



## **1. THE NETWORK – WHO WE ARE**

The Marie Curie Initial Training Network titled “Proton Ionization Molecular Mass Spectrometry” (PIMMS) was a multidisciplinary and intersectoral analytical chemistry programme which trained 15 Early Stage Researchers (ESRs) within a collaborative academic and commercial challenging research network involving 12 beneficiaries: University of Birmingham; Open University; Ionicon Analytik GmbH; Fondazione Edmund Mach-Instituto Agrario di S. Michele; Universität Innsbruck; University of Naples; Institute of Food Safety, Wageningen University and Research Centre; Zurich University of Applied Sciences; Centre de la Recherche Scientifique; Forschungszentrum Jülich GmbH; University of Rostock and the Helmholtz Zentrum Munich. Associated Partners involved with this ITN were KORE Technology Ltd (Ely, UK), Tracer Measurement Systems Ltd (Birmingham, UK), and the University Hospital Birmingham. This intersectoral nature of the PIMMS network combined commercial (both manufacturers and “end-users”), governmental and academic concerns using a range of state-of-the-art analytical techniques to address a number of topical analytical issues in an interdisciplinary cooperative with tangible societal and economic benefits. The intersectoral aspect of this Action ensured that real practical needs were addressed and that scientific and technological advances engendered by knowledge transfer initiated by PIMMS were disseminated within Europe to maximum benefit.

## **2. WHAT DID THE PIMMS NETWORK DO?**

The research training was focused on the conduct of internationally leading analytical research projects which, whilst centred upon the development and adoption of Proton Transfer Reaction Mass Spectrometry (PTR-MS) as an analytical tool, provided training across a wide variety of analytical technologies that are complementary to PTR-MS, thereby delivering a broad interdisciplinary and intersectoral experience for the ESRs in analytical chemistry. Training was provided through an integrated partnership involving all commercial companies within the EU who are developing, manufacturing and commercialising PTR-MS, a private “end-user” sector organisation, 3 governmental research institutes, 6 universities 2 hospitals, and two communication consultancy companies.

PIMMS has trained a new generation of analytical scientists in the skills necessary for the use, development and adoption of (primarily) Proton Transfer Reaction Mass Spectrometry (PTR-MS) for the detection of trace gaseous compounds in complex chemical environments. Furthermore, PIMMS has enhanced our understanding of the crucial role these chemicals play in different ecosystems, whilst providing the underpinning science needed to

develop PTR-MS in order to address the analytical challenges for compound detection and identification.

Research training was delivered and organised through five interlinking work packages (WPs) each containing research projects at the forefront of analytical science particularly concentrating on newly emerging fields that are inherently supradisciplinary in nature: viz. WP1: *Fundamental Research and Development*; WP2: *Food Sciences*; WP3: *Environmental Sciences*; WP4: *Health Sciences*; and WP5: *Homeland Security*.

## **2.1 Key Objectives of PIMMS**

The overall objectives of the PIMMS ITN, all of which were achieved, were as follow:

### **WP1. Fundamental Research and Development (Managed by UoB)**

- *determination of best reagent ion type and PTR-MS operational parameters;*
- *an improved understanding of how to exploit the ion chemistry;*
- *development of inlet systems for breath, particle & liquid samples.*

### **WP 2. Food Sciences (managed FEM)**

- *to develop suitable reliable, and reproducible nose-space sampling methods;*
- *improved understanding of the link between inter-individual differences in flavour release and food properties during food and drink intake;*
- *provision of data on intra-individual differences.*

### **WP 3. Environmental Sciences (managed UIBK)**

- *improving our knowledge of emissions of biogenic VOCs & photochemical transformation;*
- *development of PTR-MS for OH reactivity measurements in the laboratory and in the field;*
- *development and validation of a particle collection-thermal desorption inlet.*

### **WP 4. Health Sciences (managed by UR)**

- *development of breath sampling methods in humans and mice;*
- *provision of VOC profile for healthy controls (normal ranges etc.);*

- *time-resolved monitoring of breath VOCs during a well-defined physiological challenge;*
- *to investigate the diagnostic potential of breath analysis for patients suffering from liver disease and ischemia/reperfusion injury;*
- *use of mouse models to aid human studies on metabolic dysfunctions and the annotation of gene functions related to disease*

## **WP 5. Homeland Security (managed by ION)**

- *improvement in the applications of PTR-MS for security applications, i.e. suitable inlet and pre-concentration systems needed for rapid cycle times;*
- *to investigate the optimal reagent ion conditions and operational conditions (e.g. E/N).*

## **2.2 PIMMS Individual Research Projects**

The above objectives were all achieved through 15 individual PhD projects incorporated into the network all of which involved training and collaboration within the network:

1. Applications of PTR-MS to Disease Monitoring
2. Trace Detection of Threat Agents
3. Emissions of biogenic VOCs from flora and their potential as a measure of atmospheric stress
4. Fundamental & Applications of Proton Transfer Reactions
5. Improvements in Sampling Inlet Systems
6. Improvement of a direct aqueous injection inlet systems
7. The role of flavour compounds in the perception of food by nose-space analysis
8. Analysis of organic particulate matter by PTR-MS
9. Diet and breath metabolites: investigating dietary fibre action on gut microflora
10. Analytical chemometric tools for the characterization & authentication of agricultural produce
11. Towards a consumer-centric and individualized aroma science and their link to diet and health
12. Determining OH reactivity in various environments
13. Emissions of biogenic VOCs & their photochemical transformation
14. Application of volatile biomarkers for medical diagnosis

## 15. Evaluation of short & midterm changes of VOCs in the breath gas of mice and humans with a metabolic disease phenotype

In addition to the research training that the ESRs received, PIMMS also provided a wider applied training in mass spectrometry, analytical and statistical techniques, vacuum systems, fundamental quantum chemistry, and instrumental diagnostics, and more generic training in project management, communication (public and scientific), IT, proposal writing, thesis writing, creativity, IPR, project and time management, and entrepreneurial skills etc. These training programmes have enhanced the employability of the PIMMS's ESRs and not just for jobs only in analytical chemistry.

### 2.3 Timeliness of PIMMS

PIMMS was timely because of the need to accurately identify trace compounds in complex chemical environments related to the food, security, environmental and health issues. Synergies have been established through research and industrial activities that are fostering interdisciplinary and intersectoral communities and providing a forum for the important exchange of developments, best practices, ideas and knowledge across disciplines and sectors in order to overcome the current challenges that are limiting PTR-MS.

## 3. WHAT HAVE WE DISCOVERED?

There have been major step changes in the use of PTR-MS in the five key research areas, all of which are or will have significant impact on the instrumentation and its areas of applications. Major advances have been made in particular in the areas of atmospheric chemistry, health sciences and homeland security. Details on these advances and key discoveries for the individual workpackages are summarised below.

### WP 1 Fundamentals and Development

We have improved our understanding of the ion-molecule chemistry occurring in PTR-MS instruments. This is leading to an improved control on the reaction processes and fuller exploitation of PTR-MS instruments so that they are beginning to achieve their full potential. Work has been done to standardise VOC analysis so that PTR-MS yields the same results as other analytical techniques e.g. SIFT-MS and GC-MS. The developments in inlet and sampling systems for threat agents and particles have led to methods for detecting compounds with extremely low vapour pressures (WP5). Sampling systems have been

developed for breath analysis which has improved the reliability of data used for breath analysis. PTR-MS has been significantly developed from simply being a gas-phase analytical tool to one which will be able to sample liquids and particles (WP 3), Improvements in the generic analytical software for acquisition, analysis and operation have been achieved.

## **WP 2 Food Sciences**

Flavour analysis at an individual level and during the actual situation of eating and drinking has been developed. More detailed information on intra- and inter- individual differences, both on the perception of flavour and aroma and the effect of specific diets, have been obtained. Studies have successfully characterised and classified different teas, coffees and cocoa beans/chocolates by rapid and non-invasive head-space analysis in addition to investigating coffee secondary shelf life. Aroma formation during coffee roasting in an industrial scale has been successfully monitored. Furthermore, insights on the reflectance of botanical/geographical origin of cocoa beans on the final composition of chocolates were obtained.

## **WP 3 Environmental Sciences**

An inlet system to study particulate/aerosol material of environmental significance using a PTR-MS has been successfully developed and is currently being characterised. Measurements of OH reactivity using the Comparative Reactivity Method have been successfully deployed in two contrasted environments in the Mediterranean area and have helped to better understand the budget of reactive species. The new plant chamber facility 'PLUS' was characterized and applied to study real plant emissions and their photochemical transformation in the atmosphere simulation chamber SAPHIR (Simulation of Atmospheric Photochemistry in a large Reaction chamber - [http://www.fz-juelich.de/iek/iek-8/EN/Expertise/Infrastructure/SaphirPlus/SaphirPlus\\_node.html;jsessionid=B5FBB5C1BCC E6CAE07DCCBD57E09F69C](http://www.fz-juelich.de/iek/iek-8/EN/Expertise/Infrastructure/SaphirPlus/SaphirPlus_node.html;jsessionid=B5FBB5C1BCC E6CAE07DCCBD57E09F69C)). PTR-MS based measurement techniques for particulate phase constituents and for OH reactivity measurements have been compared to other analytical methods in SAPHIR.

## **WP 4 Health Sciences – Breath Analysis and Clinical Applications**

Mice studies have improved our understanding of how VOCs are linked to metabolic pathways. Non-invasive breath sampling procedures with minimal impact on animal welfare have been developed and characterised to provide reliable and reproducible measurements.

We have determined the feasibility of pilot applications of online breath gas analysis in unrestrained mice by PTR-MS with the aim to introduce and implement this test assay into high-throughput mouse phenotyping pipelines. We have established biomarkers in mice associated with different states of obesity, and metabolic fuel utilization in mice. Our mouse work clearly showed that the volatilome is influenced by genuine processes of mouse metabolism, as well as microbial actions especially of the gut microbiome, interactions of both, and last but not least exogenous mostly inhaled substances circulating in the body. Despite this complexity, the comparison of volatilomes of healthy mice and e.g. mice with impaired glucose homeostasis revealed changes that could be indicators/predictors for disease. We have discovered an exogenous compound that has the potential for use as a biomarker for early stage liver disease in humans.

With regards to lung function and breath gas analysis, we have addressed the following questions in detail:

- Is there any immediate effect of different breath holding manoeuvres on composition of exhaled breath?
- Do the effects of breath holding on exhaled VOC concentrations depend on physico-chemical properties of the substances?
- If marker concentrations change due to physiological effects, in which timeframe do they return to the original levels?
- Should the dependency on breathing patterns be taken into account if exhaled substances are to be interpreted in terms of biomarkers?
- Are there any immediate effects of different body positions on exhaled VOC profiles?
- Is there any significant relation between the time profile of hemodynamic parameters and exhaled VOCs?
- Do concentration changes depend on the physico-chemical properties of the VOCs?
- Is there any immediate physiological effect of FEV manoeuvre on VOC exhalation?
- Are those effects substance specific?
- Do such effects depend on ventilation and hemodynamic parameters?
- Can these changes be used to monitor lung functions or hemodynamic parameters?

With the support of an ESR, a fruitful collaboration between Fontem Ventures ([www.fontemventures.com/](http://www.fontemventures.com/)) and IONICON Analytik GmbH was established. With a sophisticated novel sampling system it is now possible to analyse e-cigarette mainstream

aerosol, as well as aerosol in exhaled breath following inhalation and mouth-hold. This collaboration has already led to two publications and will continue as real-time puff-by-puff analysis is of utmost importance in this field.

(Fontem Ventures is the parent company of blu eCigs, the number one e-cigarette brand in the United States and a market leader in the United Kingdom. Headquartered in the Netherlands, but present on two continents, Fontem Ventures is an innovative consumer goods company, founded in 2012, which is committed to developing and growing a portfolio of products which meet consumer needs today and tomorrow, including the highest quality electronic vaping products (EVPs).)

## **WP 5 Homeland Security**

We have developed inlet/pre-concentration sampling systems which have low memory effects so that PTR-MS could be used in security areas to check people and bags rapidly. We have improved the levels of chemical specificity to rapidly detect trace threat chemicals (explosives, chemical warfare agents, illicit drugs) in highly complex environments in real time thereby providing high-level confidence data of use for instant decision making and reduction of false positives and negatives.

## **4. HOW MIGHT OUR RESULTS BE USEFUL?**

The synergies and complementarities associated with the various applications and disciplines within the network provided the foundation for extremely fertile and innovative high quality collaborative research programmes with widespread applications. PIMMS has brought about the tools, knowledge and integration of methodologies resulting in a fuller exploitation of PTR-MS technologies, enhancing its use in health, food, environmental and security applications.

### **4.1 Impact and Societal Implications of PIMMS**

The four major areas in which PIMMS was involved - the environmental, food and health sciences and homeland security - have immense social implications. Environmental issues relate to the ability to identify and monitor pollution and to improve our understanding of atmospheric chemistry. Accurate identification of volatiles in the food sciences is important for understanding the perception of flavour, and for food classification, quality and processing. Health science is an exciting area with the potential of using volatiles in breath,



urine and faeces as biomarkers to screen, diagnose and monitor specific diseases and procedures both in rodents modelling human disease as well as in human patients. The accurate identification of traces of threat agents (e.g. explosives, toxic industrial chemicals, drugs and chemical warfare agents) in complex chemical environments is of considerable importance for national security. We have shown that PTR-MS can support security personnel by quickly and accurately identifying hazardous compounds. PIMMS key outcomes are as follow:

- PIMMS has trained 15 Early Stage Researchers who have received both a comprehensive analytical training and a more focused training in proton transfer reaction mass spectrometry (PTR-MS), thereby making a direct and significant impact in an emerging area of analytical chemistry. These 15 ESRs are now at a level where they can be employed by prestigious universities, companies and/or research institutes and significantly contribute to science with their expertise.
- In terms of research and development PIMMS has driven innovative analytical techniques which have benefitted the scientific areas of Ion-Molecule Chemistry Health Sciences, Homeland Security, Atmospheric Chemistry and Food Sciences.
- Fundamental research has improved understanding of the ion-molecule chemistry occurring within the drift tube environments of PTR-MS instruments, which has resulted in an improved control on the reaction processes leading to an improved exploitation of the technology.
- Sampling systems for breath analysis and particulate matter have been developed. The ability to study environmental particulate and aerosol compounds has led to a significance advance in broadening the scope of PTR-MS instrumentation and has improved our understanding of the formation of secondary organic aerosols. The particle inlet system developed by UIBK will be commercialized by IONICON. IONICON will thus be able to enter a new market segment (particle analysis; in addition to gas analysis). The project has thus contributed in strengthening a local SME which operates on the global market.

It is anticipated that the newly developed inlet in combination with PTR-MS instrumentation will become an important analytical tool in atmospheric particle research. Particulate air pollution has deleterious effects on public health and the improved analytical technique is needed unravel the mechanism of particle toxicity.

- Breath analysis for use in medicine has been advanced through key work at Rostock and the University of Birmingham. A notable advance has been the discovery of biomarkers in breath for liver disease. The results of our study show that three volatiles, which can be detected by PTR-MS, are related to the diseased liver. We have minimised confounding factors by using the patients' companions as controls. We have minimised the risk of false discovery by using a two stage process to reduce the variable set so we have not relied on unsupervised multivariate analysis. The most important volatile is limonene, which is of ubiquitous dietary origin and is metabolised by P450 cytochrome enzymes. Reduced levels of CYP2C19 have been shown in cirrhosis so we propose that elevated limonene in the breath is a marker for reduced enzymatic production, and is therefore, a proxy for liver function.

We have thus demonstrated that exhaled breath analysis holds great promise for the development of non-invasive, diagnostic and monitoring clinical tools

The early diagnosis of liver disease will improve the quality of life for the patient and reduce patient management cost.

A detailed review of our work by a Dr. Jonathan D. Beauchamp, Fraunhofer Institute for Process Engineering and Packaging IVV, Freising, Germany, stated the following:

“The study is unique, not only due to its complementary (two-stage) approach, but because the cirrhosis-related breath volatiles discovered are, contradictorily, by no means unique, yet appear to reflect the functionality of the liver to a high degree. Most breath research focusses on searching for biomarkers generated *by* the illness, but rather than attributing these compounds to a production in the diseased liver, the authors hypothesise that their elevated concentrations in the breath of cirrhotic patients represent an accumulation in the body due to a lack of metabolic breakdown by the impaired liver. This very plausible explanation, which is supported by

considerations on the biochemistry and physicochemical properties of these biomarkers, makes for a strong and compelling case on the potential utility of breath analysis in diagnosing liver cirrhosis and monitoring organ function after transplantation via these markers. Moreover, the authors propose a prospective pharmacokinetic-based test for assessing liver function, whereby known quantities of limonene are administered to a patient and its subsequent wash-out from the body is monitored in breath. This latter approach has a realistic potential in joining the handful of approved breath screening tests that have found their way to routine clinical use, offering a positive outlook in tackling this chronic disease. I, for one, will follow the developments with bated breath.”

Another breath analysis paper from PIMMS recently published in a Nature journal has highlighted how endogenous volatiles might assure quality of lung function tests. It is proposed that analysis of exhaled volatile concentrations could provide additional information on physiology of respiration and gas exchange.

As a complementary approach, breath gas analysis in mouse models for human disease was established and evaluated in the Energy Metabolism & Diabetes Lab of the German Mouse Clinic, Helmholtz Zentrum Munich. Here, breath gas analysis may provide a non-invasive and minimally burdened and stressful online access to monitor metabolic processes. As mutant mouse lines are the predominant model organism to annotate mammalian gene functions, novel phenotyping test assays such as breath gas analysis are of eminent importance to obtain data on genotype related effects on biological processes (metabolism, inflammation, cancer, etc.) especially if a high translational potential for diagnosis and monitoring of human disease can be expected.

- Homeland security applications have been advanced through the development of a thermal desorption unit, making the sensitivity of PTR-MS competitive with current technologies used in security areas, such as airports, but with substantially improved selectivity.

**Proton Ionisation Molecular Mass Spectrometry (PIMMS) was a Marie Curie Initial Training Network funded by the European Commission’s 7<sup>th</sup> Framework Programme under Grant Agreement Number 287382.**

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