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The proton transfer reaction mass spectrometer and its use in medical science: applications to drug assays and the monitoring of bacteria

A. Critchley[a], T.S. Elliott[b], G. Harrison[c], C.A. Mayhew[a,*], J.M. Thompson[a], T. Worthington[b]

[a] *Molecular Physics Group, School of Physics and Astronomy, University of Birmingham, Birmingham B15 2TT, UK*
[b] *Department of Clinical Microbiology, Queen Elizabeth Hospital, University Hospital Birmingham NHS Trust, Birmingham B15 2TH, UK*
[c] *Featherstone Department of Anaesthesia and Intensive Care, Queen Elizabeth Hospital, University Hospital Birmingham NHS Trust, Birmingham B15 2TH, UK*

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Abstract

Proton transfer reaction mass spectrometry (PTR-MS) enables monitoring of trace gases in air with high sensitivity without major gases interfering. In this paper, we present the potential use of a proton transfer reaction mass spectrometer for two medical applications; the monitoring of drugs and bacterial infection. The first study illustrates a feasibility trial to monitor the intravenous anaesthetic agent 2,6-di-isopropyl phenol (propofol), and two of its metabolites, on the breath of patients in real-time during surgery. Propofol is a commonly used intravenous anaesthetic. However, there is no method of instantaneously monitoring the plasma concentration of the agent during surgery, and therefore determining whether or not the plasma level is of such a value to ensure that the patient is correctly anaesthetized. That propofol and its metabolites were monitored in real-time using the PTR-MS suggests possibilities for routine intravenous anaesthesia monitoring analogous to that for volatile anaesthetic agents. In addition to the above work we also investigated proton transfer to another anaesthetic, sevoflurane. Comparisons between PTR-MS and selected ion flow tube (SIFT) investigations are presented. The second study presented in this paper investigated the volatile organic compounds emitted by microbial cell cultures. The objective was to show that different microbial cultures could be readily distinguished from the resulting mass spectra recorded using the PTR-MS. The initial results are encouraging, which taken together with the real-time analysis and high sensitivity of the PTR-MS, means that proton transfer reaction mass spectrometry has the potential to characterise bacterial infection rapidly.

Keywords: PTR-MS; Monitoring of drug; Breath analysis; Bacterial infection; VOCs

1. Introduction

Markers of disease and drugs in the body can pass through the thin barrier between the blood capillaries and alveoli in the lungs, and then be carried out in the breath. Volatile organic compounds (VOCs) that originate in the body have been identified on the breath at concentrations ranging from below parts-per-billion by volume (ppbv) to parts-per-million by volume (ppmv), many of which are known to be characteristic markers for disease. Similarly, many drugs and their metabolites will be present on breath at such minute concentrations.

Breath analysis thus has the potential for disease diagnosis, health status and drug monitoring by "finger-printing" the pattern of trace volatile marker substances in the breath with sampling and analysis taking place simultaneously. This provides the potential for the development of drug therapy

* Corresponding author. Fax: +44 121 4144577.
E-mail address: c.mayhew@bham.ac.uk (C.A. Mayhew).

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control, the early-stage non-invasive diagnosis of cancers, heart, liver, kidney and respiratory disease, organ failure and diabetes-associated conditions, as well as the opportunity to develop new rapid drug monitoring methods. Current and standard technologies which could be used for this are limited in many aspects, including costly laboratories, intricate and time-consuming sample preparation, low sensitivity, and lack of transportability, and are thus not in widespread use. The proton transfer reaction mass spectrometer (PTR-MS) overcomes many of these difficulties because of its size and its ability to be used in situ and in real-time.

Soon after the development of the PTR-MS for on-line monitoring of volatile organic compounds at a few parts-per-trillion by volume (pptv) levels, a major review of the apparatus and some of its applications was published by its inventors. In the review, Lindinger et al. [1] illustrated the use of the elegant PTR-MS technique for medical applications, food control and environmental research. Since the publication of that article there has been a huge increase in the use of the PTR-MS for trace gas analysis, with the major growth areas being those in the environmental and food sciences. This is exemplified by the number of talks and posters presented at the 1st International Conference on Proton Transfer Reaction Mass Spectrometry and its Applications (Igls, Innsbruck 2003). Of the invited talks, fourteen were dedicated to environmental science and technology, and eight focussed on food technology. Only three talks discussed possible applications of the PTR-MS to the medical sciences, predominantly on its use in breath analysis. This is in stark contrast to the dramatic increase in the number of publications over the last decade connected with the diagnostic potential of breath analysis, in which many groundbreaking results have been reported. For example, Phillips et al. [2] in the United States have used gas chromatographic (GC) methods to monitor VOCs on the breath of patients suffering from lung cancer. Their results not only show that patterns of VOCs can be used for biomarkers of the disease, but also that they can be used to monitor the stage of the disease. Whilst the use of GC technology has clearly illustrated the potential of breath analysis for medical diagnosis, there are a number of drawbacks associated with its use—and particularly in the hospital environment. For example, preconcentration of the breath samples is needed, which could lead to contamination problems. The measurements are not taken in situ, are not in real-time and are time consuming. Furthermore, breath profiling is not possible. Many of these technical problems are overcome by the use of the PTR-MS. A number of groups are now performing preliminary studies to demonstrate how powerful the PTR-MS is for medical diagnosis. For example, Rieder et al. [3] in Austria have used the PTR-MS in the area of medicine and have demonstrated that VOCs could serve as non-invasive biomarkers of diseases such as hyperlipidiemia and metabolic disorders.

It is perhaps in the area of medical science that the PTR-MS could have the most impact on our society in terms of quality of life, because this type of technology has the potential for the early diagnosis of diseases, without the need for biopsies or blood samples. The application of the PTR-MS to medicine is of course not just limited to breath analysis, but also to the analysis of emissions from skin (e.g., for the diagnosis of skin cancers), and bodily fluids such as urine. Smith et al have demonstrated the potential applications of proton transfer reactions to the identification of prostrate cancers through the analysis of the headspace above urine [4]. They analysed the presence of formaldehyde in the headspace of urine from bladder and prostate cancer patients using selected ion flow tube (SIFT) mass spectrometry. Their studies showed that formaldehyde is clearly elevated in the headspace of the urine from cancer patients as compared with the formaldehyde in the headspace of urine from healthy patients. Similarly, and again using a SIFT technique, Bierbaum and coworkers [5] in the United States have observed elevated levels of formaldehyde in human cancer cells for MCF-7 breast cancer, K562 leukaemia, and HeLa S3 cervical cancer.

Diagnosis of disease is only one area in which the PTR-MS could be useful to medical science; others include detection of bacteria and drug monitoring. Recently Mayr et al. [6] have used the PTR-MS for the detection of bacteria on meat. The purpose of this article is to illustrate recent proof-of-principle studies of relevance to medical science both in terms of breath analysis and for emissions from bacterial cultures. These studies have been performed by the Molecular Physics group at the University of Birmingham in collaboration with colleagues at the Queen Elizabeth Hospital, University Hospital Birmingham NHS Trust. Our first example below is associated with the monitoring of the intravenous anaesthetic 2,6-di-isopropyl phenol (propofol) via breath analysis of patients undergoing surgery.

2. Drug monitoring via breath analysis: real-time breath monitoring of the intravenous anaesthetic agent propofol and its volatile metabolites on patients undergoing surgery

Total intravenous anaesthesia has been in common practice for many years, having been facilitated by introducing drugs that are rapidly and completely metabolized, allowing for rapid recovery after anaesthesia. The short half-life of such compounds necessitates giving them by infusion, but one problem that has always been present with the use of continuous infusions of intravenous anaesthetics is that there has been no method of determining their plasma concentration during the operation to ensure an appropriate level of anaesthesia. In contrast, for volatile anaesthetic agents minimum alveolar concentration values have been determined and monitors are routinely used to measure anaesthetic agent vapour concentrations within the anaesthetic circuit. Here we demonstrate the use of the PTR-MS for drug monitoring; namely for the detection of the intravenous anaesthetic propofol and its two volatile metabolites (2,6-di-isopropyl quinone

and 2,6-di-isopropyl quinol) on the breath of patients undergoing surgery.

Propofol is an interesting aromatic compound with a distinct smell, indicating its volatility (vapour pressure: 3.3 Pa at 25 °C). Its partition coefficient between blood and air has not been measured. Despite this uncertainty, we considered that, with the development of the sensitive PTR-MS, it should be possible to measure it in patients' breath during propofol infusions in real-time.

Prior to any hospital trials, we established that the PTR-MS was capable of monitoring propofol at the low levels of concentration expected on the breath. This was achieved by sampling the air (headspace) above a sample of blood serum taken two weeks previously from an anaesthetised patient. The resulting mass spectrum, with background peaks subtracted, is illustrated in Fig. 1. A strong peak at 179 amu dominates, with weaker mass peaks at 95, 137 and 193 amu being distinguishable. The 179 amu peak is protonated propofol:

$$H_3O^+ + C_{12}H_{18}O \rightarrow C_{12}H_{18}OH^+$$

The 95 and 137 amu peaks are also fingerprints of propofol, because they represent fragment ions resulting from collision-induced dissociation (CID) of the 179 amu ion in the PTR-MS. At the operating voltage of the drift tube, 600 V, the CID signals had an intensity of approximately 10% of that associated with the protonated propofol signal at 179 amu. Therefore, if the concentration of propofol on a patient's breath is to be accurately determined, the CID peaks must be taken into account. The peak observed at 193 amu is the protonated quinone metabolite. A weaker peak at 195 amu is assigned to be protonated propofol quinol. These measurements were verified by sampling air above pure propofol, which provided a much greater signal in our PTR-MS for us to experiment with, thereby allowing the ion masses to be readily identified.

In addition to this study with the PTR-MS, we used our selected ion flow tube apparatus for evaluating the reactions of H_3O^+ ions with propofol in the absence of an electric field gradient. This allowed us to determine that the reaction rate coefficient is collisional. In addition the SIFT studies were used to confirm that the only product resulting from the reaction of H_3O^+ with propofol is solely protonated propofol, and therefore the 95 and 137 amu masses observed in the PTR-MS studies are indeed a result of CID rather than from a dissociative proton transfer channel. Protonated 2-(1-methylethyl1)-phenol is the mass at 137 amu and protonated phenol is the mass at 95 amu (which is likely to be derived from CID from 137 amu), in each case a C_3H_6 molecule is lost from the cation.

Further confirmation that masses 95 and 137 amu result from CID comes from investigating the intensity of the spectral peaks of these masses relative to that of protonated propofol as a function of electric field strength in the drift tube of the PTR-MS instrument, as illustrated in Fig. 2. The intensities of the peaks associated with 95 and 137 amu ions relative to protonated propofol dramatically reduce with decreasing electric field strength—a clear indication of CID processes occurring. However, it is not advisable to operate the drift tube much below 600 V because of the increase of protonated water clusters, e.g., $H_3O^+ \cdot H_2O$ and $H_3O^+ \cdot (H_2O)_2$ in the reaction chamber.

Following the success of the laboratory measurements, the PTR-MS was taken to the Birmingham Women's Hospital NHS Trust. All of the patients monitored and

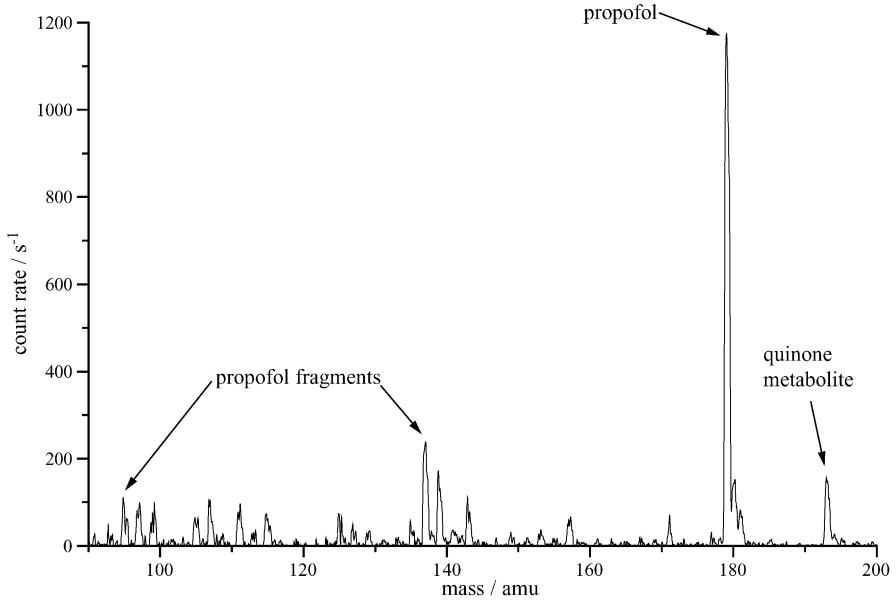


Fig. 1. Mass spectrum of the air sampled over serum taken from a patient anaesthetised with propofol. This spectrum represents the average of ten scans with the background subtracted. The peaks, which can be uniquely defined to propofol, are identified. The other mass peaks at 97, 101, 107, 111, 113, 115, 135, 139 and 141 amu remain unassigned.

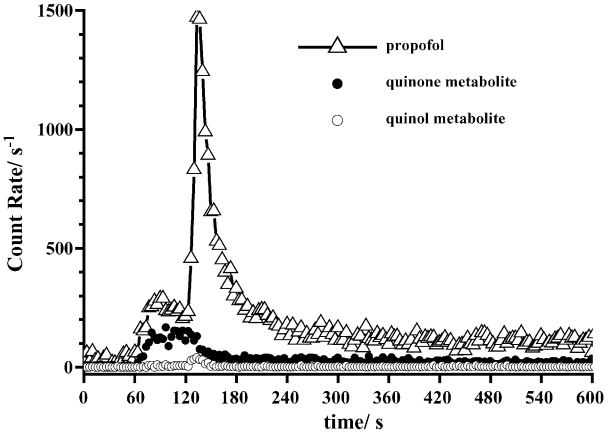


Fig. 2. Real-time propofol and metabolite trace gas measurements for the first 10 min of breath sampling from a patient undergoing surgery. This patient showed an initial surge of propofol shortly after the operation started, corresponding to approximately 50 ppbv levels and then steadily decreasing to a value of about 5 ppbv within about 10 min of the operation.

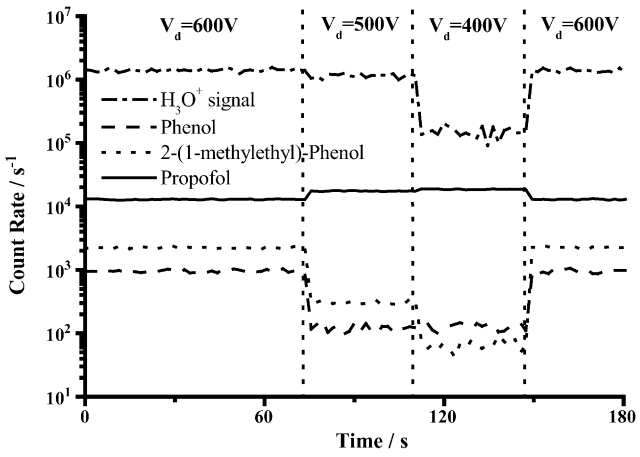


Fig. 3. Relative intensities of protonated propofol, 2-(1-methylethyl1)-phenol and phenol generated in the PTR-MS as a function of the voltage applied to the drift tube. The marked decreases in the protonated 2-(1-methylethyl1)-phenol and phenol ion intensities is a clear indication of collision induced dissociation, in which the energy gained by the protonated propofol from the electric field in the drift tube is used to break it up via a collision with a neutral air molecule.

participating in this study were undergoing gynaecological surgery. They received standard continuous intravenous anaesthesia. Anaesthesia was induced with fentanyl (1–2 $\mu g\,kg^{-1}$), followed by propofol delivered by a target controlled infusion device set initially to deliver an estimated 8 $\mu g\,ml^{-1}$ blood plasma level, until loss of eyelash reflex. Intubation was facilitated with atracurium (0.5 $mg\,kg^{-1}$). Patients were ventilated throughout surgery with a 50/50 O_2/N_2O mixture. Muscle relaxation was maintained using intermittent atracurium boluses, according to clinical need. During surgery, the target-controlled propofol infusion rates were gradually reduced to achieve 4 $\mu g\,ml^{-1}$ in plasma. Propofol infusion was stopped at the end of surgery and muscle relaxation was reversed by a mixed injection of neostigmine (2.5 mg) and glycopyrrolate (500 μg). Patients were ventilated with 100% oxygen until breathing spontaneously and able to be extubated. For the hospital measurements, we were not permitted to be in the operating theatre during surgery. Therefore, a patients' breath was side-streamed sampled from the anaesthetic circuit expiratory limb via a 4 m length of 1.6 mm o.d., 0.5 mm i.d., Kel-F unheated tubing into the PTR-MS.

In total, we monitored five surgical procedures; during which the PTR-MS was used in either selected-ion monitoring or mass spectral scan (0–200 amu) modes to record VOCs on patients' breath. The spectral scans were used to establish which masses should be monitored for the selected ion mode. For selected ion monitoring, the ion masses chosen included protonated propofol (179 amu) and its two CID products (95 and 137 amu—see below), together with those for the protonated propofol quinone and quinol metabolites at 193 and 195 amu, respectively.

Despite the expected significant loss in signal, because of the use of a long and unheated sample line, propofol and its metabolites were clearly identified on the breath of the all the patients monitored. Fig. 3 illustrates the variation in the concentration of the propofol and its two metabolites on the breath of one of the anaesthetised patient as a function of time during the early phase of an operation. Time $t = 0$ refers to the time the patient was taken into theatre. The total propofol count shown on the figure is the sum of the protonated propofol ion count and those for its two CID product ions, protonated 2-isopropyl phenol and protonated phenol. Also shown in the figure are the variations in the counts for the protonated quinol and quinone metabolites. Fig. 3 shows that this patient had an initial surge of propofol shortly after the operation started, corresponding to approximately 50 ppbv levels on the breath and then steadily decreasing to a steady value of about 5 ppbv within about 10 min of the operation.

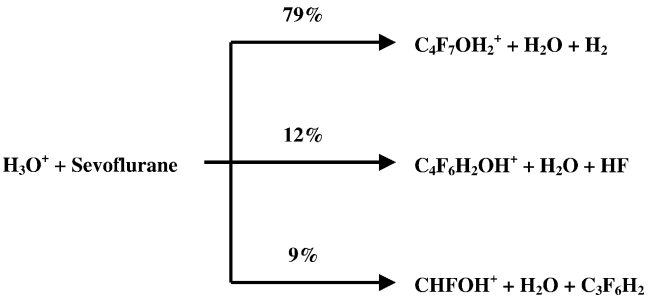
The data shown in Fig. 3 represents the most dramatic response we found. There was significant biological variation from one patient to the next, and therefore a full clinical study is required before the technique could be of benefit to reliably determine the propofol–blood concentrations of anaesthetised patients from an analysis of concentrations on their breath. Nevertheless, this proof-of-principle study has demonstrated the possibility of real-time Point-of-Care breath monitoring of propofol and its early metabolites. It opens the door not only to further experimentation that might enable the development of an instrument dedicated to the indirect measurement of plasma propofol levels during anaesthesia, but also to other applications in drug metabolism, pharmacokinetics and pharmacodynamics involving drugs and their metabolites, providing they are even just slightly volatile. If all of this is to be achieved, it is apparent from our study that better designs for breath sampling systems to minimise surface effects are needed, not only to improve the signal strength at the detector, but also, and critically, to determine the concentration of the drug on the patients' breath.

The major conclusion from this study is significant, that routine measurement of intravenous agents, analogous to that for volatile anaesthetic agents, may be possible.

3. Monitoring of the volatile anaesthetic sevoflurane: differences between SIFT and PTR-MS studies

In addition to the work on the intravenous anaesthetic propofol, we also investigated another anaesthetic, sevoflurane. Unlike propofol, this is a volatile anaesthetic for inhalation administered by vaporization. This anaesthetic has been investigated by Rieder's group, in which the breath of operating staff and patients were monitored. Through the observation of protonated sevoflurane, they concluded that the PTR-MS could be used as a simple and rapid method to determine the occupational exposure to sevoflurane [7]. In addition to this Wang et al. [8] have shown that H_3O^+ and O_2^+ ions can be used to detect sevoflurane on the breath of medical staff.

We have investigated the reactions of H_3O^+ with this molecule using both the SIFT and the PTR-MS instruments, with the results shown in Fig. 4a and b, respectively. Of interest are the differences in the results obtained between the two techniques. For the SIFT investigation, which uses a flow tube technique, the dominant reaction channel is dissociative proton transfer leading to $C_4F_7OH_2^+$. In addition to this, two other but minor dissociative proton transfer reaction channels were observed as illustrated in Fig. 4a, with branching ratios determined as shown below:



These three channels were also observed in the SIFT study by Wang et al. [8], but with varying branching ratios dependent on the reaction length used in their study. In contrast, the reaction monitored in this study with the PTR-MS predominantly progressed down the dissociative proton transfer route leading to $C_4F_6H_2OH^+$, resulting from the elimination of HF from the ion–molecule complex, which is different from the results obtained by Rieder et al. This may be a result of detecting sevoflurane on the breath rather than using the sample directly.

The difference in the branching ratios obtained from the SIFT measurements and PTR-MS measurements is surprising, given that in other proton transfer reaction processes studied, results obtained on the SIFT and PTR-MS are similar, i.e., within experimental error comparable branching ratios

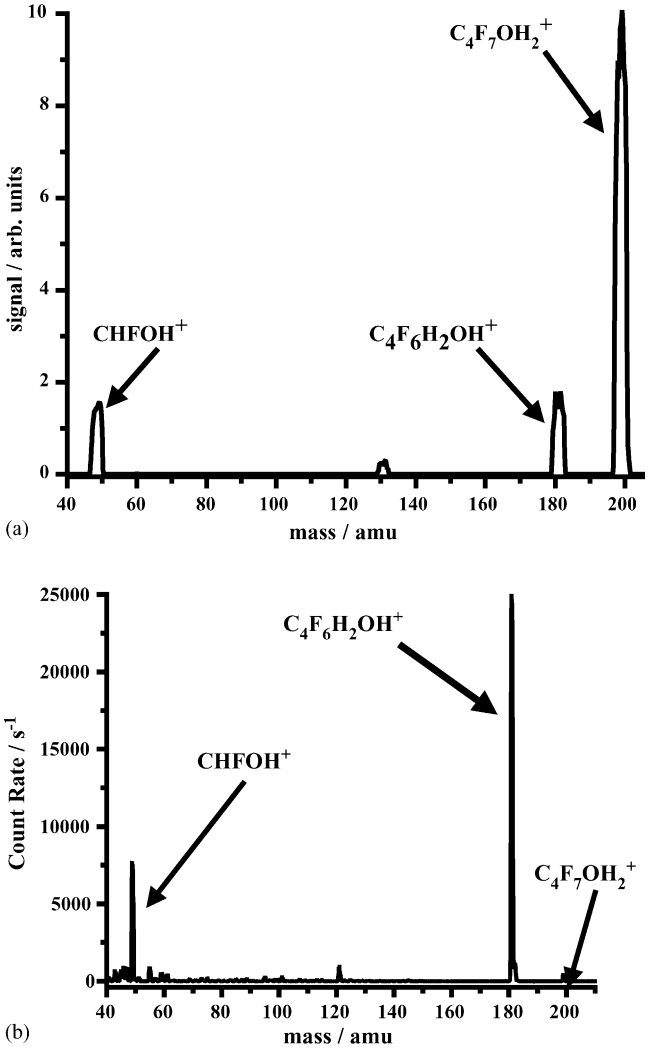


Fig. 4. The mass spectra resulting from the reaction of H_3O^+ with sevofluorane in (a) a selected ion flow tube and (b) the PTR-MS. Note the differences in the branching ratios.

should have been obtained. There are of course major differences in the experimental techniques used on the PTR-MS and SIFT; namely in the former there is an electric field which accelerates the ions through the reaction chamber, whereas in the SIFT the ions are convected through the reaction region by means of a helium buffer gas. The presence of an electric field can lead to CID, as described above for the study with propofol. However, CID cannot explain the intensity of the $C_4F_6H_2OH^+$ signal. The expected structure of protonated sevoflurane would seem to exclude any collisional process which could result in the elimination of HF. In addition no non-dissociative proton transfer channel was observed as the electric field strength in the drift tube of the PTR-MS instrument was reduced. Differences in the collisional processes occurring with the SIFT and PTR-MS with the transient protonated species must be leading to the observed differences. This is intriguing, and may be indicative that the properties of the PTR-MS have not yet been fully characterised.

4. The PTR-MS and the monitoring of bacteria

Different microbial cell cultures are known to emit characteristic aromas. Therefore, we should expect different mass spectra recorded on the PTR-MS used to analyse the headspace above different cell cultures. The ultimate aim of such studies is not necessarily to use the PTR-MS for the automatic analysis of cell cultures, although it could be used for that purpose with a complete automatic system which is computer controlled. Rather it is to exploit and use its real-time and in situ capabilities to identify bacteria quickly after any serious outbreaks of, for example, microbial contamination of food leading to food poisoning, or to monitor food routinely for bacterial infection. Another example of such a use for the PTR-MS includes the rapid diagnosis of hospital infections, which can be life threatening—especially when the time is factored in as to how long it takes to grow cultures, which at the very least is 24 h. Here we illustrate that the PTR-MS is capable of selecting different bacterial cultures, which ultimately could lead to a rapid screening procedure.

Headspace air was sampled from a closed container successively over pure blood agar cultures of *Pseudomonas aeruginosa* and *Streptococcus milleri*. Blank spectra were obtained of the headspace air over uninoculated blood agar in the same container. The blank spectra were averaged and subtracted from averaged spectra from each culture. The results are shown in Fig. 5a for *S. milleri* and in Fig. 5b for *P. aeruginosa*. The comparison between the two mass spectra is dramatic. Whilst a number of mass peaks are common, the intensity scales are very different. In addition, there are a number of different mass peaks in the two spectra.

This study has clearly demonstrated the potential of the PTR-MS for this type of analysis and its use for discriminating different bacterial species.

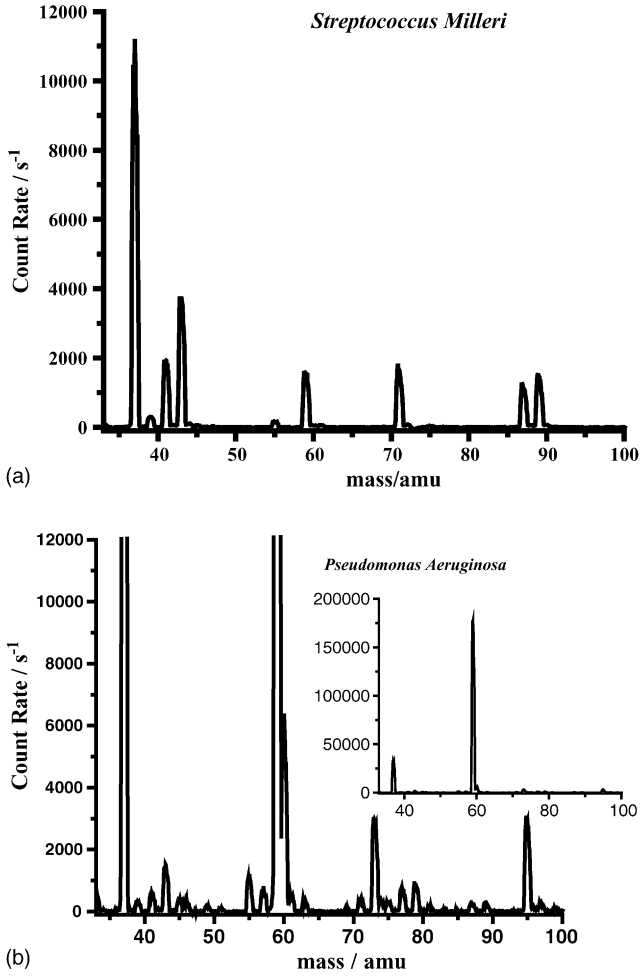


Fig. 5. The mass spectra resulting from the reaction of H_3O^+ with the headspace emitted from a cell culture containing (a) *Streptococcus milleri* and (b) *Pseudomonas aeruginosa*.

5. Summary

The overall success and general applicability of mass spectrometric approaches and instruments used for trace gas detection have been limited by a number of inherent problems; these include poor reproducibility, lack of selectivity, false positives/negatives, slow response time, and non-linearity. Underlying all these problems is an inadequate understanding of the elementary chemical processes involved which leads to a lack of development of new apparatus with improved sensitivity, selectivity, capability and flexibility. Furthermore, mass spectrometry used for trace gas detection is usually laboratory based often requiring preconcentration of any sample before analysis. This is time consuming, costly and leads to possible sources of contamination. If it is not laboratory based, transportable mass spectrometers have been specifically designed for the detection of certain trace gases and have therefore limited applicability. The recently developed PTR-MS goes a long way to overcome many of these inherent difficulties.

This work has demonstrated the potential of the PTR-MS for drug and bacteria monitoring by "finger-printing" the pattern of trace volatile marker substances emitted in the breath or from bacterial cultures with sampling and analysis taking place simultaneously, for fast and real-time detection.

The use of the PTR-MS technique for non-invasive medical diagnostics and drug monitoring based on human breath analysis of trace levels of organic chemicals has great potential. Major development and investment is required to develop the PTR-MS further, not only for the type of applications mentioned in this paper, but also for diagnosis of disease. Ultimately this could lead to the routine use of breath analysis for medical screening in hospitals, in outpatient clinics and potentially general practitioner surgeries, providing patients with better quality care.

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