Selected ion flow tube mass spectrometry for on-line trace gas analysis in biology and medicine

P. Španěl and D. Smith

J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejškova 3, Prague, 18223 Czech Republic

Keele University, Stoke-on-Trent, United Kingdom

Selected ion flow tube mass spectrometry (SIFT-MS), is a technique for simultaneous real-time quantification of several trace gases in air and exhaled breath. It relies on chemical ionization of the trace gas molecules in air/breath samples introduced into helium carrier gas, using H_3O^+, NO^+ and O_2^+ reagent (precursor ions). Reactions between the precursor ions and the trace gas molecules proceed for an accurately defined time, the precursor and product ions being detected and counted by a downstream mass spectrometer. Absolute concentrations of trace gases in single breath exhalation can be determined by SIFT-MS down to parts-per-billion (ppb) levels, obviating sample collection into bags or onto traps. Calibration using chemical standards is not required, as the concentrations are calculated using the known reaction rate constants and measured flow rates and pressures. SIFT-MS has been used for many pilot investigations in several areas of research, especially as a non-invasive breath analysis tool to investigate physiological processes in humans and animals, for clinical diagnosis and for therapeutic monitoring. Examples of the results obtained from several such studies are outlined to demonstrate the potential of SIFT-MS for trace gas analysis of air, exhaled breath and the headspace above liquids.

Keywords: selected ion flow tube mass spectrometry (SIFT-MS); breath analysis, trace gas analysis, breath metabolites, flowing afterglow mass spectrometry (FA-MS)

Introduction

The origins of selected ion flow tube mass spectrometry (SIFT-MS) can be traced back to the SIFT technique, which was conceived and developed in 1976 for the study of ion–molecule reactions in the gas phase at thermal energies. A specific objective was to provide the kinetic data, i.e. the rate coefficients and product ion distributions, for those ionic reactions that lead to the observed interstellar molecules and, thus, through kinetic modeling, to quantitatively describe the chemical routes to the observed molecules. Thus, the idea of absolute measurement is very much embedded in the flow tube approach to the study of ionic reactions. In the SIFT technique, rate coefficients are determined by observing the decay rate of a swarm of mass selected reactant ions, which are being convected along a flow tube by fast flowing helium carrier gas, with reactant molecules that are introduced at a controlled flow rate into the carrier gas. A downstream analytical mass spectrometer/ion counting system detect both reactant and product ions (see Figure 1). SIFT-MS essentially inverts this procedure in that if the rate coefficient and product ions are known for the reaction of a given reactant—now reagent—ion with particular reactant gas molecules, then the analytical mass spectrometer data can be exploited to determine the flow rate of the reactant gas into the carrier gas and, hence, gas analysis is achieved. Significantly, when the product ions for the reactions of the reagent ion with several gases can be identified unambiguously, then several different gases in a mixture can be analysed simultaneously. Reaction times in SIFT-MS are measured in milliseconds and so rapid changes in the concentrations of the components of gas mixtures can...
Figure 1. A schematic diagram of the SIFT-MS instrument indicating the main components. Direct breath samples may be analysed, as illustrated. The ion chemistry occurring is illustrated for the analysis of ammonia using H$_3$O$^+$ precursor ions and acetone using NO$^+$ precursor ions.

Chemical ionisation in SIFT-MS

SIFT-MS is distinguished from other analytical methods in that it employs chemical ionisation (CI) using three selected reagent ions, H$_3$O$^+$, NO$^+$ and O$_2^+$, coupled with fast flow tube technology and quantitative mass spectrometry. The chosen reagent ions do not react rapidly with the major components of air, viz. N$_2$, O$_2$ and Ar, and also CO$_2$ and water vapour, which are major components of exhaled breath. However, these ion species do react rapidly with most other gases and vapours, as numerous studies using SIFT and other techniques have shown. So the introduction of an air or breath sample into helium carrier gas in which a swarm of H$_3$O$^+$, NO$^+$ or O$_2^+$ ions exist, initiates rapid reactions between the trace gases in the sample but not so with the major components. A crucial difference between SIFT-MS and those other techniques that use CI and only one reagent ion species such as proton transfer reaction mass spectrometry (PTR-MS) that exploits only H$_3$O$^+$ ions, is that all three reagent ion species can be used to analyse a given sample, simultaneously, for collected (bag) samples and sequentially for single breath exhalations. This has several advantages as follows:

1. Because of the very different ionic reactions that occur for these three ion species, the product ions resulting from their reactions with a given compound are usually very different.

For example, the reaction of acetone with H$_3$O$^+$ proceeds via proton transfer producing CH$_3$COCH$_3$H$^+$ at an m/z value of 59. With NO$^+$, the resulting product ion is the NO$^+$. CH$_3$COCH$_3$ adduct at m/z 88. With O$_2^+$, charge transfer with partial dissociation results in the product ions CH$_3$COCH$_3^+$ and CH$_3$CO$^+$ at m/z 58 and 43, respectively. Thus, all three can be used to analyse acetone to check on compound identification and accurate quantification.

2. Distinguishing between isobaric compounds: the reactions of H$_3$O$^+$ ions with a species M often, but not always, result in the product ion MH$^+$ only. So, for example, the reactions with the isobaric compounds acetone and propanal both result in product ions at m/z 59 and so, when these compounds coexist in a given mixture, analysis is complicated. But NO$^+$ reacts very differently with these two compounds, producing the ion at m/z 88 for acetone (NO$^+$.M), whereas for propanal, hydride ion extraction occurs producing an ion at m/z 57 (M–H) allowing easy analysis. This is applicable to other compounds, as is seen in the many published papers relating to the construction of the SIFT-MS kinetics database (see the references in Reference 3).

3. The reactions of some classes of organic compound with H$_3$O$^+$ do not lead to MH$^+$ ions only. Such is the case for alcohols (other than methanol and ethanol) which, on protonation may lose a water molecule. The reaction of H$_3$O$^+$ with the two structural isomers of propanol results in both C$_3$H$_7$OH.H$^+$ and C$_3$H$_8^+$, but the branching ratio for the two ion products differs, offering the opportunity to distinguish between isomers. The reaction with NO$^+$ results in only C$_3$H$_7$O$^+$.N.

4. O$_2^+$ is also a valuable addition to the SIFT-MS analytical armory in that it charge transfers with some compounds with which neither H$_3$O$^+$ nor NO$^+$ react, good examples being...
nitric oxide, NO, and nitrogen dioxide, NO$_2$. O$_3^+$ also reacts with ammonia to produce NH$_4^+$, which gives a very valuable check on ammonia quantification obtained using H$_2$O$^+$ reagent ions.

**Absolute quantification**

Absolute concentrations of trace gases and vapours in air, including volatile organic compounds and water vapour, can be calculated in real time using SIFT-MS, by considering the flow tube geometry, ionic reaction time, measured flow rates and pressure and ion–molecule reaction rate coefficients. A combination of SIFT-MS with GC-MS is also showing some promise for absolute quantification of vapours after their separation in the GC column.

**Case studies**

SIFT-MS has wide-ranging applications in many areas of research where real time trace gas analyses are desirable, including environmental science, animal husbandry, physiology and medicine and related cell biology. Outlines of the results obtained from several studies are outlined below to illustrate the versatility of SIFT-MS.

**Aldehydes and other compounds in exhaust gases: respiratory irritants; asthma.**

Pilot studies have been carried out to analyse the compounds in petrol and diesel engine exhaust gases using the full scope of SIFT-MS. Compounds detected and quantified included aliphatic and aromatic hydrocarbons, aldehydes, alcohols and acetone. The combined use of H$_2$O$^+$ and NO$^+$ was essential to distinguish between isobaric compounds, especially to positively identify the various aldehydes. The O$_3^+$ reagent ions were vital in quantifying the large amounts of NO and NO$_2$ present. NO was much more abundant in the diesel exhaust than in the petrol exhaust. Samples of exhaust gas were taken into pre-evacuated stainless steel vessels and analysed directly. In a more recent study, SIFT-MS was used on-line to a large diesel engine using three types of fuel, viz. ultra-low sulphur diesel, rapeseed methyl ester and gas oil, principally to quantify the emission of aldehydes, including acrolein, which are known respiratory irritants. SIFT-MS analysis of the chemical species generated by chemical ionisation in the exhaust gases from a petrol combustion engine up to $m/z = 60$ was also carried out in New Zealand, showing that CI data are required from more than one precursor ion.

**Volatile compounds in rumen gas**

This research was carried out in collaboration with a group concerned with the welfare of dairy cows. Samples were taken into bags from the headspace of the rumen liquor of three lactating cows prepared with rumen vistulae. H$_2$S, (CH$_3$)$_3$S and CH$_3$SH were the dominant gases detected using SIFT-MS. The concentration of these sulphur-containing gases decreased during the period after feeding, as did the ammonia content of the rumen liquor reflecting their common origin (fermentation of sulphur–amino acids). These large amounts of organosulphur compounds need to be added to the atmospheric burden when considering global temperature modifications. Low concentrations of fatty acids were also detected in the rumen liquor headspace.

**Metabolites present in breath; their distribution in the healthy population.**

In order to recognise abnormal levels of breath metabolites that may be indicative of disease, it is important to know their levels in the breath of the healthy population. It is surprising that these levels have not been established so far, even though the first pioneering work in breath analysis was done more than 30 years ago and active research in this field using various techniques is ongoing. Thus, on-line real time analyses of single breath exhalations have been carried out using SIFT-MS. The initial studies focused on a 30-day study of the common breath metabolites ammonia, acetone, isoprene, ethanol and acetaldehyde in the breath of five healthy volunteers. These exploratory studies showed that the concentrations of these compounds were approximately described by normal distributions with coefficients of variation typically 0.3. Subsequently, a similar, more extensive longitudinal study has been carried out of the breath of 30 healthy volunteers over a six-month period including the metabolites methanol, propanol and acetaldehyde. This study confirmed the general results of the pilot study, but the larger amount of data allowed a better description of the data and indicated that the distributions were log normal with a geometric standard deviation of typically 1.6, indicating that these distributions are quite “tight”. The median concentrations are: ammonia 833 parts-per-billion (ppb) acetone 477 ppb, methanol 461 ppb, ethanol 112 ppb and isoprene 106 ppb. SIFT-MS studies have revealed that these levels can be very much higher and well outside the normal range in the diseased state. For example, in end-stage renal failure, the breath ammonia levels can be as high as 13,000 ppb and in diabetes the breath acetone levels can exceed 5000 ppb even under insulin control.

**Volatile compounds in urinary headspace during ovulation**

In a most exciting and rewarding exploratory SIFT-MS investigation, the volatile compounds that appear in the headspace of urine from a normally ovulating volunteer was studied over three complete menstrual periods. Mid-stream urine samples were collected in the morning before breakfast. The startling result obtained for all three menstrual periods is that, concurrent with the time of ovulation, a 3–12-fold increase in the urine headspace acetone was observed. This phenomenon has subsequently been observed in seven normally ovulating volunteers; significantly, it is not seen in post-menopausal volunteers. This may well be an important phenomenon during ovulation and might be a method of checking that ovulation has occurred. It also illustrates...
the power and potential of SIFT-MS analyses in this area of human biology.

**Real time studies of ethanol metabolism via breath analysis**

A valuable feature of SIFT-MS is that analyses can be obtained in real time on time scales of seconds. Thus, it is possible to follow rapid changes in the level of volatile breath compounds and single breath exhalations can be analysed for several compounds, simultaneously, allowing relationships between compounds to be explored. To illustrate this, a study of the kinetics of ethanol metabolism and the production of acetaldehyde has been carried out following the ingestion of a small amount of ethanol (typically 5 mL in 500 mL of tap water). Breath samples were analysed on line every 2 min for about 100 min, focusing on ethanol, acetaldehyde, ammonia and acetone. The time plots of the ethanol concentrations were sufficiently well defined to demonstrate that the loss of ethanol occurred via first order kinetics and that the acetaldehyde correlated well with the ethanol levels indicating, as expected, that it was indeed a product of ethanol metabolism. This well demonstrated the potential of SIFT-MS for accurate non-invasive studies of physiological processes. The amounts of ethanol in breath and in blood have been compared using SIFT-MS in New Zealand.22

**Monitoring of breath during haemodialysis for patients with end-stage renal failure**

It is well known that toxins build up in the blood of patients suffering from end-stage renal failure and this must reflect the appearance of volatile compounds in exhaled breath. An early SIFT-MS study was to measure the levels of common breath metabolites in the breath of several patients before and during haemodialysis sessions.18 The most startling result was the very high levels of breath ammonia prior to dialysis, often exceeding 10 parts-per-million (ppm) as compared to a typical value for the healthy population of about 1 ppm (see the section on “metabolites present in breath” above). In addition to this, the acetone levels in the breath of the diabetics were about ten times greater than those of the healthy population. The levels of both these metabolites decreased toward normal levels during dialysis but, in some cases, the breath isoprene levels actually increased. This is tentatively ascribed to bio-incompatibility of blood and the dialysis membranes. Thus, breath analysis using SIFT-MS could be a valuable monitor of the efficacy of dialysis.

**Volatile markers of infection and tumours in urinary headspace**

There is a growing interest in exploiting gas analysis to assist in the early detection of tumours. Obviously, this requires that tumours are directly or indirectly responsible for the production of volatile species in the blood and hence into the breath or the urine. For practical reasons (sample acquisition) the first SIFT-MS contribution to this topic involved the search for volatile compounds in the headspace of urine provided by patients suffering from prostate and bladder cancer. This resulted in the detection of formaldehyde in the headspace but not in the headspace of urine from healthy controls.23 During these studies, it was observed that copious amounts of nitric oxide gas were present above the acidified urine from some patients and this is attributed to bacterial action, which provides a rapid non-invasive test for urinary bacteria.24

**Acetaldehyde released by cancer cells in vitro**

As an extension to the search for volatile biomarkers of tumours, SIFT-MS studies have been carried out on the emissions from lung cancer cell lines.25 These were grown in calf serum and the headspace concentrations of several volatiles were measured for varying numbers of cells in the medium. A wide range of organic species were seen to be present in the headspace, including methanol, ethanol and acetone, but the important observation was that acetaldehyde was present at a concentration in close proportion to the number of cells in the medium. The number of acetaldehyde molecules generated was of the order of 10⁶ per cell per minute. The next phase of this work (and that reported in the section on “volatile markers of infection and tumours in urinary headspace”) is to search for these aldehydes and other compounds in the exhaled breath of patients suffering from lung cancer using on line SIFT-MS breath analysis. Note that previously formaldehyde levels were measured in human cancer cells in vitro using preconcentration-chemical ionization mass spectrometry performed with a SIFT instrument.27

**Emissions from bacterial cultures associated with cystic fibrosis**

In collaboration with paediatricians, a SIFT-MS programme has been initiated to search for biomarkers of lung diseases, such as asthma and cystic fibrosis (CF). As a first step, volatile emissions from bacterial cultures grown from cough swabs from children with CF have been investigated26 with the startling result that hydrogen cyanide is seen to be emitted specifically from *Pseudomonas aeruginosa* (PA), which colonises the lungs of those with CF. This suggests a breath test for the presence of PA in the lungs by SIFT-MS, which could greatly assist treatment.

**SIFT-MS and flowing afterglow mass spectrometry for quantification of deuterium in water vapour and the measurement of total body water.**

An important development that stemmed from SIFT-MS is flowing afterglow mass spectrometry (FA-MS),28 which is exploited for the on-line, real time analysis of the deuterium content of breath water vapour and the headspace of aqueous liquids. This technique relies on the accurate measurement of the of the water cluster ions signal ratio \(H_2O^+(H_2O)_2HDO/\text{H}_2O^+(H_2O)_2H_2^{18}O\) as generated in an afterglow plasma from \(H_2O^+\) precursor ions and the mixture of \(H_2O\) and HDO molecules present in a breath/headspace introduced into the plasma. Thus, following a known dose of \(D_2O\), the deuterium disperses as HDO throughout the
Concluding remarks

SIFT-MS is making a valuable contribution to trace gas analysis, especially to breath analysis, as the examples given in the above indicate. With the availability of smaller SIFT-MS instruments, which can be located in the clinic and at the bedside, rapid, non-invasive analyses of breath can be made, without the need for sample preparation such as water vapour removal, providing a valuable additional tool for clinical diagnosis and therapeutic monitoring.

Acknowledgment

This work was partially funded by GACR project number 202/06/0776.

References


Received: 28 November 2006
Revised: 1 March 2007
Accepted: 1 March 2007
Publication: 19 March 2007