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From molecules in space to molecules in breath

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Abstract:

The evolution of the selected ion flow tube, SIFT, used to study ion-molecule reactions of interstellar significance, to the selected ion flow tube mass spectrometry, SIFT-MS, analytical technique is described briefly. Focus is placed on the application of SIFT-MS to breath analysis and its potential for the detection of volatile biomarkers of disease including respiratory pathogens. Typical concentrations of particular volatile breath biomarkers are given and their associations with specific diseases are indicated. A special case is the identification and quantification of gaseous hydrogen cyanide that is elevated in the exhaled breath of patients with cystic fibrosis and is now taken as a biomarker of *Pseudomonas aeruginosa* infection of the airways.

Introduction:

This short paper is intended as homage to a good friend and colleague, Professor Warren Lenney, a senior paediatric respiratory physician of great repute, on the occasion of his retirement. The title of this paper is perhaps curious but, hopefully, it will be understood by the way it is structured and developed and how the content is relevant to respiratory medicine and to Professor Lenney's legacy.

The ultimate focus of this story is the real time detection and quantification of volatile trace compounds in exhaled breath and how they can be exploited as non-invasive biomarkers of normal physiology and patho-physiology and of respiratory pathogens and metabolic disease. Realization of these desirable objectives has been greatly facilitated by the development at Keele University of a new analytical technique that we call selected ion flow tube mass spectrometry, SIFT-MS. The origin of this novel analytical method in the 1970s resides in the need to explain how the complex molecules observed in interstellar clouds, which includes many biogenic-type molecules, can be synthesised under the extreme conditions of very low temperatures and pressures that pertain to interstellar clouds [1]. It was hypothesized that the chemistry largely involved the reactions of positively charged ions with neutral atoms and molecules. To study this ion chemistry new instrumentation was required that could provide the desired kinetics data on ion-molecule reactions to facilitate the quantitative modelling of this chemistry. Thus, the selected ion flow tube technique, SIFT, was born in the laboratory of the author (in collaboration with Nigel G. Adams) at Birmingham University, UK.

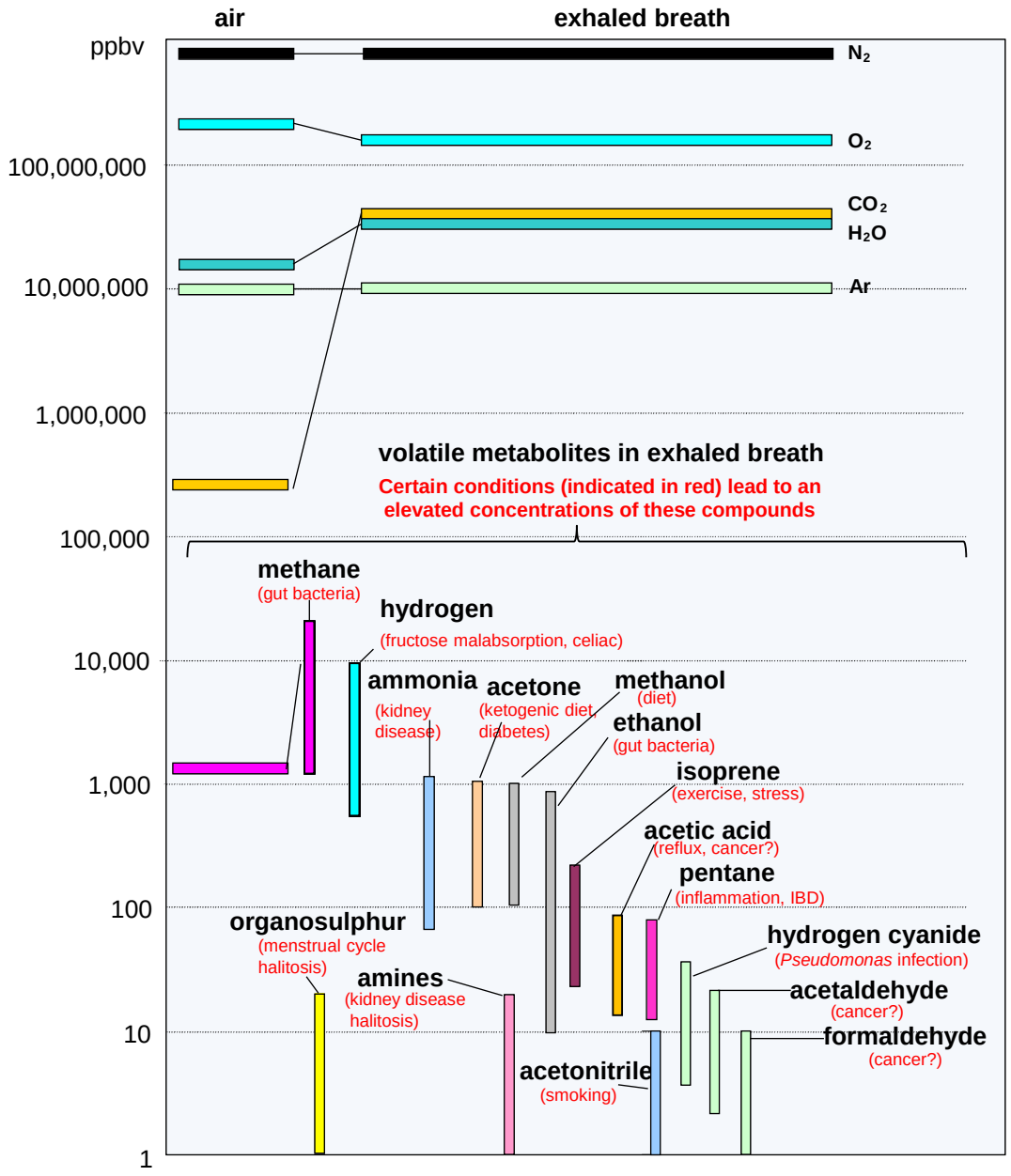

Figure 1 Composition of air and exhaled breath. The concentrations of major breath compounds and the nominal **concentrations** of some **volatile metabolites** are shown in parts per billion by volume, ppbv, on a logarithmic scale. See the listed references for further details.

SIFT and SIFT-MS:

In the SIFT the ions whose reactions are to be studied are generated in an external ion source and injected via a quadrupole mass filter into fast flowing helium carrier gas. Thus, a wide variety of ions, selected according to their mass-to-charge ratio, m/z, can be injected into flowing helium and their reactions studied with a wide range of molecular species at thermal energies, to include many reactions that are judged to be important in naturally occurring media such as the terrestrial ionosphere, other

planetary atmospheres and interstellar gas clouds [2]. The large amount of data acquired using SIFT instruments, especially the rate coefficients, *k*, for hundreds of ion-molecule reactions, has contributed greatly to the understanding of interstellar chemistry and resulted in the adoption of this technique in many laboratories worldwide.

Being cognizant of the discovery of trace biogenic molecules in exhaled breath in the 1990s, the author (with Patrik Spanel) realised that the SIFT technique could be inverted to form the SIFT-MS analytical method by which the concentrations of trace molecules could be determined in real time in ambient air and exhaled breath if the *k* values were known for specific and relevant reactions of biogenic molecules. Thus, SIFT-MS has been demonstrated to be a valuable and versatile analytical method that uniquely compliments those available to analytical science. The timeline for this development and the challenges that had to be met are described in depth in a recent paper [3]. Ambient air or exhaled breath can be directly sampled at a known rate into the SIFT-MS instrument, obviating sample collection into bags or onto traps, and analysed in real time with the results immediately available to the scientist or clinician for rapid interpretation. Breath is exhaled at the sample entry port of the SIFT-MS; on inhalation, the ambient air is sampled and analysed, as is important in breath analysis research. Details of the SIFT-MS method, the physics and ion chemistry involved, a line diagram showing the structure of the instrumentation and its application to breath analysis, have been given in recent authoritative reviews [4, 5].

Breath analysis:

The remainder of this short paper focuses on breath analysis aimed at supporting clinical diagnosis and therapeutic monitoring. Professor Lenney and his clinical colleagues have made and continue to make important contributions to this new subject area, principally by exploiting SIFT-MS. It is often stated that hundreds of trace biogenic molecules are present in exhaled breath even of healthy individuals. However, relatively few have been quantified accurately enough to be useful as reliable biomarkers of physiological status; even fewer have had reference ranges established for healthy individuals. The important point to be made is that the data acquisition rate using SIFT-MS can be very large. Since breath sampling is carried out directly, non-invasively and painlessly without time-consuming sample collection and preparation, accurate breath analysis can be carried out for significant cohorts of healthy volunteers and patients in short times. In a recent study, the exhaled breath of some 70 healthy individuals was analysed for two different compounds simultaneously in a single morning. Some of the trace compounds detected and quantified in breath by SIFT-MS are indicated in Figure 1. The association of particular trace metabolites with disease states are also indicated together with the concentration reference ranges for ostensibly healthy individuals resulting from longitudinal inter-individual and intra-individual studies [4, 5]. Careful scrutiny of Figure 1 reveals that the trace biomarkers are present in concentrations ranging from sub-ppbv (parts-per-billion by volume) to a few ppmv (parts-per-million by volume), i.e. many orders-of-magnitude in concentration below that of carbon dioxide and water vapour. Much more could be said about the data in Figure 1; rather, references to the results of individual studies of the specific molecules indicated and their biogenic significance are given in the listed review papers. However, it is worth noting the results of just two SIFT-MS studies that reveal the potential of SIFT-MS

for breath analysis in medicine. The close association of exhaled breath pentane with inflammatory bowel disease, IBD, both Crohn's disease and ulcerative colitis [6], and the association of breath acetic acid with gastro-intestinal reflux disease, GERD [7] are impressive examples. These studies involved significant cohorts of patients and healthy controls and identified just single biomarker organic compounds for these disease conditions.

HCN and PA:

Finally, focus is given to those areas of breath research to which Professor Lenney and his younger colleagues (notably Drs. Will Carroll, Beth Enderby and, most recently, Fran Gilchrist) have made a very important contribution. This is the discovery that volatile hydrogen cyanide, HCN, (which happens to be one the interstellar molecules studied by SIFT) is released by the *Pseudomonas aeruginosa, PA*, bacterium that colonises the respiratory tract of those suffering from cystic fibrosis, CF. This was first discovered during the SIFT-MS analysis of the headspace of plate cultures of PA [8]. This initiated a study over a decade guided by Professor Lenney that has ultimately established that gaseous HCN is elevated in the nose-exhaled breath of CF patients. Thus, a non-invasive biomarker of airways colonisation by PA has been identified that can be used to track the eradication of this bacterium to the benefit of the CF patients. The timeline for these thorough and extensive studies, which involved parallel *in vitro* and *in vivo* measurements, is reported in a recent paper [9]. These studies, so imaginatively conceived by Professor Lenney, are being continued and expanded at Keele by his successor clinicians supported by SIFT-MS scientists. The most recent work involves *in vitro* studies of the trace compounds emitted by other common respiratory bacteria, such as *S. aureus*, *S. pneumoniae* and *H. influenza*, in the search for and the identification of volatile non-invasive biomarkers of these pathogens [10].

Acknowledgements: The breath analysis work at Keele outlined in this paper would not have been possible without both the crucial contribution of Patrik Spanel to the development of SIFT-MS and the proving research and the close collaboration between the physical scientists and the clinicians who have been ably directed by Warren Lenney.

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