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Medical applications of proton transfer reaction-mass spectrometry: ambient air monitoring and breath analysis

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Abstract

The analysis of volatile organic compounds (VOC) has witnessed an enormous boost over the past decade, as this substance group has received broad attention regarding indoor air quality and disease screening. This review addresses the medical applications of proton transfer reaction-mass spectrometry (PTR-MS) in the fields of risk environment monitoring and diagnostic breath sample analysis.

The major advantages of PTR-MS are its ability to perform rapid measurements as needed for the quick evaluation of screening samples, and even on-line VOC profile assessment. This may be of considerable importance in the investigation of rapidly changing air contamination patterns. Sources of contamination could be unveiled in operating theatres, post-anesthesia care units, and medical sterilization wards using PTR-MS.

In the field of breath analysis, PTR-MS has been employed to investigate exhaled concentrations of isoprene, methanol, potential tumor markers, and volatile anesthetics.

In conclusion, PTR-MS offers highly sensitive and rapid determinations of VOC profiles as compared to other methods of detection. PTR-MS therefore basically meets the needs of a screening method. The potential of directly mirroring serum concentrations of diverse substances in real-time makes breath analysis a highly promising field of research.

Keywords: Proton transfer reaction-mass spectrometry; PTR-MS; Medicine; Risk environment

1. Introduction

The analysis of volatile organic compounds (VOC) has witnessed an enormous boost over the past decade, with the number of manuscripts published annually in connection with VOC analysis rapidly increasing. For example, a simple MEDLINE search for the term VOC revealed 17 manuscripts published by 1980, 84 publications by 1990, and 1108 hits by 2004. The medical importance of VOC has been acknowledged in the context of ambient air analysis [1]. Furthermore, an enormous potential of VOC analysis in breath samples has been suggested in attempts to develop screening methods for metabolic [2] and neoplastic diseases [3]. It is the aim of this review to present the applications of proton transfer reaction-mass spectrometry (PTR-MS) in the surveillance of risk environments, and diagnostic breath analysis.

2. Supervision of medical risk environments

Most people in industrialized countries spend a large proportion of both their work and free time indoors. Over the past decade, however, the quality of indoor air has been the subject of numerous studies. VOC, a collective of hydrocarbons abundantly present in ambient air, has been conjectured as one of two major substance categories responsible for sick

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building syndrome and decreased air quality [1]. VOC assessment is especially important in areas associated with inadvertent occupational exposure to volatile agents, as, e.g., operating theaters [4].

2.1. Occupational exposure to volatile agents in medical settings

It is widely acknowledged that personnel working in operating rooms and post-anesthesia care units are exposed to a variety of noxious agents [4]. Due to the specific nature of general anesthesia, and despite improved ambient air control, medical personnel working at operating rooms is still permanently [5] exposed to traces of volatile anesthetics [6]. Although it has not been ruled out that these halogenated agents, related to chemical solvents, may cause adverse health effects upon medical personnel, they still enjoy widespread use [7]. The potential risks of occupational exposure to trace anesthetic gases are still controversially discussed [4,6]. One of the first reports to spawn serious concerns about the workplace safety of volatile anesthetics was the description of higher rates of spontaneous abortions in Russian anesthetists [8]. The potential of interactions between volatile anesthetics and human reproduction has since been the subject of numerous studies [9–11]. Husum et al. and Hoerauf et al. reported increased rates of sister-chromatid exchange elicited by exposure to concentrations of volatile anesthetics in vivo and in vitro [12–14]. Moreover, halogenated anesthetics have been implicated in peripheral lymphocyte apoptosis and may thus, in principle, contribute to perioperative leukopenia [15]. Finally, recent evidence has conjectured volatile anesthetics in the pathogenesis of multiple sclerosis [16]. Therefore, national public health authorities such as the National Institute of Occupational Safety and Health (NIOSH) have introduced threshold values for ambient air regulating occupational exposure over a given time period [17]. Current literature states that strict adherence to these limits should ensure satisfactory workplace safety [18]. However, poor [19] or faulty [6] ventilation may lead to undesirably high levels of exposure. Unfortunately, even today, a high number of health care facilities still seem to fall into the latter category [8]. Therefore, it should be considered imperative to enhance surveillance of risk environments [6]. Occupational exposure to volatile anesthetics has been previously determined by gas chromatographic and infrared spectrometric measurements of ambient air [6,17]. However, thus far, direct in vivo measurements of volatile anesthetic kinetics have only been carried out in intubated patients during controlled ventilation in order to determine approximate end-tidal concentrations [20] and, utilizing gas chromatography, in urine of exposed personnel [21,22], even though the importance of both real-time measurements in ambient air and measurements of exhaled air had been emphasized [22,23]. Recently, studies utilizing PTR-MS contributed to both of these areas.

2.2. PTR-MS and occupational exposure to volatile anesthetics

PTR-MS was employed to monitor occupational exposure of personnel to volatile anesthetics in post-anesthesia care units (PACU) [6]. These workplaces are of considerable interest since postoperative patients continue to exhale volatile anesthetics, and ventilation may not be as closely monitored as in operating rooms [19]. Online analysis of ambient air in two PACU revealed an occupational burden of volatile anesthetics correlated with patient turnover [6]. Short-term peaks could be retrospectively linked to times of patient arrival and/or extubation at the PACU. Of notable interest in this study was the observation of increasing concentrations of anesthetic in ambient air during the night-time and a sudden decrease at a constant time-point in the morning (see Fig. 1). This was subsequently ascribed to a faulty programming of room air exchange capacity, which had not been spotted by the single control measurements [6].

Similarly, PTR-MS has been employed for surveillance of medical sterilization units [24]. Also in this case, a considerable source of pollution in the form of a leaking sterilization oven was demonstrated. The latter findings highlight one of the major advantages of PTR-MS analysis, namely the ability to correlate on-line measurements with activity protocols [6]. Thus, sources of indoor air pollution can be unveiled far more effectively than using cumulative measurements [6,24,25].

However, it has been suggested that the increasing technical possibilities for supervision of risk environments may lead to a considerable increase in costs. These costs would arise from more elaborate devices employed for supervision itself, and from subsequent adaptations in ambient air control systems [26]. Yet, in the context of ambient air analysis, such

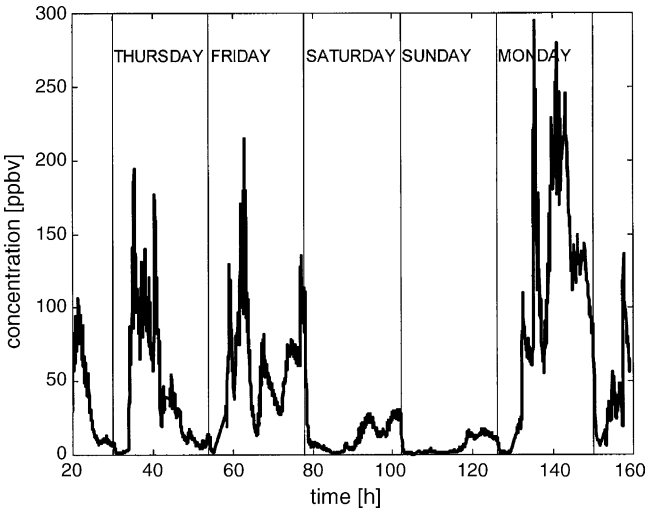

Fig. 1. Concentration of sevoflurane at a postanesthesia care unit (PACU) during routine service, taken with permission from Rieder et al. [6]. Vertical lines indicate 6:00 a.m. Faulty room air exchange programming lead to unrecognized system shutdown during the night with subsequent increase of trace gases (see text).

an assumption may be premature, as it has been calculated that coupling of on-line ambient air monitoring systems such as PTR-MS to room air exchange systems may, in fact, decrease costs [6]. This may best be deducted from the tracing of the time-course of sevoflurane in Fig. 1. Note the long intervals of minimal occupational exposure to sevoflurane, interrupted by rapid spikes in ambient air concentration. The authors calculated that during about 40% of the time, ambient air turnover would not need to be at maximum capacity, and hence costs on ventilation may in fact be economized. If the weekend with its decreased patient turnover were included in the aforementioned analysis, this share would even rise to 70% [6].

In analogy to the latter study, PTR-MS has also been described to allow for precise measurements of volatile anesthetics in operating rooms [27]. Also in this environment, the real-time nature of recordings allowed for a correlation between rapidly changing concentrations of volatile anesthetics and the source of contamination, i.e., anesthetic interventions such as endotracheal intubation and mask ventilation [27].

However, as mentioned above, PTR-MS is not only a valuable tool in the surveillance of room air exchange, but its sensitivity also allows insights into the pollution profile of medical procedures such as the securing of a safe patient's airway. For example, Rieder et al. compared the oropharyngeal leakage of trace anesthetic gases of two commonly used airway management devices, the laryngeal mask airway and the uncuffed endotracheal tube [28].

Based on the listed findings, may be deducted that single isolated measurements of ambient air quality, as they are widely performed to assure satisfactory control, are not sensible, since rapidly changing concentrations are lost in the crude temporal resolution of, e.g., intermittent measurements over the course of 1 day [6]. In this sense, PTR-MS real-time ambient air biomonitoring may represent one valuable alternative to determine concentration and time-course of volatile organic compounds at risk environments.

The evidence yielded from ambient air studies would remain circumstantial without in vivo measurements correlating these concentrations to effects. This was until recently impeded by methodological problems in the assessment of pulmonary volatile anesthetic excretion [22]. However, the ability to investigate this route of excretion would be of considerable interest since it is predominant in vivo [22].

Summer et al., therefore, employed PTR-MS to detect exhaled concentrations of the halogenated anesthetic sevoflurane in exhaled air of OR personnel [5]. The principal result of this study was the first depiction of sevoflurane uptake and excretion dynamics in exhaled breath of operating room personnel. After completion of operating room duty, operating room staff featured concentrations of sevoflurane significantly elevated as compared to baseline values and control group measurements (Fig. 2).

Similarly, Roithmeier et al. investigated changes in the exhaled profile of the volatile anesthetics sevoflurane and isoflurane in day-case surgery patients [29] before surgery and

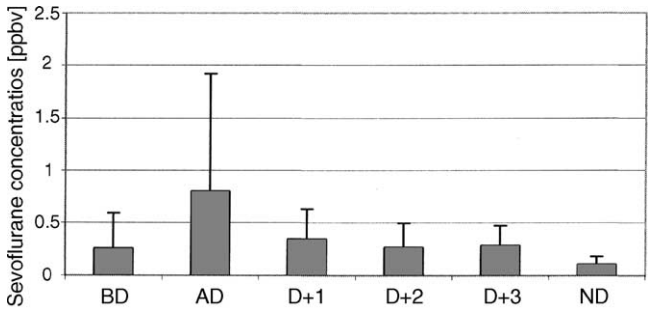

Fig. 2. Time-course of sevoflurane in exhaled air of OR personnel, taken with permission from Summer et al. [5]. Analysis of variance with adjustment for within correlation showed a highly significant time-effect. Data are given as mean ± S.D. *Abbreviations:* BD; before duty, AD; after duty, D + 1/2/3 duty plus 1/2/3 h, respectively, ND; next day.

during the postoperative days 1–5. It could be demonstrated that expiratory concentrations of sevoflurane were significantly higher than those of isoflurane at all time points. Nevertheless, parallel physical and psychological performance tests yielded superior results in the sevoflurane group, especially in the early postoperative period. These results indicate that sevoflurane may be the preferred volatile agent in day care anesthesia [29]. The higher concentrations of sevoflurane in exhaled air seem to affect patients less severely than the (lower) isoflurane trace concentrations.

Thus, the possibility to accurately measure volatile anesthetics in exhaled air coupled with, e.g., psychometric tests can be expected to significantly increase insight into concentration-dependent effects of these agents upon body function in the early postoperative period. Complementing urinary measurements, which may offer advantages in retrospective analysis of occupational exposure over periods of hours and days [22], rapid analysis of volatile anesthetics in exhaled air may serve as a mirror of short-term (minutes, hours) changes in metabolic pathways [5]. As one prominent feature of volatile anesthetics with a low molecular weight (i.e., below 200 Da) is a quick traversion of the alveolar membrane, direct correlations of exhaled anesthetics with corresponding serum levels are conceivable and will thus be the subject of further investigations [5].

3. Diagnostic sample analysis

3.1. Potential implications of diagnostic breath sample analysis

It has been common knowledge among physicians since the earliest days of medicine that various medical conditions are associated with characteristic odors. The substances principally involved in the generation of odors are the aforementioned volatile organic compounds (VOC). The latter are present in body fluids and detectable in human breath in patterns depending upon nutrition [30], disease [31,32], and physical activity [33]. Furthermore, exhalation rates of

individual, blood-borne, VOCs in human breath are dependent upon Henry's constant and, therefore, molecular weight and hydrophobicity [34]. Therefore, determination of VOC profiles in exhaled breath may represent a valuable diagnostic screening tool for a variety of metabolic [21,35], and neoplastic [3,33] diseases. This is especially tempting with regard to PTR-MS, since this appliance would allow for a rapid analysis of large number of samples, and could hence be employed as a screening tool [33].

3.2. Diagnostic sample analysis of methanol and isoprene

Methanol and ethanol were among the first substances to be investigated in depth using PTR-MS [30,36]. Whereas ethanol had been studied previously, simultaneous analysis of methanol was, until recently, virtually impossible using conventional mass spectrometric methods, since the ensuing fragmentation superposed individual molecular rudiments [36]. Methanol has been shown to be a multipotential carcinogenic agent [37]. Therefore, additional means of elucidating its role both in colonic physiology, neoplastic disease, and liver degeneration are of significant importance [36]. Breath-based analysis by PTR-MS may, in principle, be employed to test inborn variations in the metabolic pathways of alcohol breakdown [36]. Similarly, the endogenous production of methanol subsequent to the ingestion of fruit has been investigated using PTR-MS [30]. Investigations using PTR-MS could reaffirm the previously stipulated [38] endogenous methanol production within the human body of some 0.3 mg/l/h of methanol [30]. It could further be demonstrated that this endogenously yielded amount is significantly increased following the consumption of fruit. These increases subsequent to ingestion of fruits containing pectin have been proposed as an alternative hypothesis on the pathogenesis of non-alcoholic liver disease [30].

3.3. Isoprene analysis in exhaled breath

Another compound readily accessible to analysis using PTR-MS is isoprene, one of the most abundant VOC [2]. Taucher et al. first described the detection of isoprene in exhaled air using PTR-MS [39]. Isoprene is most probably synthesized via the isoprenoid pathway, and its synthesis most probably predominantly takes place downstream of the pacesetter enzyme regulating cholesterol biosynthesis, the hydroxyl-methyl-glutaryl-co-enzyme A reductase (HMG-CoA reductase) [35,40]. Based upon these assumptions and the readiness of isoprene to cross the alveolar barrier, a "mirror" function of isoprene for in vivo biosynthesis of cholesterol has been proposed [2,33,39,41]. Karl et al. investigated the response in breath isoprene concentration subsequent to treatment with a lipid-lowering drug of the statin class, which affects cholesterol biosynthesis at the HMG-CoA reductase level [2]. The decrease in blood low-density lipoprotein could be correlated to a concomitant decrease in breath isoprene (Fig. 3), although the exact mechanism of this consensual decrease could only be speculated upon.

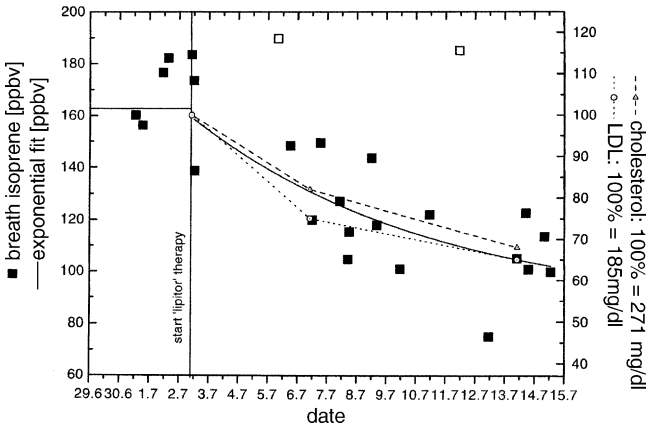

Fig. 3. Breath isoprene and serum cholesterol and low-density-lipoprotein (LDL) levels in an individual undergoing treatment with atorvastatin (Lipitor), taken with permission from Karl et al. [2]. Isoprene levels are plotted vs. decreases in blood cholesterol levels.

In turn, PTR-MS analysis could further reveal that the determination of breath isoprene content is associated with several potential pitfalls, the most important of which is probably due to its low Henry's constant (0.029 M/atm [2]). This constant governs the readiness of a given substance to cross the alveolar membrane. Therefore, a rapid reaction to alterations in cardiovascular parameters characterizes exhaled isoprene concentration was demonstrated using real-time PTR-MS breath analysis [2]. Subsequently, studies investigating isoprene should be guided by strict and comparable sampling procedures to minimize the influence of cardiovascular parameters upon test results [33,35]. One recent study into VOC kinetics carried out by Capodicasa et al. found dramatic rises in the VOC isoprene by a factor of up to 2.7 in end-stage renal disease patients undergoing hemodialysis (HD). Isoprene had been controversially discussed as a marker of oxidative stress [42,43]. Lirk et al. aimed to investigate in detail the kinetics of isoprene in 50 patients scheduled for elective hemodialysis using PTR-MS for sample analysis. In concordance with previous literature, a highly significant elevation of breath isoprene levels following HD could be demonstrated; with a mean quotient of 1.84 ± 1.41 comparing values before and after HD. Large interpersonal variations in isoprene kinetics could be observed. The mandatory resting times introduced to assure basal blood pressure and breathing rate values lead to lower values of isoprene than previously determined [2]. Taking into account the variables ambient air, lipid-lowering drugs, heart rate and blood pressure, and membranes of different biocompatibility, the previously reported increase in breath isoprene following hemodialysis could be confirmed. Direct influence of respiratory variables on isoprene exhalation was conjectured as the most probable cause for this increase based upon the results obtained using PTR-MS [35]. This would be in concordance with previous findings indicating that the previously stipulated nocturnal increase in breath

isoprene could similarly be explained by the influence of cardiopulmonary parameters such as breathing rate [2].

3.4. Neoplastic disease screening

Finally, diagnostic breath sample analysis has been proposed to recognize discrete changes in metabolic pathways elicited by neoplastic disease [3,33,44]. Examples of substances conjectured in the screening of lung cancer are *ortho*-toluidine [32] and metabolites arising from accelerated catabolism of alkanes and mono-methylated alkanes [3]. Rieder et al. investigated the diagnostic potential of *ortho*-toluidine in gynecological and hematological cancer patients using PTR-MS and found significant differences in breath content of this marker as compared to exhaled concentrations in healthy patients [33]. This would imply that o*rtho*-toluidine is not only a marker for lung carcinoma, but also for other malignancies. Considering the rapidly growing number of publications in the field of neoplastic disease screening, diagnostic breath analysis is sure to evolve into a highly promising field of investigation.

4. Conclusion

PTR-MS biomonitoring of substances in ambient air and exhaled breath is conceivable as a simple and rapid real-time method to determine the concentration and time-course of volatile organic compounds at risk environments. This is highly desirable since only real-time measurements offer the possibility to directly correlate intermittent contamination with activity in the surveyed area. As demonstrated for the cases of a post-anesthesia care unit and a sterilization ward, information such as this can be immediately applied to unveil and eliminate sources of contamination.

In the field of diagnostic sample analysis, PTR-MS offers highly sensitive and rapid determinations of VOC profiles as compared to other methods of detection. PTR-MS therefore basically meets the needs of a screening method. The possibility to directly mirror serum concentrations of substances as diverse as isoprene, methanol, etc. warrants the conduction of more detailed studies taking into account physico-chemical properties of these substances across the alveolar membrane. These studies could, in principle, help to elucidate the role of these components sensitively, non-invasively, and in real-time.

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