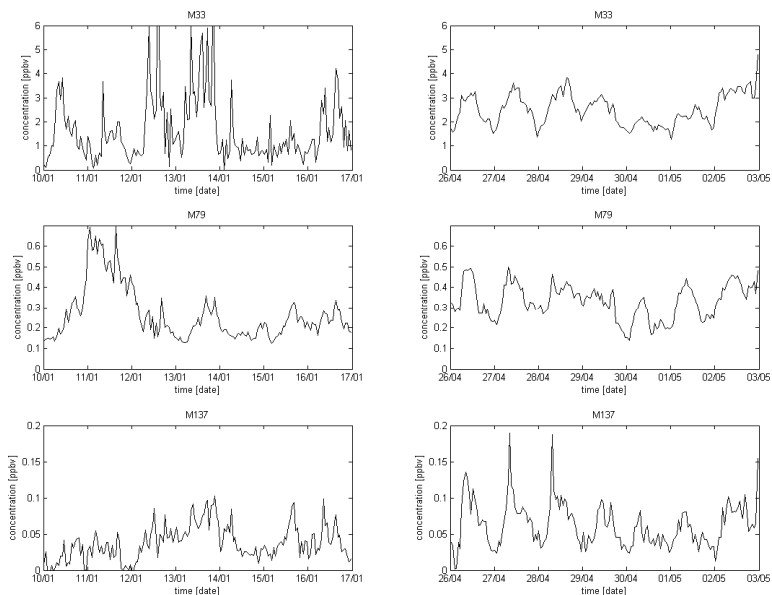


Figure 1: Concentrations of *M33*, *M79* and *M137* during one week in winter and in spring.

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PTR-TOFMS to monitor in real time volatile aroma compounds in food and to access isobaric compounds

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Abstract

Over the past ten years, PTR-MS has been step-wise upgraded to address scientific topics in food science. Due to its high performance in volatile compound analysis it is very versatile for in-vitro or in-vivo aroma characterization. Nevertheless this technique is limited to unit mass resolution due to the use of a quadrupole mass filter and therefore doesn't allow separating isobaric compounds. In food products like coffee, yoghurt, chocolate, ice cream etc. a rich composition of different volatile compounds is responsible for the overall aroma perception. A superposition of several aroma compounds to single ion traces analyzed by PTR-MS can often be observed when monitoring the aroma release of such products on-line. Fragmentation and occurrence of isotopes makes the interpretation of obtained spectra even more complex. 2,3-butanedione and 2(or 3)-methylbutanal are key aroma compounds released from the mentioned products and present in considerable concentrations such as they can be easily perceived by the human nose. As they have the same molecular mass at unit level they rise to superposition to the same protonated molecular ion trace at m/z 87 but are very different in their odour qualities. Headspace analyses of coffee brew and nosespace analysis during consumption have shown that the recently developed PTR-TOFMS technology [1] allows to clearly distinguish between the two ion traces of the mentioned molecules due to the high mass resolution. Further, the nosespace analysis of coffee shows differences in the in-mouth aroma release behavior for both molecules which opens up possibilities to understand the dynamic perception of individual compounds. The sensitivity for aroma compounds in the mass range of m/z 40 to 100 was compared between PTR-MS and PTR-TOFMS and shows similar performance. However for aroma compounds in the mass range above m/z 100, PTR-TOFMS shows higher performance in sensitivity.

Introduction

The aroma of a freshly brewed coffee contains more than 800 compounds [2]. The wide range in individual concentration, chemical stability and volatility makes the on-line analysis of coffee brew complex. Only a fraction of about 30 key compounds [3], [4], are recognized as key odorants. Due to the low concentrations some of these compounds require complex sample preparation including enrichment and chromatographic separation before mass spectrometric detection. PTR-MS on-line analysis permits to monitor some of the compounds in real time due to their major abundance in coffee headspace or to their selective protonated molecular ion $[MH]^+$. Among these we can find acetaldehyde (m/z 45), methanethiol (m/z 49), dimethylsulfide (m/z 63), propanal (ion m/z 31), N-

methylpyrazine (m/z 95), dimethylpyrazine isomers (m/z 109), ethylmethylpyrazine isomers (m/z 123) etc.

Since PTR-MS uses a quadrupole mass filter, some of volatile odorant compounds are monitored on the same ion trace like 2(3)-methylbutanal and 2,3-butanedione on m/z 87. Their odour qualities are malty and buttery respectively and participate in a large part to the overall coffee perception. With TOF mass resolution of about 5000, isobaric compounds can be separated on their exact protonated ion molecular mass which are for 2,(3)-methylbutanal and 2,3-butanedione m/z 87,0810 and m/z 87,0446 respectively. The comparison between these two detection systems was achieved by in vitro headspace analysis above the cup and nosespace analysis of coffee brew.

Experimental Methods

Sample preparation

The same analytic procedure and set-up was used as described by Lindinger et al., [5] and Prazeller et al., [6] for in vitro and in vivo analysis. A standard coffee machine was used to prepare coffee brews, using 5g R&G coffee, extracted with 25 ml water. The coffee was either transferred into the glass vessel connected to the on-line measurement set-up or directly consumed by the tester connected via a nosespace transfer line heated at 80°C to the gas analyzer. PTR-MS high sensitivity and PTR-TOFMS (both from Ionicon Analytik Ges.m.b.H, Innsbruck, Austria) were used.

In vitro aroma release

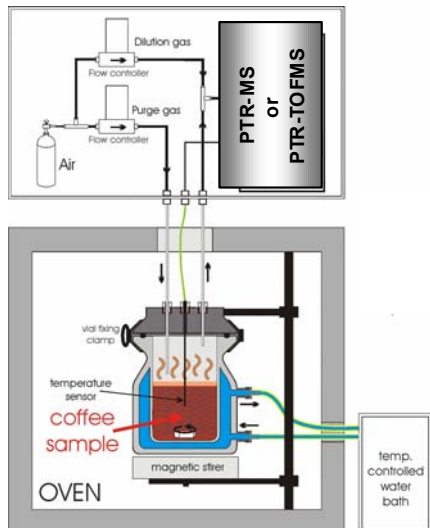


Figure 1: Headspace sampling set-up

The headspace cell was placed in an oven maintained at 80°C to avoid any condensation on the top of the cell or on connectors and outlet tubing going to the PTR instrument. The double jacket cell was maintained at 50°C with a circulating water bath. After preparation of the coffee sample, it was transferred into the headspace cell and connected to the system. The headspace above the coffee was purged with 200sccm of pure air and diluted prior going into the analyzer with 5000sccm with pure air to prevent humidity saturation in the instrument.

The data acquisition was set to scan mode from m/z 20 to 160 at 0.2s dwell time in case of PTR-MS and from m/z 0 to 425 at 0.35s/cycle in case of PTR-TOFMS. The drift pressure was set to 2,3mbar and the drift voltage to 550V.

In vivo aroma release

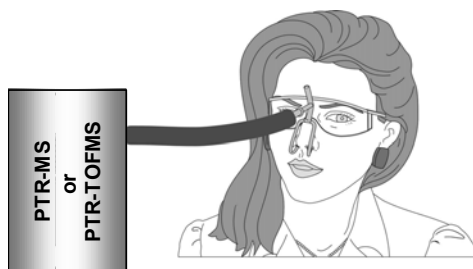


Figure 2: nospace setup

A nospace sampling system for on-line analysis of in-mouth (in-vivo) aroma release was developed at the Nestlé Research Center. In our experiments, a fraction of 80 ml/min of breath-air is drawn up continuously for analysis into a heated deactivated stainless steel tubing of 0.53 mm inner diameter. These 80 ml/min are split into two fractions: 40 ml/min is introduced into the drift tube of the PTR-MS or PTR-TOFMS, and the remainder is released through a pressure controller and membrane pump into the laboratory air. All tubings are heated to 70°C to prevent condensations.

Results and Discussion

In vitro measurement

To ensure comparable measurements with PTR-MS and PTR-TOFMS and to minimize the variability of the experiment, the same batch of coffee and the same coffee machine was used. Similar setups, one at Nestle Research Center (PTR-MS) and one installed at Ionicon Analytik Ges.m.b.H.(PTR-TOFMS) were used for comparison of the headspace release profiles of coffee.

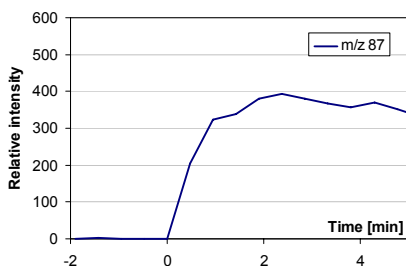


Figure 3: measurement of 2,3-butanedione and 2(3)-methylbutanal, both on m/z 87

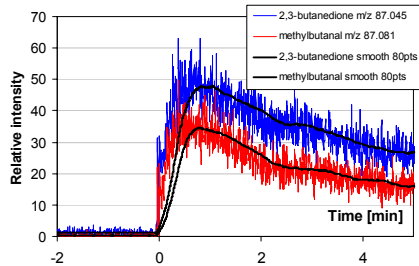


Figure 4: PTR-TOFMS measurement of 2,3-butanedione and 2(3)-methylbutanal.

Figure 3 shows overlapped release of 2,3-butanedione and 2(3)-methylbutanal measured by PTR-MS and figure 4 shows the individual release of both compounds measured by PTR-TOFMS. Due to the fast time acquisition of PTR-TOFMS an increase in time resolution at similar signal to noise ratio (S/N) can be observed. S/N ratio of 429 was calculated at maximum intensity on m/z 87 in case of PTR-MS and S/N of 190 and 330 for 2,3-butanedione and 2(3)-methylbutanal respectively in case of PTR-TOFMS.

Additionally the sensitivity difference between PTR-MS and PTR-TOFMS was tested for compounds with m/z values above 100. In case of ethylmethylpyrazine (m/z 123) an increase of S/N value from 23 to 211, and in case of 4-ethylguaiacol (m/z 153) an increase from S/N 13 to 84 was found when comparing datasets obtained from PTR-MS and PTR-TOFMS. This result shows a better sensitivity for higher m/z values (data smoothing was applied to compare at same time resolution for both instruments). This effect is mainly due to the better transmission coefficients in case of the TOF mass spectrometer.

In vivo measurement

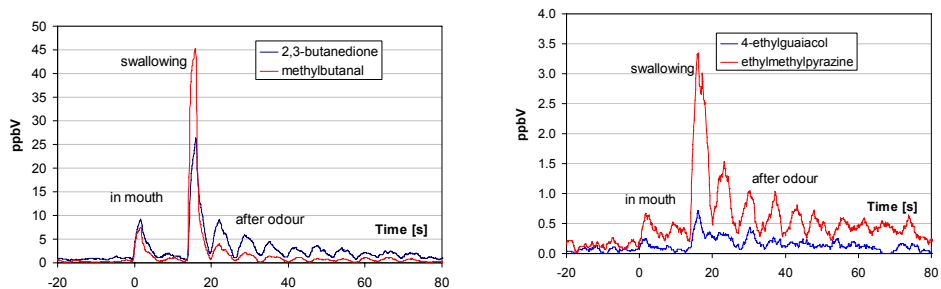


Figure 5: Coffee nospace example with few compounds analyzed with the PTR-TOFMS.

The comparison of the nospace release of the odorant compounds 2,3-butanedione and 2(3)-methylbutanal shows different time intensity profiles when drinking coffee. (figure 5-left). Methylbutanal is more intense in the swallowing breath compared to 2,3-butanedione, which is longer lasting in the after odour.

Figure 5-right, shows two ion traces of protonated 4-ethylguaiacol and ethylmethylpyrazine isomers which can typically not be measured with the PTR-MS because of the lower sensitivity.

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New Applications of SIFT-MS in the fields of Environmental Monitoring and Food and Flavour Analysis

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Abstract

SIFT-MS is an analytical system for real-time quantitative trace gas analysis. The technique features limits of quantitation in the low parts-per-trillion range, as well as the ability to rapidly switch between an array of reagent ions. For the analysis of air samples, the reagent ions of most utility are H_3O^+ , NO^+ and O_2^+ . The availability of NO^+ and O_2^+ allows the detection of compounds that are non-reactive with H_3O^+ and also enables the differentiation of compounds that produce ions of equal mass upon reaction with H_3O^+ .

Over recent years great advances have been made in the performance of SIFT-MS instrumentation. The Voice²⁰⁰®, manufactured by Syft Technologies, reflects the culmination of this work, and is now being applied across an ever-increasing range of applications. This presentation will describe a selection of applications in the areas of environmental monitoring, oil and gas exploration and in the food and flavour industry, which have recently benefited by employing this technology. These include:

- An oil exploration application for the real-time measurement of a suite of branched and straight-chain hydrocarbons and reduced sulfur compounds has enabled an increase in the number of compounds that can be simultaneously monitored during drilling as well as providing unprecedented depth resolution in the data collected.
- Coffee is considered to have one of the most complex aromas of any food or beverage. SIFT-MS analysis of coffee headspace provides a simple solution for classifying coffee flavours, and demonstrates the utility of the multi-reagent-ion system for differentiating between isomeric species even in extremely complex sample matrices.
- SIFT-MS readily detects and quantifies a range of odorous compounds in the workplace environment, including mercaptans and amines. Compound profiles provide a fingerprint of the source of the odour allowing subsequent assessment of odour origins.

High time- and mass-resolution breath gas analysis

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Abstract

Breath gas of several test subjects was analysed with a high mass resolution proton transfer reaction time-of-flight mass spectrometer (PTR-TOF, [1]) using Buffered End-Tidal (BET) online breath sampling (BET, [2]). In this paper we demonstrate the first online breath measurements with the combined method and address compelling advantages.

The PTR-TOF combines the soft ionisation of PTR and the high duty cycle of a TOF which allows to record a full mass spectrum in sub second integration time. The high mass resolving power of $\sim 5000 \Delta m/m$ (FWHM) and a high mass accuracy of 10ppm or better allow the separation of isobaric ions. The limit of detection is < 100 pptv for one second integration time.

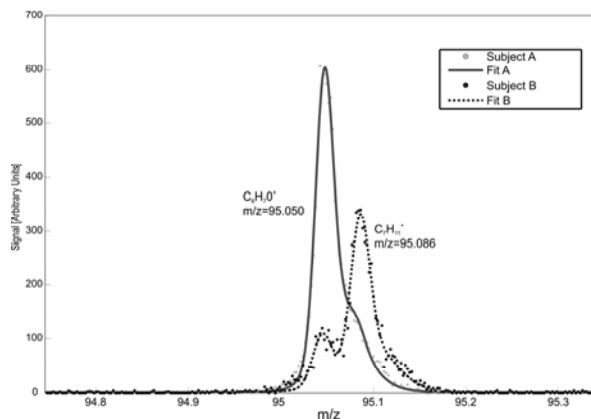


Figure 1: Part of a breath gas spectrum of two different test subjects. While the mass spectrum at nominal mass m/z 95 is dominated by C_6H_6O for subject A, subject B emits more C_7H_{10} .

BET sampling is a method for online breath analysis, where a test subject applies a single exhalation into a sampling tube. This tube has an inner volume of 40 ml and is heated to 80 °C to avoid condensation. It buffers the end-tidal part of the breath gas and allows for an extended

analysis time of several seconds, significantly improving the signal quality. During this time the subject can breathe normally, which reduces the risk of hyperventilation.

We performed online breath tests on several subjects using BET sampling and PTR-TOF for VOC analysis. Every second we recorded three full mass spectra up to $m/z = 320$. Each test subject supplied five exhalations into the BET Sampler, which led to an overall integration time of about 40 seconds for end-tidal breath. In the spectra we found over 100 significant mass peaks and we determined their exact mass. Studying an unknown trace gas composition in a complex matrix the high duty cycle of the PTR-TOF for mass scans is of great advantage. It allows for catching the whole suite of detectable VOCs in relatively short time thus keeping the stress for study subjects tolerable and the gain of information at maximum. In figure 1 selected data of two test subjects are shown. While subject A has a main peak at $m/z = 95.050$ ($C_6H_7O^+$) and a minor peak at $m/z = 95.086$ ($C_7H_{11}^+$), the ratio flips for subject B. With a quadrupole version of a PTR-MS the two peaks would not be separated and the signal of the two subjects would seem almost equal.

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A multimethodological approach to study the spatial distribution of air pollution in an Alpine valley during wintertime

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Abstract

In order to investigate the spatial distribution of air pollutants in the Inn valley (Tyrol, Austria) during wintertime, a joint field campaign of the three research projects ALPNAP (Monitoring and Minimisation of Traffic-Induced Noise and Air Pollution Along Major Alpine Transport Routes), INNAP (Boundary Layer Structure in the Inn Valley during high Air Pollution) and INNOX (NO_x-structure in the Inn Valley during High Air Pollution) was carried out in January/February 2006. In addition to continuous ground based measurements, vertical profiles of various air pollutants and meteorological parameters were obtained on six selected days. For in-situ investigations, a tethered balloon was used to analyse the lowest atmospheric layers (0-500 m above the valley bottom (AVB)), and a research aircraft sampled at 150-2200 m AVB. An aircraft equipped with an aerosol backscatter lidar performed nadir measurements at 3000 m AVB. Combined results from a typical day show a strongly polluted layer up to about 125 m AVB in the morning. Around midday concentrations on the valley floor decrease indicating some vertical air exchange despite thermally stable conditions. Strong vertical and horizontal gradients with enhanced pollution levels along the sunny side of the valley up to 1300 m AVB were observed in the afternoon. However, this vertical mixing due to thermally or dynamically driven slope and valley winds was not strong enough to renew the valley air volume. The observed small scale distribution of pollutants constrain the representativeness of point measurements and clearly show the need for fast measurement techniques.

Application of the PTR-MS for the emission test of building products

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Abstract

During the emission testing of building products a large variety of organic compounds can be detected in air. The PTR-MS principally allows monitoring the concentration development (“emission profile”) of these compounds with a high time-resolution. In practice, the interaction of several compound spectra inhibits the reliable quantification of organic pollutants because the concentration of the measured ions results from a superposition of several compounds. If a compound with high fragmentation is present in the emission test chamber, that’s breakup cannot be prevented by changing the measuring conditions, the PTR-MS data is associated with a high error in quantification.

Introduction

The emission measurement of building products using emission test chambers is a common technique for the quality assessment of manufacturers and for registration purposes. Emission test chambers provide controlled environmental conditions, like air exchange rate, temperature, and relative humidity. Actual standards for the emission analysis like the ISO 16000-6 [1] define a measurement procedure using sampling on Tenax TA and analysis via thermal desorption/gas chromatography/mass spectrometry. This method provides analytical results several hours after sampling. In case of a standard emission test the sampling takes place at defined intervals after insertion of the material. For long-term emission tests, like the emission test according to the AgBB-scheme [2], sampling takes place after 3 and 28 days. This low amount of data points complicates the characterization of the sample regarding its emission properties. Especially less volatile compounds, that need a high sampled volume and a long-term sampling respectively, might show a non-constant emission profile during sampling. This considerably increases the measurement uncertainty. Therefore, online-techniques are necessary to provide rapid information about the concentration in the chamber without extensive gaps between the measurement points. Nowadays, these techniques are broadly available but they are also associated with some disadvantages. The online measurement of formaldehyde in air needs a constant derivatization before quantitative determination. Other types, like electrochemical sensors (mostly for inorganics) and chemical noses are specific for a singular substance or a class of compounds. Therefore, direct mass spectrometry of organic compounds in air via PTR-MS provides in

principle a rapid and efficient technique for the precise characterization of building products emission characteristics.

This paper highlights some approaches on the use of a PTR-MS to measure the release of organic compounds from wood and the analytical tasks that show the application limits of this system.

Material Methods

Measurement of volatile organic compounds were performed in a 1 m³ glass emission test chamber that fulfils the specifications of ISO 16000-9 [3]. The environmental conditions were 23°C and 50 % relative humidity. The air exchange rate of the chamber was 1 h⁻¹.

Experiment A: A high sensitivity PTR-MS (Ionicon) was attached to the chamber and several ion masses were recorded with a dwell time of 5 s (*m/z* 16, 69, 81, 83, 89, 95, 109, 117, 137, and 143). The blank of the chamber was recorded for 2 h. Then, the chamber was loaded with two pine board panels (30 cm x 80 cm) that had been stored in a large pile of panels for several weeks. The panels were placed on edge in the chamber to achieve a loading factor of 1 m²/m³. Sampling on Tenax tubes (3 L) was performed 15 h, 20 h, 46 h, and 70 h after loading of the chamber. After 4 days the panels were removed from the chamber and the concentration decay was monitored for another 6 h. The results of the Tenax measurement after 70 h were used to calibrate the PTR-MS.

Experiment B: A self-developed dosage apparatus for VOC was used to establish constant concentrations of the target compounds in an empty chamber. The device consists of six flasks: Two in a row are filled with the same target compound to saturate the ingoing air (channel). In total the device can be filled with three different pure compounds. The saturation system is located in an isolated box that is tempered to 19°C. The temperature of the interior is monitored by a temperature logger (Rotronic). The flow in every channel can be controlled by a valve to change the composition of the air concentrations. The total flow through the device was chosen to 5 mL/min. For this experiment the dosage apparatus was filled with pure liquids of ethylhexanol, dichlorobenzene, and methyl ethyl ketone (MEK). The apparatus was started up and the composition of the gas was changed after 46 h by reducing the amount of MEK. The air concentration of the target compounds was monitored by the PTR-MS and by sampling on Tenax TA. The Tenax tubes were measured via thermal desorption (ATD 400, Perkin-Elmer) and gas chromatography-coupled mass-spectrometry (GC/MS, Agilent Technologies). The calibration was done on the basis of original standards. The ions monitored via the PTR-MS were chosen from the fragment ion list provided by Ionicon. The following ions were used: *m/z* 57 (ethylhexanol) and *m/z* 73 (MEK). For dichlorobenzene *m/z* 149 was chosen on the basis of a performed spectrum scan.

Results

Regarding the emission test of pine wood panels high VOC concentrations were monitored. The fragment ions *m/z* 81, 95, and 137 are linked to several terpenes (e.g. α/β -pinene, limonene, 3-carene). The PTR-MS is limited to the measurement of the sum of terpenes. Consequently, the detected concentrations of the Tenax measurement are summed up to compare the results. Selected results of the chamber experiment are shown in Figure 1. The emission profile of the sample shows a rapid increase of concentration within the first two hours after loading of the chamber. The terpenes reach a concentration of 9000 $\mu\text{g}/\text{m}^3$. Then, the concentration drops

without reaching a stable equilibrium concentration within the following 80 h. After removing of the sample the concentration decay in the chamber needs 10 h to reach the blank concentration. The ions of m/z 69, 83, 89, 117, and 143 show concentrations below 10 ppb and are excluded from the figure. The correlation between tenax samples and PTR-MS on the basis of only one calibration point is high. The data illustrates the emission profile of the different compounds with a high time resolution that cannot be delivered from a detector that only measures the total volatile organic compounds (TVOC). The delayed release of compounds due to decomposition of the material or its additives should be detectable with this method.

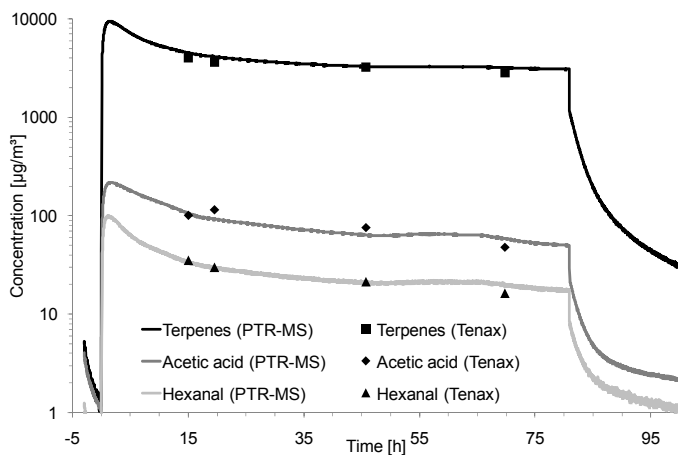


Figure 1: Development of air concentrations during an emission chamber test of two pine wood panels using the PTR-MS data of m/z 81 (terpenes), m/z 61 (acetic acid), and 83 (hexanal).

The experiment, that artificially installs a high VOC concentration in the chamber, has the advantage of only three known compounds present in the chamber air. After 20 h the concentrations of all compounds have reached nearly equilibrium (see Figure 2). The change in flow rate of the MEK channel considerably reduces the MEK concentration by $\sim 1500 \mu\text{g}/\text{m}^3$ while the other concentrations are slightly affected. Due to the slight increase in flow of the other channels the concentration of dichlorobenzene increases while the concentration of ethylhexanol keeps constant. The reference measurement using Tenax TA allows the monitoring of the concentration changes with a low time resolution.

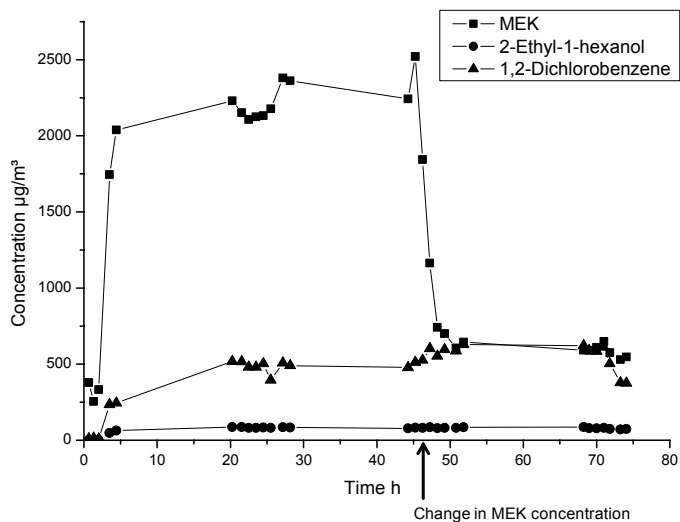


Figure 2: Results of Tenax monitoring of air concentrations in the 1 m³ chamber

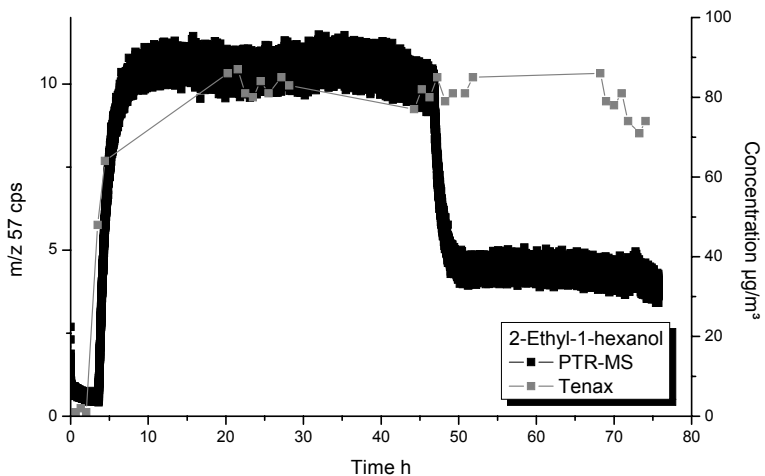


Figure 3: Comparison of PTR-MS results (raw data) and Tenax measurements of 2-ethyl-1-hexanol in the emission test chamber

Regarding the results of the PTR-MS the changes in concentration can be monitored more precisely and show the same development after changing the MEK inflow. Unfortunately, ethylhexanol deviates exceptionally. From Figure 3 it can be derived that the PTR-MS shows a considerable change in the concentration of 2-ethyl-1-hexanol while the Tenax value stays constant. The reason for this finding is the fact that MEK also shows small fragments of m/z 57. The strong drop in concentration of MEK is reflected by m/z 57 because the previous value of 10 cps was a superposition of the MEK concentration and the 2-ethyl-1-hexanol concentration.

The present data illustrates that the monitoring of a certain compound in the air of emission test chambers might contain large errors due to the overlap of the fragments of several compounds. In the case of a real sample the amount of emitted substances is very large and the superposition of different ions is most likely.

Conclusions

The presented data demonstrates the limitation of the PTR-MS for the monitoring of the emission from building products. The mixture of compounds which is emitted from a building product can reach a spectrum of ~200 compounds. The superposition of the fragments of all these compounds hinders a reliable quantification of target compounds in air. Even though just three compounds were present in the chamber air the interaction was strong enough to prevent the correct monitoring of the compound concentration.

However, the PTR-MS allows the determination of the emission profile under the given conditions with a high time resolution. This gives valuable information about the time that is necessary to reach equilibrium concentration. Additionally, different emission profiles of single compounds or compound classes can be monitored in parallel to analyze the release behavior of substance from the matrix under observation.

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Assessment of ambient VOCs levels in Belgrade semi-urban area

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Abstract

In order to assess the ambient concentrations and possible origins of volatile organic compounds (VOCs), concentrations of thirty seven compounds were measured on-line using Proton Transfer Reaction Mass Spectrometer (PTR-MS) in a semi-urban site of Belgrade. Measurements were conducting during three days in May, 2008. The one-hour mean values from on-line measurements have been calculated and used for further statistical analyze, together with ozone, NO, NO₂, NO_x and SO₂ concentrations. Meteorological parameters influence was analyzed, too. The most abundant compound was methanol. The highest correlation coefficients were observed for the compounds originated from motor vehicle exhaust emission (benzene, xylene and trans-2-butene).

Introduction

In semi-urban areas, VOC sources can be both anthropogenic and biogenic. Major anthropogenic sources include vehicle exhausts, gasoline evaporation, solvent use, natural gas emissions, and industrial processes. Benzene and toluene are compounds associated with traffic emissions; toluene is also released with the use of solvents (painting, printing, dry cleaning, etc.). Biogenic emissions, whose main sources are terrestrial plants, are globally the main sources of the VOCs found in the atmosphere. Among biogenic VOCs, isoprene and monoterpenes are highly reactive in lower atmosphere [1]. Methanol, acetone and acetaldehyde, oxygenated VOCs also have significant biogenic sources and may play substantial role in tropospheric chemistry as a source for OH radicals. By day, OH-initiated oxidation and, to a lesser extent, O₃ are the main pathways for the chemical removal of most of the VOCs, while at night, NO₃ is an important sink, especially for biogenic VOCs [2].

Experimental Methods

VOC concentrations were measured on-line using Proton Transfer Reaction Mass Spectrometer (PTR-MS) from Ionicon Analytik, Innsbruck, Austria. The air was sampled at 4m above ground, at the platform of the Institute of Physics, ($\varphi = 44^{\circ} 51' N$, $\lambda = 20^{\circ} 23' E$, $H_s = 92$ m) Zemun, 10 km northwest of Belgrade centre (Serbia), in the semi-urban area and 100 m far from the right bank of the Danube River. It was conducted to a PTR-MS system through a Teflon tube. Ozone levels were monitored using Dasibi Environment Corporation instrumentation (Dasibi 1008-AH), based on the UV radiation absorption by O₃ at 254 nm. NO, NO₂, NO_x and SO₂ concentrations were continuously recorded by the Institute of Public Health of Belgrade. Meteorological parameters including temperature, relative humidity, rainfall, wind direction and speed were

provided by the Meteorological Station of the Hydro-Meteorological Institute of the Republic of Serbia ($H_s = 132$ m, $\varphi = 44^\circ 48' N$ and $\lambda = 20^\circ 28' E$).

Results and Discussion

PTR MS was programmed to monitor 37 masses at 100 ms per mass. The concentrations of some VOCs and other related compounds were presented in Table 1.

Table 1: Statistical parameters of VOCs, O₃, NO, NO_x, NO₂ and SO₂ concentrations [ppbV] measured in Belgrade sub-urban area, 28 - 30 May 2008 (N=42)

	Mean	Median	Minimum	Maximum	95th Percentile	Range	Std.Dev.
Formaldehyde	0.002	0.002	0.001	0.005	0.004	0.004	0.001
Methanol	16.98	15.03	9.64	34.89	27.91	25.25	6.39
Hydrogen sulphide	0.05	0.04	0.01	0.14	0.09	0.13	0.02
Acetonitrile	0.42	0.33	0.23	1.70	0.97	1.47	0.27
Propene	4.97	4.18	2.87	10.12	9.32	7.25	1.98
Acetaldehyde	6.59	4.64	2.80	21.59	15.63	18.79	4.75
Ethanol	4.62	4.55	3.48	6.64	6.12	3.16	0.76
2-Propenenitrile	0.03	0.03	0.00	0.12	0.08	0.12	0.03
1,3-Butadien	2.33	2.02	1.14	4.39	4.00	3.25	1.00
Trans-2-Butene	1.09	0.74	0.31	3.69	3.55	3.37	0.93
Acetone	3.81	3.38	2.70	8.42	5.97	5.73	1.20
Acetic Acid	3.63	3.36	2.22	7.38	5.24	5.16	1.15
Dimethyl sulphide	0.21	0.20	0.11	0.35	0.29	0.23	0.05
Pyrolle	0.01	0.01	0.00	0.15	0.03	0.15	0.02
Isoprene	0.25	0.21	0.06	0.83	0.56	0.78	0.17
MVK	0.41	0.32	0.12	1.26	1.09	1.14	0.29
Butanal	0.61	0.51	0.29	2.06	1.16	1.77	0.35
Benzene	0.24	0.14	0.03	0.91	0.85	0.88	0.22
Pyrazine	0.12	0.07	0.03	0.84	0.28	0.81	0.14
2-Methylfuran	0.11	0.09	0.03	0.46	0.26	0.44	0.08
Cyclohexane	0.09	0.07	0.02	0.35	0.19	0.33	0.06
Pentanal	0.15	0.13	0.07	0.40	0.26	0.33	0.07
Toluene	0.51	0.35	0.15	3.14	1.06	2.99	0.52
Phenol	0.05	0.04	0.01	0.18	0.14	0.17	0.04
Styrene	0.02	0.02	0.00	0.07	0.06	0.07	0.02
Xylene	0.30	0.19	0.09	1.12	0.91	1.02	0.24
Monoterpenes	0.04	0.03	0.00	0.40	0.09	0.40	0.06
O ₃	28.06	30.54	1.08	44.98	41.60	43.89	10.79
NO	26.01	7.85	1.00	282.30	150.50	281.30	61.45
NO ₂	34.98	30.00	3.00	201.00	118.00	198.00	42.47
NO _x	39.43	22.70	2.60	331.40	182.50	328.80	70.93
SO ₂	11.40	10.00	6.00	29.00	25.00	23.00	5.82

The most abundant compound measured was methanol (17 ppb), with peak value of 35 ppb, followed by acetaldehyde, propene, ethanol, acetone, propanol, 1,3-butadien, trans-2-butene. The high concentration of methanol could be related to the low photochemical reactivity of methanol.

The concentration of monoterpenes was estimated as the concentration of ions with mass 137 divided by 0.46, because a certain fractionation of non-oxygenated monoterpenes occurs during ionization in the drift tube resulting in masses 67, 81 and 95 [3] [4].

The most significant correlation ($r = 0.98$) between VOCs (Table 2) was found between benzene and xylene, both from traffic emission. Benzene was also, very significantly connected with trans-2-butene, MVK and other compounds related to traffic emissions. MVK is photo-oxidation product of isoprene. Correlation of isoprene with common vehicle exhaust tracers indicated mainly anthropogenic origin of isoprene (from vehicular emissions).

Table 2: Pierson's correlation coefficients between some VOCs, O₃, NO₂, SO₂, wind speed and temperature

	Methanol	Propene	Acet- aldehyde	Acetone	Benzene	Toluene	Xylene	Mono- terpene	O ₃	Wind Speed	Temp	NO ₂	SO ₂
Methanol	1.00	0.86	0.51	0.94	0.70	0.82	0.75	0.27	-0.69	-0.58	0.19	0.54	0.69
Propene		1.00	0.73	0.84	0.87	0.72	0.89	0.32	-0.64	-0.50	0.34	0.60	0.76
Acetaldehyde			1.00	0.44	0.72	0.32	0.67	0.53	-0.43	-0.65	0.59	0.40	0.56
Acetone				1.00	0.62	0.89	0.70	0.23	-0.58	-0.42	0.10	0.44	0.61
Benzene					1.00	0.52	0.98	0.23	-0.71	-0.57	0.29	0.70	0.69
Toluene						1.00	0.62	0.18	-0.52	-0.31	0.08	0.40	0.55
Xylene							1.00	0.21	-0.72	-0.54	0.22	0.69	0.70
Monoterpene								1.00	-0.09	-0.37	0.51	0.12	0.33
O₃									1.00	0.57	0.16	-0.74	-0.63
Wind Speed										1.00	-0.46	-0.39	-0.48
Temperature											1.00	0.04	0.35
NO₂												1.00	0.85
SO₂													1.00

High correlation between acetone and methanol (0.94) indicated the main source of these compounds were of decaying process of dry plant material. Also, monoterpenes and pyrazine were high correlated, what was understandable since pyrazine was found in natural products of α -terpene. But, on this protonated mass 81, the signal could be, also, from hexenal dominant fragment ion with mass 81. When plants are physically damaged they emit hexenal. Monoterpenes showed, also, correlation to acetaldehyde and temperature.

There were no correlations between volatile compounds with atmospheric pressure, relative humidity and wind direction. Temperature had positive influence on concentration of acetaldehyde and ethanol, beside monoterpenes. Wind speed was significantly (negative) correlated with majority of VOCs. Volatile organic compounds (but not those emitted from

plants) were highly connected with nitrogen oxides and SO₂, markers for traffic emissions. The third group compounds, probably originated from some local source of hydrogen sulfide, pyrolle, butanediol, showed no correlation with nitric oxides.

The highest correlation coefficients with ozone were found for trans-2-butene, xylene, benzene, methanol, pentanal. Other VOCs are significantly (negative) connected with ozone, except the compounds with biogenic origin: monoterpenes, fragment with mass 81 and hydrogen sulfide. The contribution of hydrocarbons to the production of photochemical ozone was related to their reaction with hydroxyl radicals and ozone in the complex photo-oxidation mechanism.

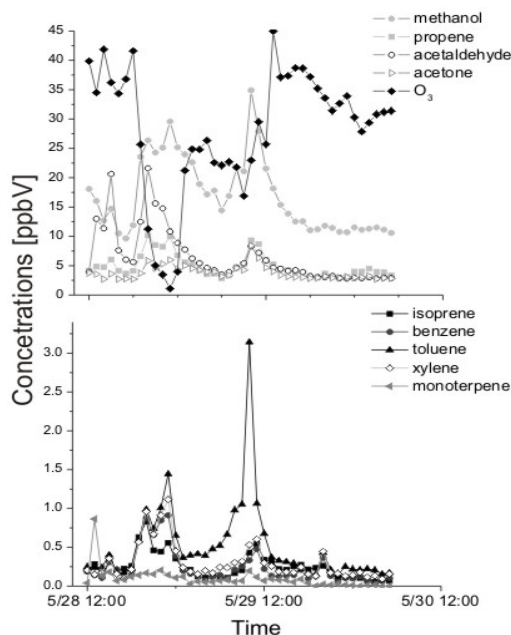


Figure 1: Diurnal variation of some VOCs and O₃ in Belgrade sub-urban area, 2008

Most of measured VOCs showed a diurnal variation with the peak concentrations observed during morning hours (about 10:00 h) and in the evening hours (20:00 - 23:00 h). Opposite patterns with ozone concentrations could be clearly seen on diagrams (Fig.1).

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Discrimination of Cancerous and Non-malignant Cell Lines by Headspace-Analysis with PTR-MS

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Abstract

Proton transfer reaction-mass spectrometry (PTR-MS) was used to analyze the volatile organic compounds (VOCs) emitted by in vitro cultured human cells. For this purpose, four human cell lines, retinal pigment epithelium cells hTERT-RPE1, lung epithelium cells A-549, immortalized human bronchial epithelial cells BEAS2B and squamous lung carcinoma cells EPLC were cultured in different specific growth media. The VOCs in the headspace of the cell cultures were sampled online with a direct connection to the culture flask and by overnight sampling with PTFE bags. The recorded mass spectra show a complex pattern in the mass range between 20 – 150 amu. Multivariate statistical methods (U-test, LDA) applied to the concentrations made it possible to separate the cancerous from the non-malignant cell lines.

Introduction

VOCs from living cells are mainly released as metabolic products during cell growth or as signaling molecules for cell-to-cell communication. The discrimination of malignant and non-malignant cells by VOCs could offer the promising possibility to early detect cancerous transformation within the lung by simply analyzing the exhaled air [1].

To find potential markers studies of in-vitro cell cultures seem to be an appropriate method. For analyzing VOCs released from in vitro cultured cells, headspace analysis offers good potentialities. VOCs produced by single-cell organisms like yeast have been examined with SIFT-MS [2] and the differentiation between bacteria strains were demonstrated by PTR-MS [2]. However, no human cells have yet been investigated in vitro with regard to their VOC release. The only studies in this respect focused on in vivo skin measurements [4,5].

In this study, we evaluated the use of the PTR-MS in combination with headspace sampling of VOCs to distinguish in vitro cultured non-malignant and cancerous human cells. Two different sampling techniques were applied: (a) direct coupling of the culture flask to the PTR-MS and (b) overnight collection of the VOCs in a bag filled with CO₂ enriched air tightly connected to the culture flask. By comparing the VOC-headspace composition from culture flasks with pure medium and cells plus medium, cell specific markers should be identified. In addition, two non-malignant and two cancerous cells lines were compared to screen the VOCs for cell line-specific markers.

Experimental Methods

Cell lines and culture conditions for growth

Four different human cell lines were used for the experiments: immortalized retinal pigment epithelium cells hTERT-RPE1 (RPE), lung epithelium tumor cells A-549, immortalized human bronchial epithelial cells BEAS2B and squamous lung carcinoma cells EPLC.

The cultivation was performed in polystyrene culture flasks with an area of 175 cm² by using an appropriate growth medium for each cell line in an incubator at 37°C and 5 % enriched CO₂ atmosphere. hTERT-RPE1 and A-549 were grown in different media, whereas BAES2 and EPLC were grown in the identical medium until semi-confluent. The medium was renewed before online measurement and in the case of the overnight bag-sampling before connecting the bag to the flask. Seeding cells and changing medium were performed under sterile conditions. Overnight-sampling of the bag-flask tandems were performed in the incubator. Table 1 summarizes cell incubation, sampling conditions and sample numbers.

Table 1: Cell incubation and sampling conditions and sample numbers.

cell line	culture medium	sampling	cells + medium	pure medium
hTERT-RPE1	RPE-specific	online	28	19
A-549	A-549-specific		15	11
BAES2B	VLE RPMI	overnight collection with bag	13	14
EPLC			23	

PTR-MS

The gas in the headspace of the in vitro cultured cells and from the bag samples was analyzed by using a proton transfer reaction-mass spectrometer (PTR-MS). In the case of the online setup the culture flask was connected to a reservoir of synthetic air containing 5 % CO₂ to maintain constant pressure in the flask during measurement. All the time the cells were kept at a constant temperature of 37°C.

For the measurements the PTR-MS was operated at a drift tube pressure of ~2.0 mbar and gas flow of 50 ml/min. Masses were scanned from 20 to 200 amu using a dwell time of 1 second per mass. Seven consecutive mass scans were performed to calculate an average count rate for every mass. Determination of transmission efficiency was performed by measuring 10 cycles of a standard gas mixture in the mass range of 20 to 200. The measured count rate per second was normalized to 10⁶ normalized counts per second (ncps) and to the sum of primary ions plus water clusters and then converted to VOC concentrations.

Results

The PTR-MS spectra of the headspace of cells covered with medium as well as the pure media show a complex pattern with relative abundances ranging over 6 orders of magnitude in the mass range between 20 and 150 amu. As the culture media contained a multiplicity of ingredients the background produced by the medium itself was large.

The first aim was to search for characteristic ion markers of the A549 - RPE and EPLC-BAES2B sets. Common to both sets we found some increased mass lines as well as decreased mass lines. An increase of the concentration corresponds to cell-produced compounds whereas a decrease corresponds to consumption of the compound by the cells. Since the count rate of most masses was not normally distributed, a two-sided non-parametric Mann-Whitney U-Test with a level of significance of 5 % ($z = \pm 1.96$) was used to identify significantly different mass lines. As an example, the U-test applied to 15 A-549 cell and 11 pure medium samples gives 17 different cell specific markers of which three VOCs seemed to be produced and fifteen seemed to be consumed by the cells.

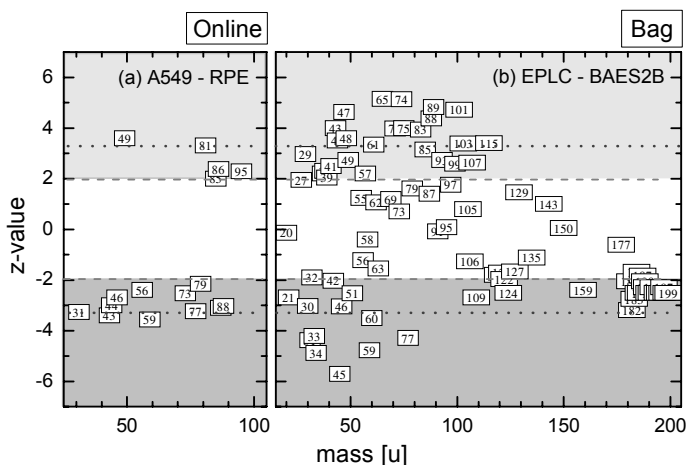


Figure 1: Results of the U-Test for the discrimination of cell lines (a) A-549 and RPE and (b) EPLC and BAES2B. Numbers denote the corresponding mass. Points above the dashed line $z = 1.96$ are significant VOCs of the cancerous cell lines A549 in panel (a) and EPLC in panel (b), whereas points below $z = -1.96$ are significant VOCs of the non-malignant cell lines RPE in panel (a) and BAES2B in panel (b).

By applying the U-test to the A549 and RPE cell lines 16 line-specific markers could be determined (Fig. 1(a)). Because the culturing was done in different media all the masses which differ in the medium spectra were skipped. Thereof, the concentrations of only five markers were higher in the headspace of lung epithelium cells A-549 and the concentration of eleven markers was significantly higher in the headspace of RPE cells than of A-549 cells. The overnight sampling in PTFE-bags of the headspace of EPLC and BEAS2B results in a higher number of significant masses over an extended range of masses of the applied U-test (Fig. 1(b)). For EPLC cell culture 26 masses are significant and with BEAS2B 32 masses. Because cell cultivation was done with the same medium no masses were excluded.

Additionally LDA analysis was performed using identified markers from the A549 and RPE culture samples by processing the concentration data with the software “R”. Only those markers outside the 5% significance level of the U-test applied and which are not related to water clusters and show no differences in the pure medium were selected. Fig. 2 shows the result of the LDA analysis applied to these 16 masses. The malignant cell line A549 is clearly separated from the non-malignant cell line RPE.

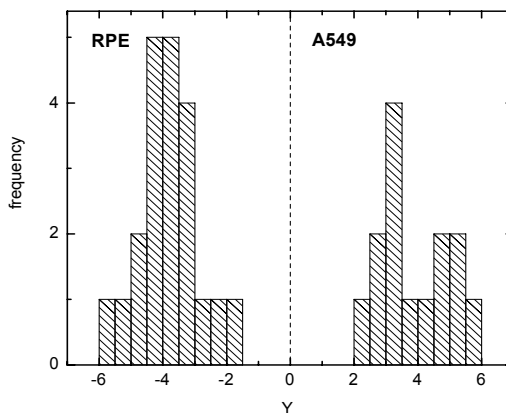


Figure 2: Separation of of human malignant RPE cells (negative Y-values) and non-malignant A-549 cells (positive Y-values) by performing a LDA on the significant masses found with the U-Test.

Conclusion

These data suggest that characteristic markers may be found for the identification and differentiation of human cells in culture by analyzing the headspace of in vitro cultured cells by PTR-MS using multivariate statistical approaches like the Mann-Whitney-Test and the linear discriminant analysis. Further investigations are required to separate the background of the medium used in cell cultivation and to synchronize the state of the cells in the culture. Thus, analysis of the headspace over cells by PTR-MS could become a fast and easy method for differentiating between healthy and cancerous tissues.

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VOC emissions from a boreal forest – direct ecosystem scale measurements by PTR-MS in 2006–2008

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Abstract

We measured volatile organic compound (VOC) emissions from a boreal Scots pine forest in southern Finland in June–September 2006, March–September 2007, and May–August 2008. These direct ecosystem scale flux measurements were conducted using the disjunct eddy covariance method and a proton transfer reaction mass spectrometer (PTR-MS) was used for the associated VOC mixing ratio measurements. Emissions of oxygenated volatile organic compounds (OVOCs) consisted of methanol, acetaldehyde, and acetone. They were of the same order of magnitude as emissions of monoterpenes. The compatibility of the measured monoterpene emissions with the traditional temperature dependent emission algorithm was reasonable.

Introduction

VOCs are involved in the formation and growth of atmospheric aerosol particles [1], which are an important factor in the climate system. Boreal forests emit large quantities of terpenoids (isoprene, monoterpenes, and sesquiterpenes) into the atmosphere [2]. In addition, they are estimated to emit significant amounts of OVOCs. To quantify OVOC emissions in the ecosystem scale and to assess their importance in comparison with monoterpene emissions, we carried out disjunct eddy covariance measurements above a boreal forest in 2006–2008. We also measured emissions in the shoot scale [3] as well as ambient mixing ratios [4–6] at the same time.

Methods

The disjunct eddy covariance method is a direct micrometeorological method for flux measurements in the ecosystem scale [7–9]. We applied it to VOC flux measurements above a boreal forest ecosystem at the SMEAR II (Station for Measuring Ecosystem–Atmosphere Relations II; [10]) station of the University of Helsinki in Hyytiälä, southern Finland (61° 51' N, 24° 17' E). The station is situated at a rather homogeneous 46-year-old Scots pine (*Pinus sylvestris*) forest. The stand also contains some Norway spruce (*Picea abies*), silver and downy birch (*Betula pendula* and *pubescens*), common aspen (*Populus tremula*), and grey alder (*Alnus incana*).

The flux measurement height was 22 m, about 6 m above the top of the forest canopy, and the flux averaging time was 45 min. The wind velocity was measured with a three-dimensional sonic anemometer (Gill Instruments Ltd., Solent HS1199) using a sampling frequency of 10 Hz. The VOC mixing ratios were measured with a PTR-MS (Ionicon Analytik GmbH; [11–12]), which was calibrated with a gas standard (Apel–Riemer Environmental, Inc.) approximately once a week. Depending on the measurement period, the PTR-MS measurement cycle contained 11–13 masses which were measured successively within 5.6–6.6 s. The measured VOC-related masses were M31 (formaldehyde, protonated mass 31 amu), M33 (methanol), M45 (acetaldehyde), M59 (acetone), M69 (isoprene and fragments of methylbutenol), M81 (fragments of monoterpenes), M87 (methylbutenol), M99 (hexenal), M101 (*cis*-3-hexenol and hexanal), M113 (?), and M137 (monoterpenes). A sampling time of 0.5 s was used for these masses. The signal of $\text{H}_3\text{O}^+\text{H}_2\text{O}$ ions, which depends strongly on the ambient water vapour mixing ratio [13], was utilized to determine the lag time between the wind and mixing ratio measurements.

Results and discussion

Emissions of OVOCs were of the same order of magnitude as emissions of monoterpenes (Fig. 1). The OVOC emissions consisted of methanol, acetaldehyde, and acetone. The median fluxes for the measurement period 13 June–15 August 2006 and 28 March–25 June 2007 were $142 \mu\text{g m}^{-2} \text{h}^{-1}$ for methanol, $38 \mu\text{g m}^{-2} \text{h}^{-1}$ for acetaldehyde, $86 \mu\text{g m}^{-2} \text{h}^{-1}$ for acetone, and $217 \mu\text{g m}^{-2} \text{h}^{-1}$ for monoterpenes.

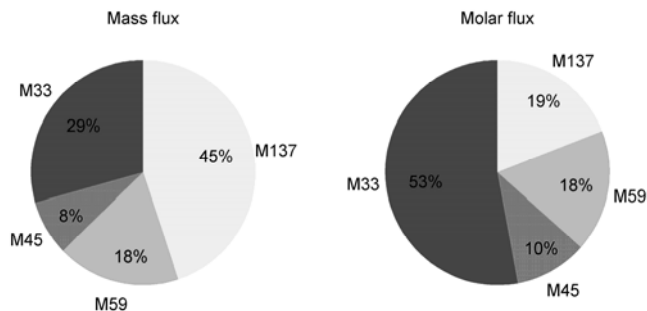


Figure 1: Relative magnitudes of the median VOC fluxes (M33 methanol, M45 acetaldehyde, M59 acetone, and M137 monoterpenes).

The compatibility of the measured monoterpene emissions with the traditional temperature (T) dependent emission algorithm $E = E_{30} \exp\{\beta(T - 30 \text{ }^\circ\text{C})\}$ [14] was reasonable (Fig. 2). A fixed temperature coefficient $\beta = 0.09 \text{ }^\circ\text{C}^{-1}$, commonly used in emission inventory models, yielded an emission potential $E_{30} = 744 \mu\text{g m}^{-2} \text{h}^{-1}$. This value agrees well with the ones derived from micrometeorological flux measurements with the gradient technique ($E_{30} = 648 \mu\text{g m}^{-2} \text{h}^{-1}$ [15]) and from shoot scale measurements with the dynamic chamber method ($E_{30} = 626 \mu\text{g m}^{-2} \text{h}^{-1}$ [16]). To determine the dependence of OVOC emissions on environmental variables, further

micrometeorological flux measurements as well as shoot scale experiments in a controlled environment are required.

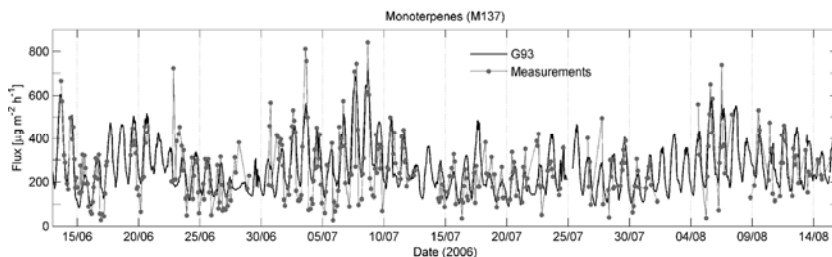


Figure 2: Monoterpene emissions in the summer 2006. The black line shows the emissions derived from the temperature dependent emission algorithm (G93).

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Authentication of traditional, dry-cured hams: volatile organic compounds measured by PTR-MS

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Abstract

Proton Transfer Reaction Mass Spectrometry (PTR-MS) was used to discriminate traditional organic hams from commercial non-organic hams. The volatile organic compounds were analyzed in quadruplicate in the mass range of m/z 21-150. The peak concentrations of the quadruplicates were averaged and subjected to Principal Least Square Discriminant Analysis in order to authenticate groups of samples: (a) organic vs. non-organic, (b) organic farm ‘De Feijterhof’ vs. all others, and (c) organic farm ‘De Feijterhof’ vs. organic farm ‘Naturlandhof Büning’. Production systems and origins of the samples were predicted with high success rates and thus few misclassifications.

Introduction

Flavor intensity is one of the most important features of dry cured hams and is related to ham quality [1]. The composition of volatile compounds highly contributes to the flavor of dry-cured hams and several studies have identified and quantified these compounds in French, Spanish and Italian dry-cured hams [2-4]. Authentic dry-cured hams produced from Bentheimer pigs by the farm ‘De Feijterhof’ have their own unique sensory features as a result of organic farming (feed) and traditional processing by dry-curing for at least one year.

The length of the dry-curing process influences the composition of volatile compounds. A range of compounds were shown to be present at higher levels in short dry-curing processes of Spanish, Italian and French dry-cured hams, whereas these can decrease during extended ripening processes. High contents of other components can be characteristic for longer processing times [2-4]. Volatile compounds are formed by reactions such as lipid oxidation, lipolysis, Maillard reaction, protein and amino acid degradation and also by the contribution of microorganisms [3,5]. Most of the components detected in the headspace of Iberian dry-cured ham are generated in lipid oxidation and in Maillard reactions [5].

Esters are very important constituents in dry-cured hams and are formed by the interaction of free fatty acids and alcohols generated by lipid oxidation in the intramuscular tissue. These esters are characteristic for the aroma composition and have been found in Italian and Spanish dry-cured ham. Concentrations of esters in hams are much lower when nitrate is added in the curing process [2,5]. Compounds with very low threshold values can have a major contribution to the desired aroma of dry-cured hams [5], this holds especially the nitrogen and sulfur containing compounds. Spoilage in ham could also be detected in Italian dry-cured ham and other pork meat and was due to an increase in concentration of particular volatile compounds [3,6].

The aim of the present study was to evaluate Proton Transfer Reaction-Mass Spectrometry (PTR-MS) for the authentication of dry-cured hams by (a) their production systems (organic vs. non-organic) and their origin (Feijterhof hams vs. others, and Feijterhof hams vs. Naturlandhof Büning). Data were initially evaluated by Principal Component Analysis (PCA). Classifications were carried out by means of Partial Least Square Discriminant Analysis (PLS-DA).

Material and Methods

In total, 45 samples were examined including 18 commercial non-organic samples, and 27 traditional organic samples. The organic samples originated from two organic farms: 19 from “De Feijterhof” and 8 from “Naturlandhof Büning”. Both farms are located at the Dutch/German border. The farm “De Feijterhof” is an organic farm, which produces authentic hams from Bunte Bentheimer pigs. The hams are dry-cured for over a year by using pepper and salt as the only preservatives. The commercial hams were purchased in the Netherlands and in Spain. Most of the commercial hams contained preservatives, such as potassium nitrate and sodium nitrite, and also antioxidants, such as sodium citrate and sodium ascorbate.

Ham samples (5 g) were taken from different areas in the ham and were placed in a 250 mL glass flask at 30°C to allow equilibration for 60 min prior to the measurement. The volatile compounds were drawn into the PTR-MS using an inlet flow (500 mL / min) by connecting the flask to the machine. A constant drift voltage of 600 V and a pressure of 2.1 ± 0.1 mbar in the reaction chamber was applied. The headspace data were collected over the mass range m/z 21-150 at a rate of 0.2 s/mass. Each ham sample was measured for 5 cycles and the 3rd, 4th and 5th cycle were taken as an average to calculate the concentrations of the different ions. The concentrations were corrected for the background by analyzing the environmental air, before each sample was analyzed. They were also transmission corrected. The average of quadruplicate measurements were subjected to multivariate data analysis (PCA and PLS-DA; Pirouette 4.01, Infometrix). For PLS-DA raw data were auto-scaled, the number of factors optimized, and the performance of the model evaluated by leave-one-out cross-validation.

Results

The headspace of non-organic and organic dry-cured ham samples was examined by PTR-MS analysis. Concentrations were calculated and two typical mass fingerprints of a commercial non-organic sample (NO15) and an organic sample of the Feijterhof (O17) are displayed in Figure 1. Mass peak concentrations of all ham samples based on averages of four replicates were first subjected to a PCA, of which a plot of the first three dimensions is shown in Figure 2. The organic samples (Feijterhof and Büning) are clustered and can be clearly distinguished from the non-organic samples.

In order to predict the production systems and the origin of the ham samples PLS-DA models were built using three separate classifications: (a) organic vs. non-organic; (b) Feijterhof vs. all others; and (c) Feijterhof vs. Büning. The models were optimized in terms of pre-processing and the number of factors. The optimal models consisted of two factors (organic vs. non-organic) or three factors (others). High success rates were obtained for the prediction of samples in their actual class and few samples were misclassified (Table 1). Further analysis of the PLS-DA score plots, in the first three dimensions, showed clear separation of the sample groups.

The scores of the first two dimensions of the classifications, (a) organic vs. non-organic; (b) Feijterhof vs. all others; (c) Feijterhof vs. Büning, are shown in Figure 3. It illustrates the distinct groupings. The samples found in the outer regions of the clusters shown in Figure 3 reveal a remarkable high abundance in the volatiles presented as the ions m/z 45 (acetaldehyde among others) and m/z 47 (e.g. ethanol) and are also somewhat higher with regard to the ions m/z 71 (e.g. 2-butenal) and m/z 89 (e.g. ethyl acetate, 3-methylbutanal, methyl propionate and propyl formate). Some compounds, like 3-methylbutanal, have been previously identified in French and

Spanish hams [7]. Others have been reported to possibly indicate a first sign of spoilage [6]. As the identification in the present study is only tentatively, and the samples were not microbiologically or assessed otherwise, spoilage could not be confirmed.

The loadings of the volatile compounds were examined for the PCA analysis and for all PLS-DA classifications and gave a clear view on the positive correlation of volatile compounds and the three different classes. Though some of the concentrations of volatiles are low, a large range can be ascribed to “de Feijterhof” samples, which could be important for the hams’ authentic flavor.

Acknowledgements

This project was funded by the strategic research funding of Wageningen UR, Theme Food Safety (project 87241701). Authors thank “De Feijterhof” for supplying sample material, and Oons Ambacht as communication platform which allowed the interaction between RIKILT and De Feijterhof. Furthermore, Ionicon (Innsbruck) is acknowledged for technical support.

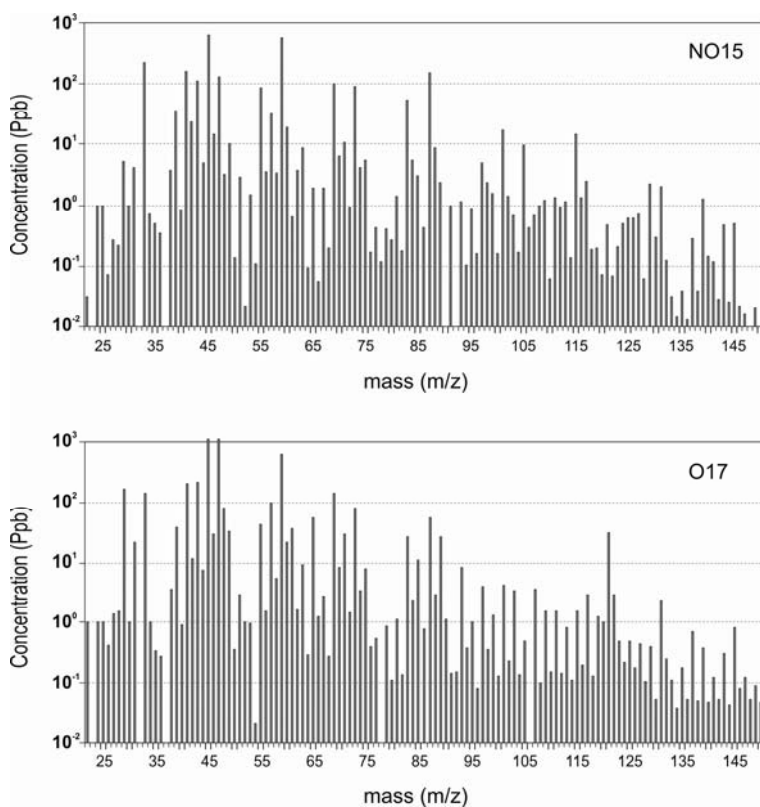


Figure 1: PTR-MS mass fingerprints of the headspace of organic and non-organic dry-cured ham. Sample NO15 is a non-organic sample and sample O17 is a typical organic sample of "De Feijterhof".

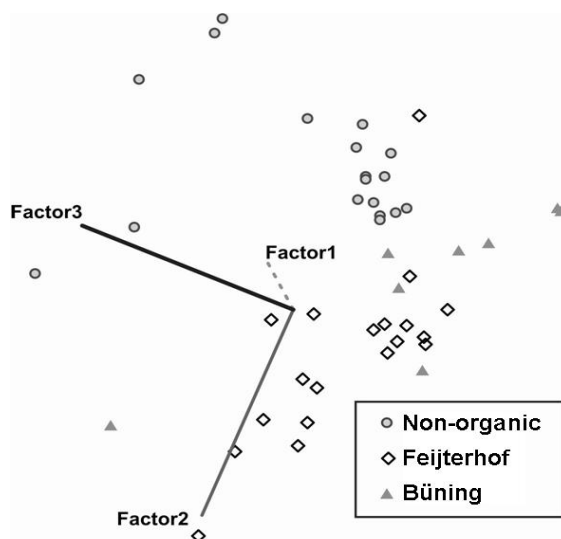
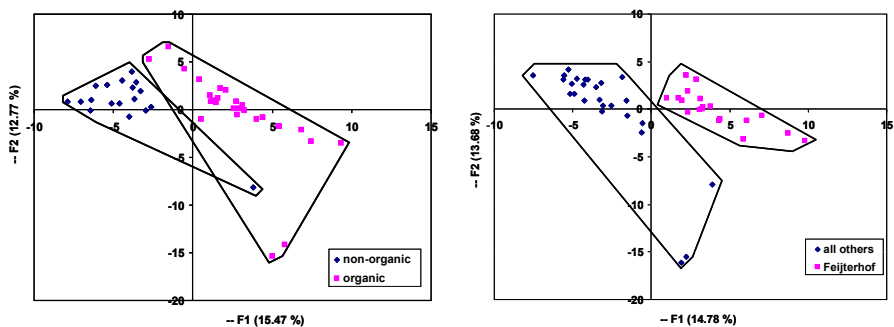


Figure 2: Plot of the first three dimensions of Principal Component Analysis on the PTR-MS headspace data of dry-cured ham samples showing the three groups non-organic, Feijterhof (organic), and Büning (organic).



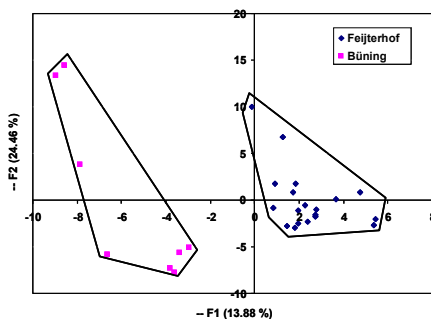


Figure 3: Scores plot of the first two dimensions of PLS-DA ($F1$ and $F2$) of three separate classifications of PTR-MS headspace data of dry-cured ham samples. The classifications concern (a) organic vs. non-organic (upper left plot), (b) Feijterhof vs. others (upper right plot), and (c) Feijterhof vs. Büning (lower left plot).

Table 1: Production system and origin of dry-cured ham samples predicted by PLS-DA. Actual classes, shown in the first column, are given for three PLS-DA classification (A, B, and C). The success rates for correct classification were calculated for each sample group.

Predicted \ Actual Class	Correct Class	Incorrect Class	Success rate
A			
Organic	26	1	96%
Non-organic	15	3	83%
B			
Feijterhof	18	1	95%
All others	24	2	92%
C			
Feijterhof	18	1	95%
Büning	7	1	88%

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Comparison of apple cultivars based on VOC release determined by PTR-MS

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Abstract

In year 2007, samples from 59 different apple cultivars were picked as they reached a ripe harvestable stage. 40 of these 59 cultivars were harvested both at Laimburg (220 m a.s.l.) and in Tarsch (670 m a.s.l.), 2 climatically very different zones of the South Tyrolean apple growing region. All fruit samples were stored in normal cool storage. After 3 months of storage fruits were kept at 20°C for a period of 6 days of shelf life before analysis. At harvest and after storage quality analysis was performed with the semiautomatic Pimprenelle robotic machine to assess firmness, sugar content and acidity. Sensory profiles of the fruits were generated using a trained expert tasting panel. In addition, PTR-MS was used to measure volatile organic compounds (VOCs) in the fruit headspace.

The aim of this work was to assess differences in the VOC profiles of different apple genotypes and of apples of the same variety grown in climatically different environments. Furthermore, the relation between instrumental analysis by PTR-MS and human perception of flavor and aroma should be investigated.

Preliminary results indicate that the 59 different apple varieties measured after shelf life were successfully distinguished. We could not detect a clear effect of the growing location due to an interaction between cultivar and site.

Preliminary results of diesel emission measurement using PTR-TOFMS

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Abstract

Diesel exhaust was measured by PTR-TOFMS using a partial flow dilution system to dilute and cool the exhaust. Sensitivity of < 10 ppb and time resolution of < 10 sec was achieved. With high mass resolution, interference by an isobaric compound can be avoided for reliable results.

Introduction

Diesel engine is most efficient internal combustion engine. Emissions from modern diesel engine for automobiles, such as NO_x and PM, were significantly reduced by progress in combustion technology and emission after treatment devices. Complex nature of diesel emission, analytical technique should have high sensitivity and capability of qualification. In addition, because emission rate varies with engine operating conditions, higher time resolution of measurement is desirable. Pioneering work of application of PTR-MS for emission measurement of diesel engine used conventional Q-MS, so time and mass resolution of PTR-MS was limited. In this work, PTR-TOFMS recently developed by IONICON ANALYTIK is used for VOC measurement of a modern diesel engine with and without diesel oxidation catalyst.

Experimental Methods

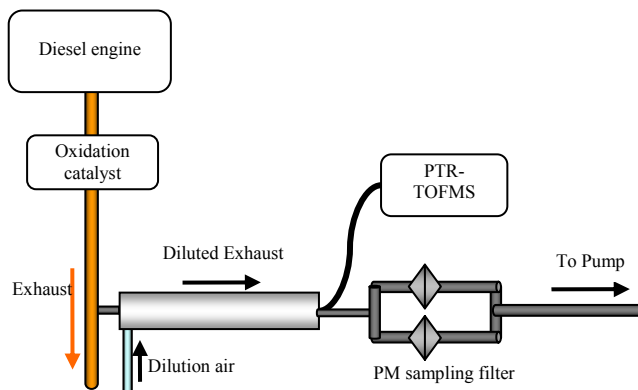


Figure 1: Exhaust sampling setup for PTR-TOFMS using partial flow dilution system

Figure 1 shows experimental setup of sampling diesel exhaust using a partial flow dilution system. Exhaust was sampled from exhaust pipe into a dilution tunnel and mixed with dilution air. Dilution ratio was set to 15:1 and flow rate of diluted exhaust was set to 80 L/min. Part of diluted exhaust was sampled from the dilution tunnel to PTR-TOFMS at flow rate of 0.2 L/min.

PTR-TOMS scans m/z up to 600 and the data were averaged for 1 sec and logged every 1 sec.

Results and Discussion

Figure 2 shows mass spectrum of diluted diesel exhaust without oxidation catalyst. Many peaks were detected but the spectrum was rather simple because of suppressed fragmentation by PTR ionization. In addition to wide range spectrum, spectra of toluene and acetaldehyde are shown. With mass resolution approx. 4000, acetaldehyde and an interfering compound can be discriminated, so quantification by PTR-TOFMS is expected to be more reliable compared with conventional PTR-MS.

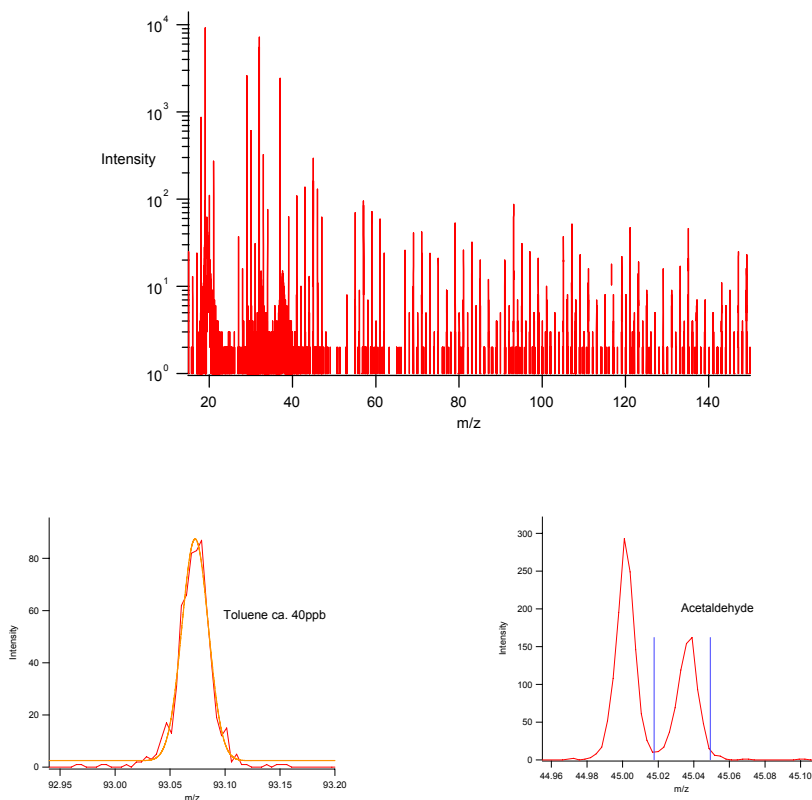


Figure 2: TOFMS spectrum of diluted diesel exhaust

Figure 3 shows time trend of concentration of benzene and toluene in diluted exhaust of diesel engine without oxidation catalyst during ESC (European Stationary Cycle). Sensitivity of less than 10 ppb is confirmed and time response is of the order of several sec.

From these preliminary results, PTR-TOFMS is very promising analytical tool for transient measurement of emission from automobiles.

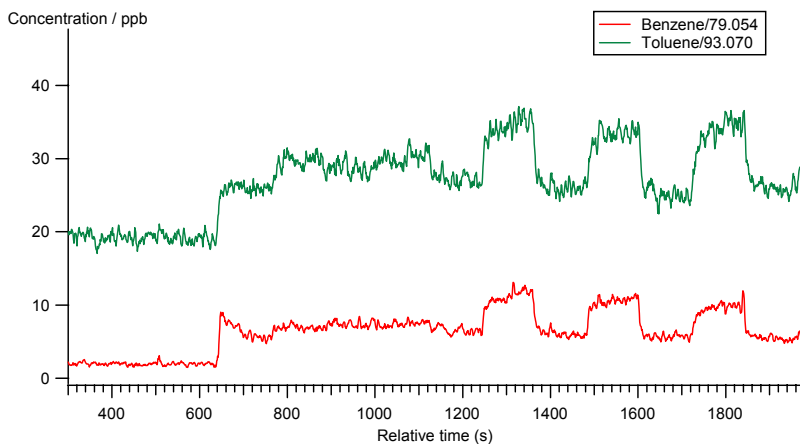


Figure 3: Concentration change of benzene and toluene emitted from diesel engine without oxidation catalyst during ESC (Dilution ratio 15, Time averaged 5sec)

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Volatile Organic Compounds in the Urban Atmosphere of Perth, Western Australia

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Abstract

A High Sensitivity Proton Transfer Reaction Mass Spectrometer (HS PTR-MS) was run in the Central Business District of Perth, Western Australia. Sixty days of ambient monitoring measurements were obtained between 14 December 2007 and 29 February 2008. The data were examined with statistical techniques and 20 masses of interest were identified either because of their high signal or because they are associated with key urban air constituents. The likely composition and statistics for some of the masses are presented. A preliminary examination of the data indicates three events of interest. There are the occurrences of:

automobile exhaust as shown by the high correlation of benzene (m/z 79) with ethylbenzene plus xylenes (m/z 107) and tri methyl benzene (m/z 121)

emissions from vegetation as evidenced by high and diurnally varying concentrations of isoprene (m/z 69) and methacrolein plus methyl vinyl ketone (m/z 71)

high concentrations (relative to typical urban levels) of pure chemicals such as acetonitrile (m/z 42) in the air unassociated with other compounds indicating a pure source.

These and other features in the data will be subject to further scrutiny. Based on this learning the PTR-MS has been deployed at a rural location where there are air quality concerns in the community.

Introduction

An ambient air monitoring study with a HS PTR-MS and US-EPA air toxic methods was conducted in the Central Business District (CBD) of Perth, Western Australia, from 4 November 2007 through to 30 March 2008. The HS PTR-MS instrument was conjunctly deployed from the Western Australian Department of Environment and Conservation - Air Quality Management

Branch (WA DEC AQMB) and the University of Western Australia (UWA). The aims of this monitoring exercise were to:

- Conduct a technology test to learn about the functioning of the HS PTR-MS for the purpose of long-term monitoring of VOCs in ambient air within the state.
- Compare the results obtained from PTR-MS with those from other air quality studies available for the Perth CBD area.
- Provide an updated insight on the levels and sources of VOCs in ambient air in the CBD during Summer 2007/2008.

Experimental Methods

The measurements were made from the Chemistry Centre of Western Australia (CCWA) building, located at 125 High Street in East Perth. This one-store building is situated at the corner of Hay St. and Plane St., two main roads at the South eastern end of the CBD, not far from the Swan River. Perth is on a coastal plain on the west coast of Australia. The Indian Ocean commences approximately 7 km west of the sampling site. The ambient air inlet for the measurements was positioned at a height of about 3.5 m from the street level on Plane St. The distance of the head of the inlet to the roof of CCWA is of about 1 metre. The PTR-MS was operated as follows: ambient air was measured continuously, VOC-free air from the scrubber inside the Gas Calibration Unit was supplied to the PTR-MS typically twice a day for 1 hour each to provide the zero level background correction. The MS was set to measure in continuous (SCAN mode), sweeping a mass range between 21-250 a.m.u. at a dwell time of 500 ms for each mass. This gave a data point measurement per each protonated mass every 115 s. The operation follows the literature descriptions of the PTR-MS method [1, 2].

Calibrations measurements were conducted using a 12 component certified VOC standard gas from Apel Reimer. The calibrations covered a broad range of concentrations ranging from 0.5 to 50 ppb. The calibration standard included p-cymene which fragments to mass 93, the parent ion of toluene [3]. At this time, toluene measurements are not presented because the correction to the calibration for this interference has not been made.

Results

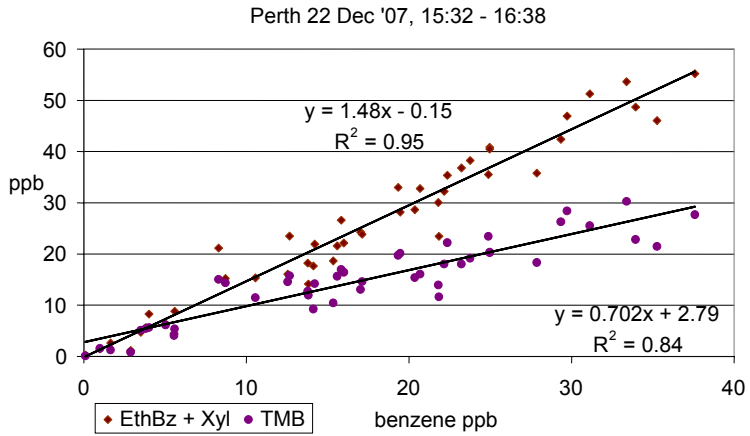


Fig 1 Event of high concentrations of benzene, trimethylbenzene and ethylbenzene + xylene

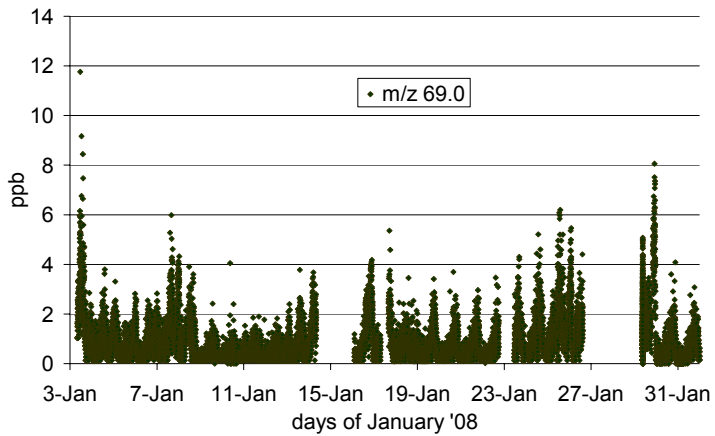


Fig 2 Patterns of isoprene variation during January 2008

Table 1. The statistics (including percentiles) of ambient concentrations (ppb) of VOCs detected as PTR-MS masses and the minimum detectable limit for Perth CBD for 14 Dec 2007 – 29 Feb 2008.

Masses (calibrated) and probable compounds	Max	95%ile	75%ile	50%ile	25%ile	10%ile	Min	MDL avg
	m/z 33 Methanol	1199.0	12.94	7.17	4.97	3.38	2.17	<i>n.d.</i>
m/z 42 Acetonitrile	417.53	2.51	0.44	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	0.22
m/z 45 Acetaldehyde	28.98	2.43	1.49	1.03	0.67	0.44	<i>n.d.</i>	0.26
m/z 59 Acetone	940.19	3.20	1.62	1.08	0.69	0.43	<i>n.d.</i>	0.11
m/z 69 Isoprene	11.76	2.48	1.13	0.55	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	0.32
m/z 71 Methacrolein and MVK	8.12	2.10	0.95	0.44	0.17	<i>n.d.</i>	<i>n.d.</i>	0.10
m/z 73 MEK	5.79	0.46	0.26	0.15	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	0.14
m/z 79 Benzene	37.59	0.66	0.25	0.11	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	0.10
m/z 107 Ethyl Benzene and Xylenes	55.17	1.07	0.42	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	0.27
m/z 121 1,3,5 Trimethylbenzene	30.28	0.69	0.29	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	0.24

Discussion

The ratios of benzene to ethylbenzene + xylene and benzene to trimethylbenzene shown in fig 1 are characteristic of motor vehicle exhausts, as observed in other studies [4, 5, 6]. This is expected in the data because the sampling is at the side of a roadway in the Perth CBD.

The regular diurnal cycle of isoprene is consistent with emission from vegetations in the hot, sunny weather that occurs in Perth during Summer.

Table 1 shows maximum concentrations for some species that are ten times the value of the 95 percentile concentrations. This is an unusual behavior for most urban pollutants. Examination of the data indicates that in some cases the maximum of the compound is uncorrelated with other species. This indicates that it is a pure compound that is being released in to the atmosphere.

Acknowledgements

This work is supported by the Western Australian Department of Environment and Conservation, Air Quality Management Branch.

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