Breath Analysis Science at PittCon 2012, Orlando, Florida

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Background

Breath analysis science was featured in three organized sessions at this year's Pittsburgh Conference and Exposition, or "PittCon 2012" (http://www.pittcon.org/). As described in previous meeting reports, PittCon is one of the largest international conferences for analytical chemistry and instrumentation, typically attracting about 20,000 attendees and 1,000 commercial exhibitors (Pleil 2010, Pleil 2011). This year the conference was held in Orlando, Florida, USA at the Orange County Conference Center, a beautiful venue centrally located a few miles from some of Florida's most famous tourist attractions including Universal Studios Florida, Walt Disney World, and Seaworld Orlando; NASA's Kennedy Space Center is only 50 miles away, and the Florida Keys start a mere 290 miles to the south with the famous Key Largo, and end 100 miles further at the legendary Key West. (We've noted that Orlando in the springtime is a particular favorite among our European colleagues).

In the past few years, breath analysis science has taken on an ever-increasing role at PittCon through the success of the International Association of Breath Research (IABR) and the Journal of Breath Research (JBR). We have also had a presence at a recent conference of the Submarine Air Monitoring and Air Purification (SAMAP) organization in Taranto, Italy, wherein breath analysis and other biomarker measurements are taking on an increasingly important role in assessing exposures and health in artificial atmospheres including submarines, aircraft and spacecraft (Pleil and Hansel, 2012).

In 2012, members of IABR organized or participated in three distinct PittCon events: a contributed technical session, a conferee networking session, and an invited symposium series. The common thread among these three different conferencing settings was the concept of "non-invasive" environmental, medical, and diagnostic biomarker assessment. Of particular interest was how breath is being considered more and more mainstream as a biological medium, and is often discussed favorably as complementary or alternative to blood and urine measurements.

Technical Session: Non-invasive Biomedical Analysis

This was our JBR/IABR signature session organized and co-chaired by Dr. Joachim Pleil (pleil.joachim@epa.gov) from the U.S. Environmental Protection Agency and Dr. Wolfram Miekisch, Ph.D. (wolfram.miekisch@uni-rostock.de) from the Rostock University Hospital in Rostock, Germany. Our speakers were carefully selected to cover a broad range on breath diagnostic techniques with some topics including urinary biomarkers, the "other" non-invasive (or at least minimally invasive) biomarker approach.

The first presentation, *"Non-invasive biomedical analysis – Dawning of a new area of diagnostic information"* was given by Prof. Jochen K. Schubert, MD (jochen.schubert@uni-rostock.de) from the University of Rostock, Germany. Prof. Schubert discussed the important role that diagnostic tests play in life-saving therapy and in early stage disease detection. He particularly stressed the increasing need for non-invasive techniques (especially for critical care medicine) and for rapid assessments preferably performed at the bedside. He discussed new technologies for instrument miniaturization for breath analysis and presented results showing the potential of real-time mass spectrometers based on proton transfer reactions (PTR). He also gave an intriguing example for a minimally invasive blood test based on a gas-phase (breath) technology wherein specially coated fibers are inserted directly into the blood stream to absorb certain compounds of interest for subsequent desorption analysis using standard solid phase micro-extraction (SPME) methods.

Dr. Jens Herbig (jens.herbig@ionimed.com) from Ionimed Analytik in Innsbruck, Austria gave the second presentation, entitled "*Applications of PTR-MS in Medicine and Biotechnology*". Dr. Herbig began his talk with an introduction to proton-transfer-reaction mass spectrometers (PTR-MS), highlighting benefits of these analytical tools for real-time analysis of VOCs in biological media, and articulating differences between available quadrupole and time-of-flight (TOF) based detections systems. He then demonstrated how, via real-time monitoring of exhaled breath, PTR-MS instruments can be used to evaluate chemical update, distribution, and metabolism in the human body. Finally, he showed applications of the PTR-MS systems for real-time monitoring of in vitro systems. An example was given of the analysis of fermentation off-gas produced by microorganisms. Together these descriptions and examples showed unique advantages of real-time monitoring equipment over classical offline tools, drawing considerable interest from session attendees.

Dr. Jon Sobus (sobus.jon@epa.gov) from the U.S. Environmental Protection Agency in Research Triangle Park, NC presented *"Statistical considerations for interpreting urinary biomarker concentrations."* He discussed how urine, like breath, is a preferred sample medium for biomedical analysis since it is available in abundant supply and collected using non-invasive techniques. He highlighted that urinary biomarker measurements are generally reported in units of concentration, and are therefore subject to variations from changing urine output, as well as changing exposure or health state. Dr. Sobus stressed that, given this dependency, careful evaluation of urinary biomarker measurements is critical for decision making in medical and public health investigations. He further proposed a mathematical approach to correct for changing urine output; correction factors, based in part on urinary creatinine measurements, used empirical observations of healthy adults made during a recent EPA study. In closing, Dr. Sobus highlighted implications of using and not using correction factors for biomedical urine analysis.

Dr. Sobus was followed by Prof. Cristina E. Davis (cedavis@ucdavis.edu) from the University of California, Davis with a presentation entitled *"Mammalian cell culture headspace volatile organic compounds hold vital clues as putative biomarkers of cellular changes."* Prof. Davis described the analysis of volatile organic compounds (VOCs) from cell culture using gas chromatography-mass spectrometry (GC-MS) to develop an odor fingerprint. She illustrated how VOC headspace analysis can distinguish between human B cells differing by a single human leukocyte antigen (HLA) gene; demonstrating how VOC analysis can illustrate changes occurring on a cellular level. Prof. Davis then showed that different viral infections result in unique fluctuations to VOC fingerprints indicating tremendous biomedical potential. She suggested the potential for such a VOC analysis as a non-invasive technique for diagnosing infections given the development of appropriate sensor devices.

Next, Dr. Tzipporah M. Kormos (kormos.tzipporah@epa.gov) from the U.S. Environmental Protection Agency presented *"Metabolomics evaluation: perturbations of organic metabolites in human breath and urine."* Dr. Kormos provided an overview of the attempts underway in method development at the U.S. EPA for applying non-targeted top-down approaches to link environmental exposures to human health effects. She described the goal of characterizing the human exposome in order to effectively link exposure to health effects. However, she also denoted how the eclectic chemical composition of the exposome poses an analytical challenge requiring a range of instrumentation to effectively characterize its components (including liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance spectroscopy (NMR), immunochemistry, and GC-MS. Dr. Kormos stressed the need for non-invasive sampling in such exposure studies as the only way to obtain a sufficient number of biological samples from the general populace in field studies.

The sixth presentation of the session, entitled *"Real-Time Measurements and Mathematical Modeling of Breath Biomarkers to Address the Impact of Physiological Effects,"* was presented by Dr. Julian King (julian.king@assoc.oeaw.ac.at) of the Austrian Academy of Sciences, Dornbirn, Austria. Here, Dr. King presented real-time measurements of VOCs in breath samples, collected during periods of rest, exercise and sleep, and determined using PTR-MS and solid phase micro-extraction (SPME)/GC-MS. Dr. King focused on acetone and isoprene as target molecules, and showed how measurements of these analytes, combined with mathematical models, can be used to explore mechanistic relationships governing the general behaviors of VOCs in breath. Dr. King suggested that these results provide a basis for experimental design and general guidance for interpreting empirical results. Specifically, he recommended breath gas analysis for evaluating metabolic processes of VOCs, namely storage, transport, and biotransformation, *in vivo*.

Matthew Stiegel (mstiegel@live.unc.edu) from the University of North Carolina at Chapel Hill, NC, gave the seventh talk, entitled *"Correlations of Inflammatory Cytokines in Blood, Exhaled Breath Condensate, and Urine."* This talk focused on multi-media biological measurements of interleukins, interferon-γ, and tumor necrosis factor-α made as part of a controlled chamber experiment using human volunteers. Here, the TH1/TH2 cytokines were evaluated as makers of inflammatory/endogenous response to various controlled exposures, including diesel exhaust, ozone, and diesel exhaust plus ozone. Stiegel showed that cytokine levels and patterns changed across media and subjects, and suggested that these changes reflect subject-specific responses to the controlled exposures. He concluded that cytokine levels in blood, EBC, and urine are

probative indicators of exposure and/or endogenous response, and thus, are useful for exposure and health monitoring.

Phillip Trefz (phillip.trefz@uni-rostock.de) from the University of Rostock, Germany gave the final talk *"Micro extraction techniques as a link between clinical application and hyphenated analytical techniques."* Mr. Trefz explained how a current hindrance to the biomedical application of breath analysis is sampling. As analytical instrumentation is not yet a bedside technique, reliable tools are required for breath collection, concentration, and storage in order to enable the detection of trace VOCs. He explained the benefits of needle trap devices (NTDs) over solid phase extraction (SPE) and SPME techniques in terms of sensitivity and sampling time. A comparison of different sorbents showed the possibility of storing samples over a week while retaining high levels of recovery and reproducibility. He then described an interesting pilot study using NTDs to sample from ventilators of patients with pneumonia.

Networking Session: Non-invasive Biomedical Analysis - The Fast, the Furious, and the Brave - Innovative Analytical Instrumentation for Breath Gas Testing

As in past PittCon meetings, the networking session was organized and facilitated by IABR's own Dr. Wolfram "Wolfie" Miekisch. The theme this year was the spectrum of novel instrumentation spanning the range from biomarker discovery, high-throughput analysis, and eventual outpatient or homecare devices. The play on words in the title (taken from recent movies) referred to the three featured underlying analytical regimes for breath – discovery using two dimensional gas chromatography and time of flight mass spectrometry (2D-GC-ToFMS), on-line assessment and screening using proton transfer reaction mass spectrometry (PTR-MS), and the use of specific chemical sensors for outpatient monitoring and continuing therapy assessment.

After an opening statement and introduction by Dr. Miekisch, Dr. Jens Herbig from Ionimed Analytik began the networking session with a brief introduction of what it takes to have an "ideal" breath analysis instrument. He stated that the PTR-MS has all four main components of this ideal instrument: sensitivity, specificity, real-time practicality, and accuracy. The fact that it has all four of these necessary elements makes it THE instrument to use when doing real-time biomarker discovery or monitoring. The discussion moved from an instrumentation perspective to a more philosophical one as audience members wondered why it was important to have the ability, or even the desire, to use a discovery tool such as the PTR-MS. Opinions given ranged from the need for top-down discovery approaches that involve pattern analysis and perturbations of normal biomarker ranges, to the use of targeted single-compound, or "low-hanging fruit", analysis that could be perceived as more effective. It was further suggested that PTR-MS is more of an operational instrument in that it trades off speed of analysis for highly resolved specificity. Other discussions revolved around the issue of "competition" with blood and urine analysis; the strength of breath analysis in real-time is that it provides access to reactive/intermediary metabolites that otherwise can only be inferred from stable compounds measurements.

Joe Binkley (joe_binkley@leco.com) from LECO Corporation in St. Joseph, MI, followed this enlightening discussion with an introduction to the details of 2D-GC-ToFMS. The instrumentation was presented as a great discovery tool that allowed for a comprehensive

analysis of difficult matrices such as that of human breath. The increased chromatographic resolution of GCxGC made possible by orthogonal modes of separation (i.e., boiling point and polarity) as well as increased sensitivity due to the cryo-focussing effects of thermal modulation have lead to increasing use of this technology amongst breath researchers. The practical applications of the instrumentation were immediately compared to those of the PTR-MS. The 2D-GC-ToFMS was shown to be excellent not only in discovery but also with quantification, however it lacked the ability to do real-time analysis, potentially losing any reactive compounds during the downtime from sampling to analysis. The PTR-MS shines in the real-time arena, however it lacks the quantification ability of the 2D-GC-ToFMS. The consensus was that neither style of instrumentation was intrinsically "better" than the other but that they both served the research needs well within their niche applications. Comments were made that 2-D GC creates so much data that it takes quite some time to interpret it, but that it is probably the best way to get an appreciation for the breath exposome.

The last segment of the session was introduced briefly by Dr. Andreas Hengstenberg (andreas.hengstenberg@draeger.com) from Draeger, Lübeck, Germany. Draeger is well known for a variety of laboratory and industrial safety products including handheld or portable instrumentation for making air measurements. Dr. Hengstenberg commented that blood analysis is still considered to be the gold standard in medical practice and that this is a difficult hurdle to overcome. He presented some thoughts as to how chemical-specific biosensors could be implemented for breath bio-monitoring applications with handheld devices that could be used for outpatient or homecare monitoring where blood analysis cannot effectively compete. Dr. Hengstenberg led the discussion to the concept of the "triangle" of breath analysis comprised of the large laboratory discovery instruments (e.g. GC-MS or ToF, LC-MS-MS or ToF, 2D-GC-ToFMS), the real-time multi-components instruments (e.g. PTR-MS or ToF, SIFT-MS), and finally the small, targeted instruments (e.g. chemical sensors, sensor arrays, optical absorption, photoacoustic). The subsequent discussions revolved around the need for all three types of methodologies to be used in parallel. Although the eventual goal is the penetration of non-invasive breath measurement into the home at the personal level, this cannot occur without the continuous discovery and validation from the other two research arenas implementing broad-based analysis. The ensuing audience discussion concluded that specific biosensors could be used to support all three arenas but that the targeted sensors were limited by their compound-specific characteristics.

Symposium: Breath Analysis as a Non-invasive Alternative for Medical Diagnostics

The symposium session for breath analysis was organized by Prof. Janusz Pawliszyn (janusz@uwaterloo.ca) from University of Waterloo, Canada. Prof. Pawliszyn is the inventor of SPME techniques for measuring trace-level organic compounds in complex gas-phase, in the headspace of liquid and solid phase matrices, and even immersed in complex liquid phases such as blood and urine. SPME is now a mainstream technique for all forms of environmental and biomarker research including blood, breath and urine metabolites. Prof. Pawliszyn gave a brief introduction about breath analysis and then proceeded to serve as the moderator for the symposium presentations.

The first speaker was Prof. Raed A. Dweik, MD, (dweikr@ccf.org) from the Cleveland Clinic in Ohio. He presented an overview entitled "*The state of breath analysis: Achievements and*

challenges" wherein he discussed some of the most pressing issues in furthering the acceptance of breath analysis for clinical practice and diagnosis. He used the development of the standardization for nitric oxide (NO) analysis as an example that the rest of breath applications methodology should follow. In particular, he described the painstaking process undertaken by the American Thoracic Society (ATS) and the European Respiratory Society (ERS) as a joint effort to develop specific guidance for NO analysis. Without such efforts and assurances of unambiguous quality, he suggests that breath analysis will not be readily accepted in the clinical and diagnostic communities. In fact, he suggests that physicians want a simple answer for a diagnosis from breath analysis methods like a traffic light where green means healthy, yellow means maybe, and red means disease; he then added, *"…but without the yellow"*.

He was followed by Prof. Anton Amann (anton.amann@i-med.ac.at) from the Austrian Academy of Sciences, Innsbruck Austria who continued with the overview theme with his presentation *"The state of breath analysis: Achievements and challenges – analytical perspective"*. He discussed recent work in his laboratory that implemented new proton transfer reaction-time of flight mass spectrometry (PTR-ToF-MS) instrumentation to monitor endogenous breath markers in real-time. As an example, he demonstrated how the metabolic pathway for isoprene in breath could be traced back to muscle tissue acting as both source and reservoir. His experiments also included analysis of breath during sleep cycles wherein he showed that exhaled isoprene is directly affected by sleep stage, increasing during deep sleep, and decreasing during REM stages.

Prof. Terence H. Risby (thrisby@jhmi.edu) from Johns Hopkins University, Baltimore, Maryland, gave the next presentation *"Methodological aspects of VOCs collection in real-time breath analysis"*. Initially, Prof. Risby explained that exhaled organic biomarkers are not unique for different diseases but all reflect specific human biochemical pathways; the probative differences between sick and healthy patients are seen in changes in patterns that depend on how the pathways are affected by the disease. He also stressed the importance of regulating and monitoring the breathing technique of patients during breath collection because humans tend to hyperventilate when they focus on their own breathing. Feedback for depth of inhalation, breath pacing, and exhalation volume is necessary to assure that samples are comparable within and between subjects and among different laboratories. This is accomplished by monitoring exhaled carbon dioxide and differential pressure. He also stressed that external analytical standards made up to simulate the breath matrix are necessary to assure that cross-laboratory results are comparable. Only if diagnostic results can be shown as consistent and accurate will diagnostic breath methods ever be broadly accepted.

Dr. Joachim D. Pleil (pleil.joachim@epa.gov) from the U.S. Environmental Protection Agency's main research campus in North Carolina presented the environmental aspects of discovery research in biomonitoring; his presentation was entitled *"Breath biomarkers in environmental health science: decoding the human exposome"*. He discussed new discovery research for distinguishing between the human pulmonary microbiome and endogenous breath biomarkers derived from human metabolism. His examples showed gas chromatography – mass spectrometry (GC-MS) results for headspace analyses of cultured anaerobic bacteria known to infect humans, and contrasted them to aerosols collected directly from humans and grown under anaerobic conditions. He also followed up on Prof. Risby's concepts regarding breath sampling by introducing new instrumentation from Loccioni Group, San Rosario, Italy, that was adapted to

collect exhaled breath condensate using feedback "coaching" of the subjects. He showed preliminary data that variability in collected volume was reduced by a factor of 10.

The final symposium speaker was Dr. Heather L. Lord (hlord@uwaterloo.ca) from University of Waterloo, Canada who presented her research implementing various novel sampling techniques for breath analysis. Her talk was entitled *"Micro-sampling/sample preparation devices for breath analysis"* which focused on micro-scale sampling of breath wherein the organic compounds are partitioned away from the bulk breath matrix either through transmission or absorption for subsequent analysis. She explained that the novel membrane and sorbent-based technologies provide a fast and efficient concentration step due to the high surface to volume ratio in contrast to other absorptive devices. This technology is expected to be well suited for miniaturization and application to handheld devices as the sampling technology is essentially passive and requires little, if any, power.

Concluding remarks:

This year's foray of IABR members into the world of PittCon and analytical instrumentation was a successful venture. We had the opportunity to interface with the broader instrument and laboratory equipment manufacturing community. Breath work was well received and we hope to continue to disseminate the message that non-invasive biomarker methodology, especially using breath as a biological medium, is a valuable tool. We intend to continue our relationship with PittCon and encourage the JBR readership to consider attending and contributing to future meetings. We also encouraged our PittCon colleagues to participate in breath-specific endeavors by looking at JBR, the IABR website, and possibly attending the next IABR meeting.

References:

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The United States Environmental Protection Agency through its Office of Research and Development has subjected this article to Agency administrative review and approved it for publication.