ARTICLE

Ion mobility spectrometry to detect lung cancer and airway infections

J.I. Baumbach[a] and M. Westhoff[b]
[a]ISAS—Institute for Analytical Sciences, Department of Metabolomics, Bunsen-Kirchhoff-Straße 11, 44139 Dortmund, Germany
[b]Lung Hospital Hemer, Theo-Funccius-Str. 1, 58675 Hemer, Germany

Introduction

Early diagnosis of lung cancer and airway infections is gaining increasing importance. We have examined if volatile metabolites occurring in human exhaled air can be correlated directly to different kinds of diseases. The analytical technique, which is the same basic technique used to detect explosives or chemical warfare agents, is being employed at ISAS—Institute for Analytical Sciences, Dortmund, and the Lung Hospital Hemer to examine hospital patients with lung cancer and airway infections. The technique, based on ion mobility spectrometry, is able to detect effectively metabolites in human breath down to the ppt_v or $pg\,L^{-1}$ region. For investigations of human breath at a comparatively high level of humidity, a combination of a Multi-Capillary Column (MCC) for partly pre-separating the analytes is used in combination with a conventional ion mobility spectrometer (IMS). An IMS coupled to a MCC allows for the identification and quantification of volatile metabolites occurring in human breath down to the $ng\,L^{-1}$ and $pg\,L^{-1}$ range of analytes within less than 600 s and without any pre-concentration. The IMS investigations are based on different drift times of swarms of ions of metabolites formed directly in air at ambient pressure. About 10 mL of breath is necessary to carry out a full analysis. The aim of this article is to report on a quick, low-cost device for human breath analysis, and in addition to investigations of blood and urine, as a potential non-invasive standard method for use in hospitals and for medical applications.

It is well recognised within the medical community that a person exhales volatile compounds that may carry important information about the health of the individual. Thus, a successful detection of the products of different metabolic processes is attractive, especially if the detection limits of the spectrometric methods used are sufficiently low and the instruments are available at moderate cost, so that they can be used as standard methods in hospitals. The vision of the authors is to contribute to the development of breath analysis as a diagnostic method for disease in support of blood and urine analysis.

Our procedure is based on miniaturised ion mobility spectrometers supported by mass spectrometric validations. The full procedure, including sampling, pre-separation and identification of metabolites in human exhaled air, was developed and implemented with a view to future use in hospitals. Metabolic profiling of the breath of healthy individuals and those suffering from different diseases, in particular lung cancer is considered at various lung hospitals and point-of-care centres.

The analytical methodology for breath analysis was reviewed recently in *Spectroscopy Europe*[1] and will be summarised here only briefly. Today, a wide range of techniques are used for scientific medical investigations and clinical trials, these include: gas chromatography coupled to mass spectrometry, different mass spectrometric methods based on proton transfer reactions, ion–molecule reactions or selected ion flow, laser spectrometric methods including infrared cavity leak-out spectroscopy or tunable absorption spectroscopy, partly in combination with quantum cascade lasers and different kinds of sensors for single molecule detection. Some of the methods need sampling in bags, some use pre-concentration techniques like Tenex resins or SPME (solid-phase microextraction). The method presented here allows one to use about 10 mL of human breath without pre-concentration to be transferred directly into a MCC, which is connected to the ionisation region of an IMS.

Ion mobility spectrometry

For the measurements described below, a custom designed IMS equipped with a ^{63}Ni β-ionisation source was used. The operating principle of the IMS and the operational details has been reported elsewhere.[2] Therefore, only a brief description will be given here. The term ion mobility spectrometry refers to the method of characterising analytes in gases by their gas-phase ion mobilities. The drift times of ion swarms formed using suitable ionisation sources and electrical shutters are normally measured, see Figure 1. The product ions formed in defined chemical reactions of neutral analyte molecules with reactant ions are characteristic of the analyte molecules, so the mobility of these product ions may be used to identify the analyte molecules. The drift velocity *v* of the ions

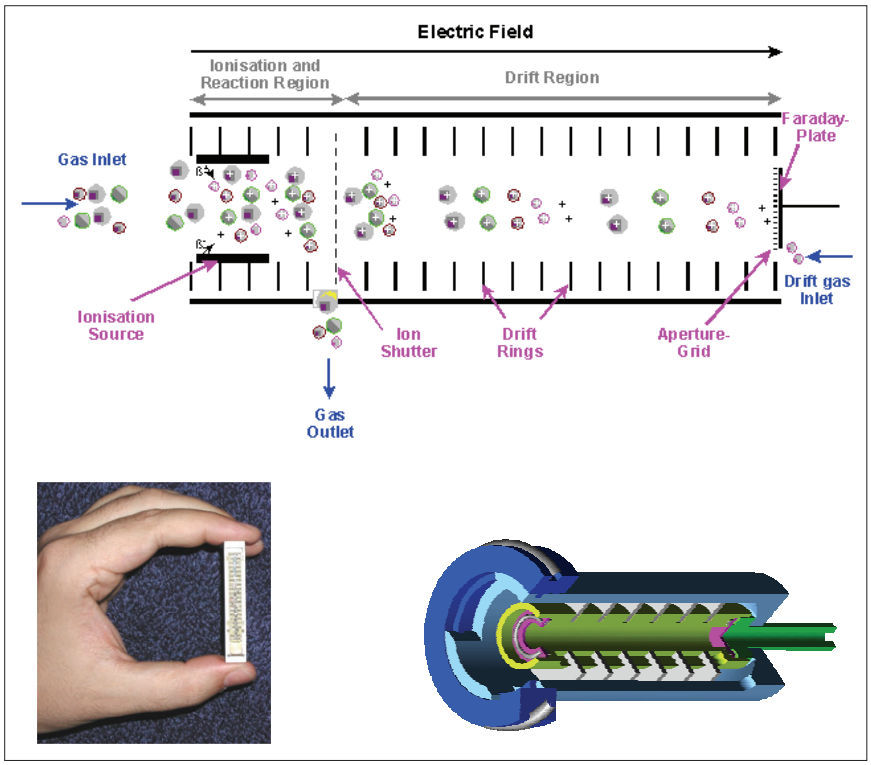

Figure 1. Top: Schematic of the working principle of a constant field ion mobility spectrometer. Bottom: Comparison of the size of a miniaturised IMS (left) and arrangement of the electrodes for the stabilisation of the electric field in the ionisation and the drift region of an IMS (right).

is related to the electric field strength E by the mobility k: $v=kE$. Therefore, the mobility is inversely proportional to the drift time, which is usually measured at a fixed drift length. Theoretical considerations show that the mobility is related to the collision rate of the ions with the gas molecules in which they are drifting (the reduced mass), the temperature, the dimensions of the ion (structural dependencies) and the collision integral. The collision integral, and therefore the mobility, is influenced by the size of the ions and molecules, their structures and polarisabilities. Therefore, a dependence of ion mobility on mass and structure is commonly observed. Thus, it is clear that isomeric forms of ions should be distinguishable.

Ion mobility spectrometry was originally developed for the detection of trace compounds in a gas, for example, gaseous pollutants in air for military applications. It combines both high sensitivity and relatively low technical expenditure with a high-speed data acquisition. The time to acquire a single spectrum is in the range of 10–50 ms. Thus, an IMS is an instrument suitable for process control, but due to the occurrence of ion–molecule reactions and relatively poor mass resolution of the ionic species formed, it is generally not good for the identification of unknown compounds. The working principle is based on the drift of ions in a buffer gas at ambient pressure under the influence of a weak applied electric field. Unlike conventional low-pressure mass spectrometry, the mean free path of the ions in the buffer gas is smaller than the dimensions of the instrument. Therefore, the different ion species that comprise an ion swarm drifting under such conditions separate in space according to their drift velocities, which are related to their different masses and geometrical structures. Collection of these ions by a Faraday plate results in a time-dependent signal corresponding to the mobility of the separated ions. Such an ion mobility spectrum contains information on the nature of the different trace compounds present in the sample gas from which the ions were formed. For the generation of ion swarms some additional components are required, including an ionisation source (normally ^{63}Ni β-radiation sources, UV lamps or discharges), an ion shutter grid and a high voltage supply that delivers voltages between a few hundred and 10,000V to establish the electric field in the drift tube.

The ions formed in the ionisation/reaction region (see below) drift under the influence of the static electric field of strength between 100 and about 1000Vcm^{-1} counter to the buffer/carrier gas flow direction. The shutter control circuit drives the ion gate. The shutter opening time is normally fixed between 10 µs and about 1 ms. Generally speaking, digital signal processing provides a better readability of the spectra obtained. Under ideal circumstances the final spectrum consists of clearly separated peaks.

The amplification realised with different types of preamplifiers is between 1VnA^{-1} and 100VnA^{-1}. The current measured lies in the range of a few nA, sometimes pA, and is converted into a signal voltage by commercial AD cards and then into digital signals for further processing. It is necessary to shield the IMS against external electromagnetic disturbances. Both the gas flow rates (normally in the range of some mLmin^{-1}) and the temperature in the ionisation and drift regions must be controlled.

The most important sections of the instrument are the ionisation and reaction regions and the drift region. A uniform external homogenous electric field is established in the drift tube using several drift rings. The carrier gas retains sample molecules within the ionisation region. There, direct ionisation can occur using UV lamps or by chemical ionisation by collisions of the analyte with ionised carrier gas molecules formed by radioactive ionisation sources, and fragmentation in the case of discharges.

The, so-called, drift gas flows from the Faraday plate/cup towards the ionisation region. Normally, if the shutter is closed, no ions can reach the drift region. The drift gas will protect the drift region and no neutral analyte molecules should enter the drift region. If the shutter is held closed all analyte molecules, neutrals and ions will pass through the gas outlet.

During the shutter open time, some ions will enter the drift region. Following several collisions with the surrounding gas molecules, a steady drift velocity will be reached. If no chemical reactions occur, total spatial separation of the various ionic species will be reached at the Faraday plate. Because of the influence that moving particles have on the current to the metal plate, the Faraday cup is shielded by using a, so-called, aperture grid. Thus, only ions moving through the aperture grid are collected and directly converted into a current and later, using a preamplifier, into a voltage. The time-dependent voltage or current plotted against the time interval referenced to the shutter opening pulse is called the ion mobility spectrum.

The type of product ions produced will differ depending on the method of ionisation. Frequently used are radioactive sources (α- and β-radiation), UV lamps with different photon energies between 8.6 eV and 11.7 eV, lasers, different kinds of discharges (including corona or, so-called, partial discharges) and electrospray. Using nitrogen or air as the carrier gas, the carrier gas molecules are normally ionised directly by the β-particles. Positive carrier gas ions and free electrons will also be formed. These primary positive ions (called reaction or reactant ions) will undergo different chemical reactions with the analyte molecules to form, so-called, product ions by: proton transfer, nucleophilic attachment, hydride abstraction and other processes. The electrons may attach to sample molecules to form negative ions (electrophilic attachment, resonant attachment, dissociative attachment). Also, charge transfer and proton abstraction may occur. Often both positive and negative ions are formed. The formation of reactant ions depends on the activity of the ionisation source. However, the number of reactant ions produced is largely independent of the design of the reaction region. All parts of the IMS that are in contact with the analytes are made from inert materials. Teflon® was used for the ionisation chamber and the drift tube. The shutter grid was built from parallel nickel wires and is enclosed by an electric field. All conducting surfaces and drift rings are constructed from brass. The rings are outside the drift tube and therefore are not in contact with the analytes. The drift tube was designed by modelling the homogeneity of the electric field. The electric field in the drift tube is established by using a high-voltage supply with a voltage divider connected to the drift rings placed at equal distance.

To realise effective pre-separation of a rather complex exhaled air mixture, a 17 cm long polar multi-capillary chromatographic column (MCC, OV-5, Sibertech, LTD, Novosibirsk, Russia) was used, made by combining approximately 1000 capillaries each with an inner diameter of 40 µm and a film thickness of 0.2 µm. This was coupled to the ^{63}Ni source IMS. The total column diameter of 3 mm allows operation with a carrier gas flow up to 150 mL min^{-1}, which is the optimum flow rate for the IMS. The heating of the column is essential to obtain reproducible results. To achieve rather comparable retention times, the MCC was held at 300°C during the breath analysis procedure. To realise isothermal separation, a simple heating construction is needed, but it is only necessary to hold the temperature constant. A combination of a MCC to an IMS sometimes allows one to identify the "hidden dimension"[3] in comparison to standard GC investigation using un-specific detectors like electroantennographic detection (EAD).

In the breath sampling process, the subject blows through a mouthpiece coupled to a brass adapter (designed by ISAS) via a Teflon® tube (¼", Bohlender GmbH, Lauda, Germany), which is connected to a 10 mL stainless steel sample loop of an electric six-port valve (Nalco, Macherey-Nagel, Düren, Germany). By switching the six-port valve, breath is transported by the carrier gas from the sample loop into the MCC. Separated compounds can be analysed directly by IMS. Results can be achieved within 600 s depending on the separation time of the compounds. This construction enables a direct and rapid sampling at a known breath volume. The schematic drawing of the sampling and detection systems for breath analysis using MCC-^{63}Ni-IMS is shown in Figure 2. The loss of any molecules of the analyte

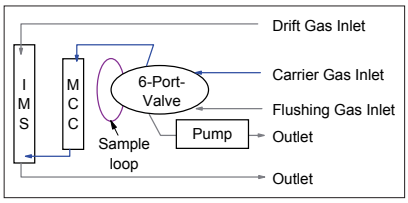

Figure 2. Sampling system including a six-port-valve and a sample loop of 10 mL volume.

in any part of the analytical system must be avoided, especially when considering the detection of traces of analytes. The effective separation of water vapour is one major advantage of the MCC. Using other techniques like humidity sorbents or membrane separation units, some of the original analytes may be lost. It should be noted, that only 10 mL of the human breath is necessary for a total analysis using IMS. By triggering the six-port valve using CO_2 or O_2 sensors to control whether only alveolar and not bronchial air will be investigated, the dead volume between the mouthpiece and the sample loop must be taken into account.

In Figure 3 a photograph of the home-made IMS including mouth piece and sampling unit used during the studies in the Lung Hospital Hemer is shown. The single transfer line between the mouthpiece and the six-port valve is made by Teflon® and so could be sterilised in the hospital.

Results of preliminary studies

A typical IMS-chromatogram as obtained by MCC-IMS investigations is shown in

Figure 3. Home-made IMS with mouthpiece and sampling unit in the Lung Hospital Hemer.

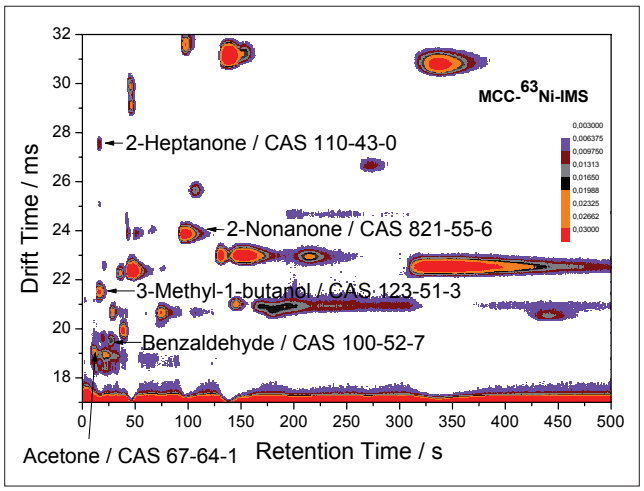

Figure 4. Typical IMS-chromatogram of human breath with indications of some metabolites by name and CAS number.

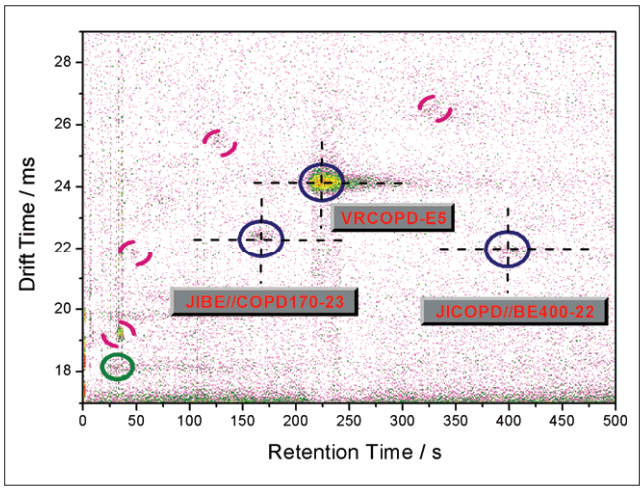

Figure 6. Major peaks indicated in IMS chromatograms of human breath for patients suffering chronic obstructive pulmonary disease (COPD).

Figure 4. An identification of the analytes can be realised using the retention time and the drift time as major parameters. In addition, the temperature and pressure values are taken into account to calculate the ion mobilities; for further details for the procedure see Reference 2.

Some of the analytes are well known, like acetone, a potential marker for some kinds of diabetes.

In a pilot study using data from 36 patients suffering with lung cancer and 54 healthy persons in a control group, a differentiation was realised as shown in Figure 5. These results were awarded the 2006 Science Price of the German Association of Pneumologists. A reduction from more than one million data points per IMS chromatogram to 25 variables enabled a classification and differentiation of these two groups with an error of 1.3%.[4,5]

During a further study, IMS chromatograms were obtained from 30 patients with different airway infections, chronic obstruc-

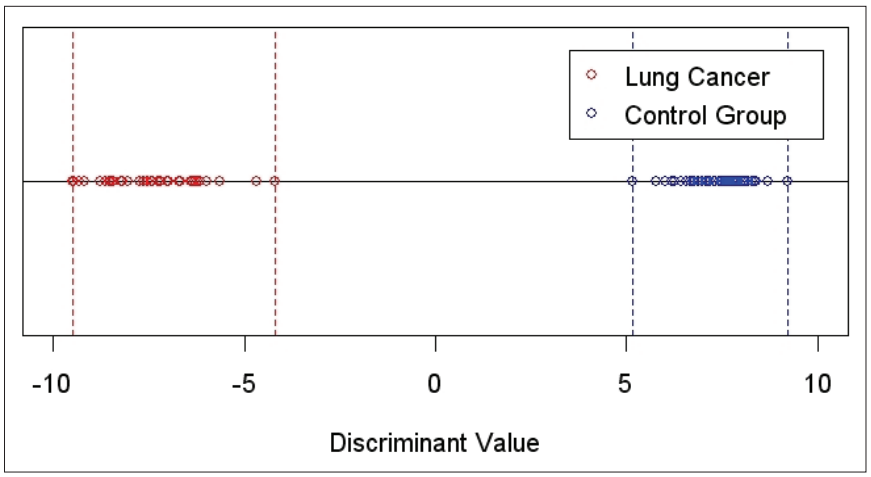

Figure 5. Differentiation between the IMS chromatograms of patients with lung cancer and a control group during a pilot study, with 36 patients suffering with lung cancer and 54 healthy persons in a control group.

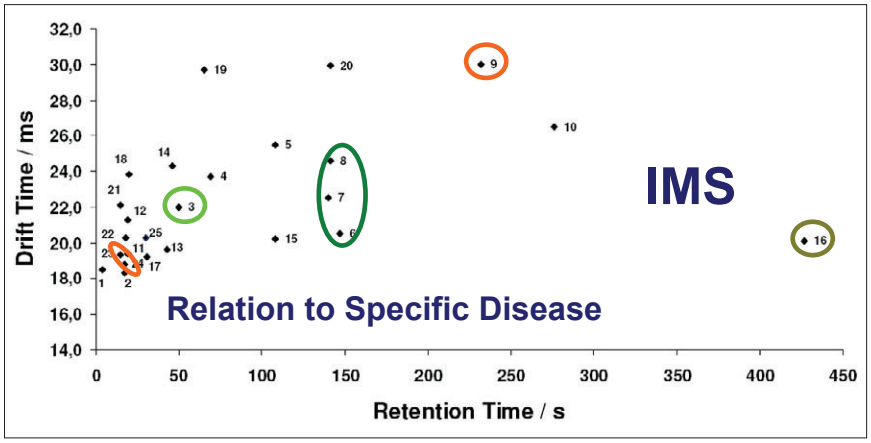

Figure 7. Relations of peaks in IMS chromatogram proposed to be related to specific diseases. The circles and ellipses indicate relationships between the analytes associated with the number and specific diseases, e.g. peak numbers 6, 7 and 8, are related directly to lung cancer on the basis of a pilot study of 137 persons at the Lung Hospital Hemer. The findings are statistically significant (error level 5%). However, for general-purpose use investigations are underway of a greater population.

tive pulmonary disease (COPD)-exacerbations, bronchiectasis, pneumonia).[6] Figure 6 shows the three major peaks identified by names as: VRCOPD-E5, JIBE//COPD170–23 and JICOPD//BE400–22. The first peak was proposed by Ruzsanyi[7] in 2005, the two others by Baumbach in 2006. It should be noted that, in some cases, all three peaks occur. But, sometimes, only two of the three peaks in different combinations could be observed, in one case only one was observed. Thus, further investigations on a greater population must be considered to support these early findings.

In a recent study of 130 patients at the Lung Hospital in Hemer more than 400 different analytes were considered in the positive mode of the IMS. Some patterns look to be assignable to certain illnesses as indicated in Figure 7. Ion mobility spectrometry seems to be a promising diagnostic tool for lung cancer and airway infections. However, these preliminary data need further confirmation by studies with larger populations.

Early diagnosis and specification of bacterial airway infection is of importance, especially in patients who are at high risk of respiratory failure, invasive or non-invasive ventilation and a prolonged hospital stay. In an *in vitro* study IMS chromatograms of different bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*) and a fungus, *Candida albicans*, were obtained.[8] The selected bacteria and *Candida albicans* could be defined and distinguished by different metabolites. In Figure 8 the relation between peak position in drift and retention time allows one to distinguish between different bacteria and the mould (selected as occurring very often in airway infections); for further details see Reference 7). Ion mobility spectrometry seems to provide a tool for precise bacterial analysis. The results of this pilot study have to be proven by an *in vivo* study, especially in patients with airway infections such as COPD exacerbation and pneumonia. A future aspect might be the implementation of a bedside test. Detailed case studies including, lung infections and lung cancer have been reported elsewhere.[9–11]

Conclusions

Volatile metabolites occurring in human exhaled air are correlated directly to different kinds of diseases. An ion mobility spectrometer (IMS) coupled to a multi-capillary-column (MCC) was used to identify and quantify volatile metabolites occurring in human breath down to the $ng\,L^{-1}$ and $pg\,L^{-1}$ range of analytes within less than 500 s and without any pre-concentration. The IMS investigations are based on different drift times of swarms of ions of metabolites formed directly in air at ambient pressure using about 10 mL of human exhaled breath.

During a pilot study IMS chromatograms were obtained from patients with lung cancer and compared with those from healthy persons. Twenty-five variables of the chromatogram were discriminatory between the two groups. IMS from bacterial cultures showed typical chromatograms of different bacteria, which could be confirmed in spectrograms of patients

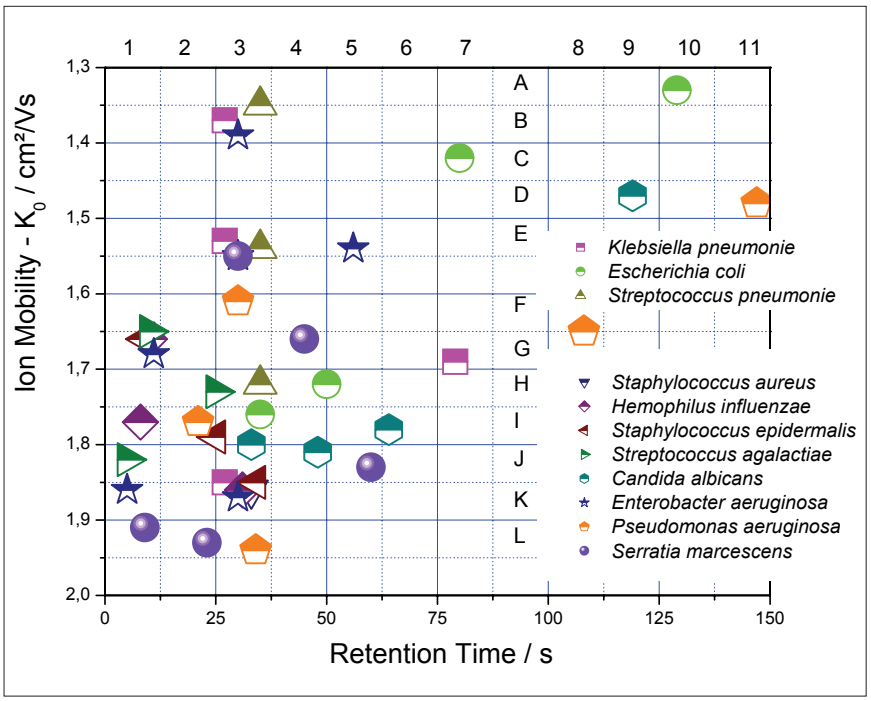

Figure 8. Correlation between peak positions in IMS chromatograms by retention time and ion mobility for different bacteria and a mould.

with distinct airway infections. This gives hope for the use of IMS in early detection and differentiation of bacterial airway infections.

These preliminary data need to be confirmed by studies with larger populations.

Acknowledgements

The authors wish to thank Mrs Sabine Bader, Mrs Stefanie Güssgen, Mrs Barbara Oberdrifter, Dr Vera Ruzsanyi, Mrs Lucia Seifert and Dr Wolfgang Vautz for their major contributions to experimental and laboratory work. In particular we express our hearty thanks for the use of data and results obtained during the work of Dr Vera Ruzsanyi at ISAS and published partly in her PhD thesis. In addition, the use of data from Martin Meier, obtained at his stay at ISAS and Lung Hospital Hemer during his diploma thesis should be mentioned with thanks. The dedicated work of Mrs Sabine Bader, Mrs Luzia Seifert and Mr Martin Meier was essential for these successful investigations. In addition, the mechanical department of ISAS headed by Mr Hans-Georg Krebs was essential and contributed to the results obtained.

We wish to thank gratefully Dr Lutz Freitag and Dr Patric Literst for their continuous support of the experiments and studies at the Lung Hospital Hemer and Dr Frank E. Dornbach, Dortmund, for helpful discussions.

The financial support of the Bundesministerium für Bildung und Forschung and the Ministerium für Innovation, Wissenschaft, Forschung und Technologie des Landes Nordrhein-Westfalen is gratefully acknowledged.

References

1. A. Amann, A. Schmid, S. Scholl-Bürgi, S. Telser and H. Hinterhuber, "Breath analysis for medical diagnosis and therapeutic monitoring", *Spectrosc. Europe* **17(3),** 18–20 (2005).
2. J.I. Baumbach and G.A. Eiceman, "Ion Mobility Spectrometry: Arriving On Site and Moving Beyond a Low Profile", *Appl. Spectrosc.* **53,** 338A–355A (1999).
3. A.N. Davies and J.I. Baumbach, "Multidimensional data analysis—quantifying the hidden dimension", *Spectrosc. Europe* **11(5),** 23–25 (1999).
4. S. Bader, W. Urfer and J.I. Baumbach, "Processing ion mobility spectrometry data to characterize group differences in a multiple class comparison", *Int. J. Ion Mobility Spectrom.* **8(1),** 1–4 (2005).
5. M. Westhoff, P. Litterst, L. Freitag, V. Ruzsanyi, S. Bader, W. Urfer and J.I. Baumbach, "Ionenmobilitätsspektrometrie—eine neue Methode zu Detektion von Bronchialkarzinomen und Atemwegsinfektionen in der Ausatemluft? Erste Resultate einer Pilotstudie", *Pneumologie* **60, S81 (2006).**
6. M. Westhoff, L. Freitag, P. Litterst, V. Ruzsanyi, S. Bader, W. Urfer and J.I. Baumbach, "Ion mobility spectrometry: A new method for the detection of lung cancer and airway infection in exhaled air? First results of a pilot study", *Chest* **155,** S128 (2005).
7. V. Ruzsanyi, *Analyse flüchtiger Metaboliten von der Ausatemluft mittels Ionenmobilitätsspektrometer*. Thesis, University Dortmund (2005).
8. P. Litterst, M. Westhoff, L. Freitag, V. Ruzsanyi and J.I. Baumbach, "Bacterial differentiation by ion mobility spectrometry: First results of a pilot study", *Chest* **128,** S375 (2005).
9. V. Ruzsanyi, J.I. Baumbach, S. Sielemann, P. Litterst, M. Westhoff and L. Freitag, "Detection of human metabolites using multi-capillary columns coupled to ion mobility spectrometers", *J. Chrom. A* **1084,** 145–151 (2005).
10. J.I. Baumbach, W. Vautz, V. Ruzsanyi and L. Freitag, "Metabolites in human breath: Ion Mobility spectrometers as diagnostic tools for lung diseases", in *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring*, Ed by A. Anmann and D. Smith. World Scientific, Singapore (2005).
11. J.I. Baumbach, W. Vautz, V. Ruzsanyi and L. Freitag, "Early detection of lung cancer: Metabolic profiling of human breath with ion mobility spectrometers", in *Modern Biopharmaceuticals*, Ed by J. Knäblein. Wiley-VCH, Weinheim (2005).