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Detection of human metabolites using multi-capillary columns coupled to ion mobility spectrometers

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Abstract

The human breath contains indicators of human health and delivers information about different metabolism processes of the body. The detection and attribution of these markers provide the possibility for new, non-invasive diagnostic methods. In the recent study, ion mobility spectrometers are used to detect different volatile organic metabolites in human breath directly. By coupling multi-capillary columns using ion mobility spectrometers detection limits down to the ng/L and pg/L range are achieved. The sampling procedure of human breath as well as the detection of different volatiles in human breath are described in detail. Reduced mobilities and detection limits for different analytes occurring in human breath are reported. In addition, spectra of exhaled air using ion mobility spectrometers obtained without any pre-concentration are presented and discussed in detail. Finally, the potential use of IMS with respect to lung infection diseases will be considered.

Keywords: Breath analysis; Volatile metabolites; Volatile organic compounds; Ion mobility spectrometry; Trace gas analysis; Metabolomics; VOCs; IMS

1. Introduction

It is well recognised in the medical community that humans exhale volatile compounds which potentially carry important information about the health status of the humans. Thus, a successful detection of potential products of different metabolic processes becomes attractive if the detection limits of the spectrometric methods used are low enough and the instruments becomes available on moderate price levels to be used as standard methods in hospitals. The vision of the authors is to contribute to use human breath as carrier of information of the health status of the body in addition to human blood and urine.

Human breath contains numerous volatile substances derived from both endogenous metabolism and external exposure to vapours and gases and their metabolites. Approximately 200 different compounds have been detected in human breath; some are correlated to various common disorders like diabetes, heart disease and evaluation of lung cancer [1].

The composition of different constituents in respired air is representative for blood borne concentrations detected through gas exchange at the blood/breath interface in the lungs [2]. Thus, the presence and also the quantitative variations of specific volatile organic compounds (VOCs) in respired air are directly linked to VOCs in the blood, which is in contact with diseased tissues or organs. On the other hand, metabolites derive from local bacterial infections in the airway system can be also detected using the breath. In hospitals, pulmonary infections carry a significant risk for people with weak immune systems especially for long-time inhaled and post-operative patients.

Investigations of breath were carried out using different techniques. The most popular sampling method is the use of Teflon bags into which the subject expels the air. The components of the exhale are then collected using a sorbent-trap or a cryo-trap followed by desorption into an analytical instrument like GC–MS, which is used in the majority of cases

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reported [3,4]. This is a rather time consuming process with numerous steps which may lead to loss of analytes [5,6]. Several substances may adsorb on the surface of the bag [7] such part can be significant, particularly if trace levels of analytes should be quantified.

In the literatures the number of the different compounds analysed from the exhaled air and their quantities can alter with the applied sampling and analytical methods. In the most cases, the major VOCs in the breath of healthy individuals found are isoprene (12–580 ppb_v), acetone (1.2–1880 ppb_v), ethanol (13–1000 ppb_v), methanol (160–200 ppb_v). All are products of the normal metabolic processes [8].

For the majority of the potential analytical methods for breath analysis, the high moisture content of the breath samples is a prominent problem. It can bother the sensitivity or the water should be frozen by using liquid nitrogen as cryo-trap necessary to use GC–MS effectively. Because of the status as laboratory instruments, which are comparatively large and expensive and offer an analysis time approx. 40–90 min (depending on the different sample preparation steps), a need for instruments applicable for direct and on-line analysis of the breath exists. If the sample handling procedure steps could be minimised, no additional laboratory steps becomes necessary and no additional carrier gas supplies like high purity nitrogen or helium as used in GC–MS are necessary for effective breath analysis procedures. In such cases, the investigations on humans could be handled direct in the hospitals by standard personal.

On the other hand, in the recent years ion mobility spectrometers (IMS) become comparatively small and effective devices to determine traces of quantities of VOCs down to the low ppb_v range, especially in air [9]. The primer advantages of IMS are that there is no vacuum required for the operation and ambient air can be used as carrier gas. As a consequence, the IMS can be miniaturised, which provides a benefit in the commercialisation of the system in comparison to other on-line techniques applied in the research for breath detection, like PTR–MS [10] and SIFT–MS [11].

World wide, more than 50,000 IMS units are in service, especially to detect chemical warfare agents, narcotics and explosives [12]. The instruments available on the market are handheld, too [13].

In the most recent years, miniaturised IMS [14–17] should be considered with respect to the potential for the analysis of rather complex mixtures of analytes as occurring in human breath.

The analysis with ion mobility spectrometers is based on characterising chemical substances through their gas phase ion mobilities in weak electric fields. Normally, an ion mobility spectrometer consists of an ionization chamber with a β- or UV-radiation source, an ion-molecule injection shutter (e.g. Bradbury–Nielsen-shutter), an ion drift tube and an ion collector (Faraday plate) [18].

A carrier gas, typically air on nitrogen transports the vapour analyte molecules into the ionization chamber, where they are ionised through a series of ion-molecule reactions. Conventionally, IMS comprise a β-radiation (typically ^{63}Ni) source. The ions are then injected into the drift tube by opening the shutter grid periodically and travelled through the drift region within a constant electric field against a counter-flow of neutral gas (e.g. drift gas) to the ion collector [19,20]. A high rate of collisions caused by such ions in the drift gas prevents the possibility to get neutral molecules into the drift region and to build clusters. Ions are then selectively detected on the basis of their unique drift times during the flow through the drift tube. The mobility of the analyte ions is determined from the drift velocity attained by ions in the electric field of the drift region at atmospheric pressure, which is in the range of 100–350 V/cm [18].

Because of the limited selectivity of the IMS, especially in the case of the detection of complex mixtures a pre-separation becomes helpful. For this reason, IMS are often coupled with standard gas chromatographic columns [21]. Actually, with respect to the analysis of exhaled air GC–IMS (or even a GC–MS) are not sensitive enough to obtain results by direct injection of the breath samples into the spectrometer, due to the very low concentration of the most breath constitutions normally occurring in low ppb_v and ppt_v ranges.

In the present case, so called multi-capillary columns (MCC) originally developed by the military of the former USSR should be considered because of the potential advantages like a comparatively high flow rate and a high sample capacity in comparison to single narrow-bore columns [22] are achieved. The MCC could be used for the direct injection of higher sample gas volume into the column, especially in the range of 10–50 mL. The high carrier gas flow, normally between 50 and 200 mL/min enables an isothermal separation of VOCs at ambient temperature. This is an important question with respect to the design of portable instruments, because an oven requires huge place, within a stable temperature program is insured.

Such MCC consists of a bundle of capillaries made from over 1000 individual capillaries, formed in a single small tube. The standard sizes of such a MCC is 50–300 mm in length and approx. 3 mm in bundle diameter which are adequate for packing portable instruments such as GC–IMS [23]. The stationary phases of the capillaries are conventional bonded silicones. Nowadays, MCC have become commercially available with a range of different stationary phases [24–26]. Thus, because of the high capacity, the gas flow conditions of MCC are comparable to IMS gas flows and retention times achieved at ambient temperature in the range of minutes [27] make the MCC favourable for combinations with IMS.

2. Experimental

2.1. Instrumentation

For the measurements described below, a custom designed IMS equipped with radioactive ionization source was used