Instrumental Tools for Express Analysis of Lacrimal Fluids

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Abstract: Analysis of lacrimal fluid is under attention of scientists and physicians as an open "window" for non-invasive assessment to relevant information about the health status. In the present work we propose an innovative method for the analysis of volatile metabolites present in the lacrimal fluid by non-invasive, fast and inexpensive technique: Ion Mobility Spectrometry coupled to a Multi-Capillary Column (MCC-IMS). Experimental protocol for lacrimal fluid collection and its further analysis by MCC-IMS was developed. For the first time this technology was used for the analysis of tears from healthy and diabetic person for a "proof of concept" purpose. Obtained experimental result showed that proposed method is suitable for the sensitive in-situ express analysis of Volatile Organic Compounds (VOCs) from lacrimal fluid and have a promising diagnostic potential.

1 INTRODUCTION

The financial costs related to the diagnostic and treatment of several diseases such as diabetes, cancer, pulmonary diseases and others are extremely important and need to be carefully controlled. Even the conventional analysis of biological matrices, like blood and urine, require long time and complex reagents that increase the cost of this analysis. Beyond that, the collection of blood samples almost every is an invasive procedure and need a specialized medical staff and conditions. Therefore, nowadays the medical community is interested in new non-invasive, accurate, time and cost saving methods of analysis for diagnostic or screening purpose.

Among the existent biological matrices, the lacrimal fluid has been shown to have the essential characteristics to perform the non-invasive analysis. The tear is an extracellular fluid that covers the surface epithelial cells and forms the anterior component of the ocular surface. The lacrimal fluid lubricates and prevents the dehydration of the eye.

Several compounds are present in this matrix such as: amino acids, glucose, proteins and electrolytes (Beuerman and Zhou, 2012). These matrix elements vary from person to person in the range of concentrations from parts-per-billion (ppb) or microgram/litre (μg/l) to parts-per-trillion (ppt) or nanogram/litre (ng/l) and are related to the health or metabolic condition of each individual.

Thus the sensitive in-situ express analysis of Volatile Organic Compounds (VOCs) present in the matrix of lacrimal fluid is a very interesting issue. Currently, this analysis is not very common in the clinical practice. Mainly due to the difficulties on analysis of analites in such low concentration, lack of information about the detected metabolites and due to the inexistence of a uniform method of analysis.

In the present feasibility study, a lacrimal fluid from patients with diabetes and from the healthy persons was analyzed in order to find common and/or discriminating volatile organic compounds. The main objective was the characterization of tears matrix by Ion Mobility Spectrometry, but not the identification of the analytes and determination of their concentration. As far as we know these are the first results of the investigations of lacrimal fluids by IMS technology.

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2 EXPERIMENTAL

2.1 Ion Mobility Spectrometry

Among several techniques that are used for direct analysis of VOCs, there is one that stands out due to its high sensitivity, low cost, portability and simplicity: the ion mobility spectrometry. The ion mobility spectrometry (IMS) is based on the drift of ions according to their mobility in the gas phase at ambient pressure, under the influence of an electric field (Stach and Baumbach, 2002).

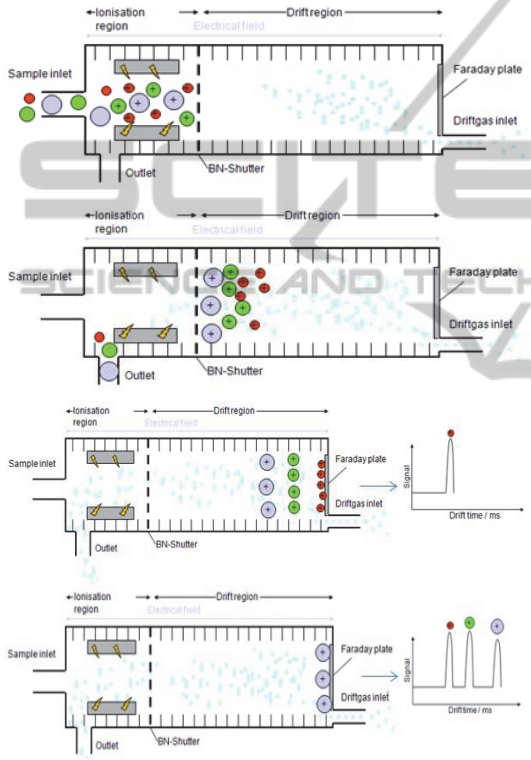

Figure 1: Physical principle of the IMS. The ionized ions enter the drift region and are separated according to their mass and structure.

The major component of the IMS is the spectrometer: a measuring tube that consists of a reaction chamber (also called ionization chamber), in which the ions are generated by a β-radiation source (3H - tritium), and a drift region, at the end of which a detector is positioned (Fig.1). The reaction chamber and the drift region are separated by a shutter grid, called Bradbury-Nielsen grid. This element is responsible for controlling the passage of the ion cloud formed in the ionization region.

In the drift region, there's an external electric field that is responsible for the movement of the formed ions in this chamber. Also, a drift gas (usually nitrogen, but also ambient air can be utilized) is pumped into the spectrometer from the detector's side.

Due to the applied electrical field and the opposite gas flow, the ions are vulnerable to collisions with the drift gas molecules and are separated according to their structure, charge and mass, reaching the detector (Faraday plate) at different times. Ideally, all the analyte molecules in the sample considered for analysis are totally separated.

The detected ions generate an ion mobility spectrum, which shows signals registered at different times (ms) with the corresponding intensities (V). Those intensities are proportionally related to the concentration of each compound, meaning that higher intensities correspond to higher concentrations.

The combination of IMS with a multi-capillary column (MCC-IMS) allows a pre-separation of the sample, through a gas chromatography technique (Baumbach, 2009). This provides an increasing of selectivity and the advantage of an immediate twofold separation of VOCs with visualisation in a three-dimensional chromatogram, as represented in the Figure 2.

IMS has a wide range of applications. Initially, it was used to detect explosives and chemical warfare agents, but the same principle has been applied for medical applications, and to give information about nutrition, oral hygiene and environmental conditions (Eiceman, 2005).

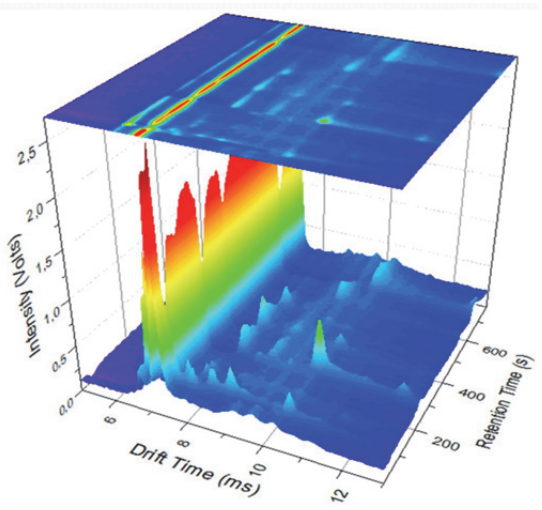

Figure 2: Typical MCC-IMS chromatogram showing characteristic signals of biological matrix.

2.2 Other Analysis Techniques

There are other techniques that are also suitable for the volatile organic compounds analysis, like GC-MS, SIFT-MS and PTR-MS.

GC-MS (mass spectrometry with gas chromatography) is a technique that enables the separation and identification of volatile organic compounds and some volatile inorganic compounds of a gas mixture (Dolan, Newman and Stauffer, 2007). This technique needs a long time to perform the analysis and pre-concentration (Blake, Monks and Ellis, 2009).

SIFT-MS (mass spectrometry associated with a selected ion flow tube) is a technique that uses precursor ions to ionize gases in a gas sample. It is a technique that cannot be miniaturized and it is less sensitive than the MCC-IMS (Spanel and Smith, 1996).

PTR-MS (mass spectrometry by proton transfer reaction) is a technique that enables the identification of volatile organic compounds mostly from natural sources. It uses a vacuum system and for that reason it cannot be miniaturized (Blake et. al., 2009).

Compared with other methods of VOC analysis, ion mobility spectrometry (IMS) stands out due to its high sensitivity, low cost, portability and simplicity. The fact that is does not require vacuum or further sample preparation and the analysis is performed in a few minutes makes this technique suitable to be used in hospitals and healthcare centers.

2.3 Materials and Methods

The samples were collected from 9 diabetic patients and 9 health individuals according to the standard protocol procedures with sterile tear flow test strips (by Sno*Strips) based on the technique of the Schirmer's Test (Zhou and Beuerman, 2012). In this test, the sterile strip is placed in the outer lower eyelid of each eye during 3 minutes. After this time, the strip is collected and stored into vials which are closed with screw caps with a silicone septum.

The reason why the strip is placed in a specific part of the eyelid is related to some facts:

- The lacrimal gland which is responsible for the production of the aqueous layer of the lacrimal fluid is located at the superior temporal portion of each eye,
- The lacrimal channels are located at the nasal portion of each eye and communicate with the lacrimal sac, which could cause a contamination of the lacrimal sample with nasal compounds.
- The cornea is an extremely sensitive eye region that could be negatively affected by the strip contact.

A strip with collected lacrimal fluid was placed in a 20 mL vial, sealed and heated in an Analogic Heating Plate from VWR®, at 60ºC during 10 minutes. The vial was connected to the MCC-IMS through a needle that was inserted across the silicone septum. Figure 3 shows the experimental scheme considered in this study

After the 10 min of equilibrium headspace the carrier gas (N_2, 25 mL min−1) transferred the injected sample to the MCC for separation. Then, the separated analyte, was driven into the ionization chamber of the ion mobility spectrometer.

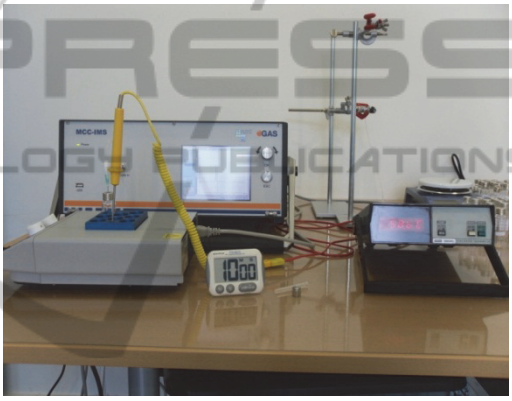

Figure 3: Experimental scheme considered in this study.

Analyses were performed on a MCC-IMS apparatus fabricated by Gesellschaft für Analytische Sensorsysteme (G.A.S. mbH, Dortmund, Germany). The multicapillary column (Multichrom, Ltd., Novosibirsk, Russia) has a length of 20 cm, a volume of 0.45 mL, providing a high sample capacity for preseparation.. The detector was equipped with a Tritium ioniztion asource (St. Petersburg, Russia) with an activity of 300 MBq. A sample inlet lets a continuous stream of nitrogen 5.0 (Air Liquide, Portugal) at 25 mL min−1 to pass through the ionization chamber where ions are formed and focused to a shutter grid. A drift gas flow rate of 500 mL min−1 was used to provide a good separation of ions and to reduce the flush time between consecutive measurements.

All experimental parameters of MCC-IMS , such as drift gas and carrier gas flow rates, injection volume, grid pulse and system temperature, were optimized in order to obtain the better spectra.

3 RESULTS AND DISCUSSION

All spectra were recorded in the positive ion mode. To avoid contamination of the system with residual traces from previous runs a system was flushing by high drift gas flow rate of 500 mL min−1 before each analysis.

The peak area was used as analytical signal. A dedicated data acquisition and analysis software LAV® from G.A.S. was used for analysis of the results.

A typical spectrum from the performed analysis is presented in Figure 4. In this map the white colour represents the highest intensity and saturation and the black colour represents a negative intensity. The middle colours vary from blue to green, and then to pink and red. It was possible to observe several peaks in the obtained spectra.

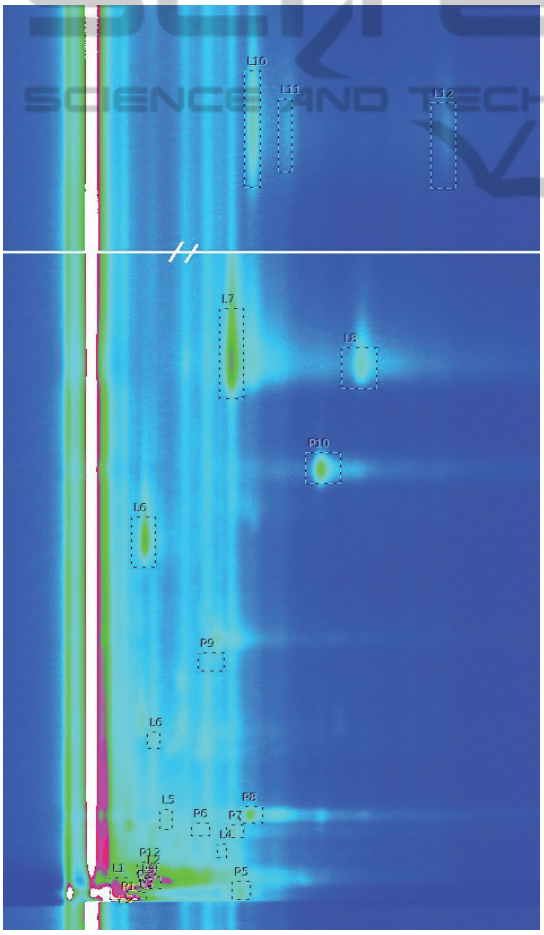

Figure 4: Example of a spectrum obtained for one of the samples analysed. Identified with a P and a number are the peaks related to the strip analysis, and identified with an L the peaks of the lacrimal fluid.

To distinguish the peaks related to the lacrimal fluid and the peaks related to the sterile strip we have also performed a separate analysis of a single strip without any tear sample. The peaks in the Figure 4 identified with P are related to the strip analysis and the peaks identified with L are related to the lacrimal fluid.

In the present study were identified 10 characteristic peaks for the strip, and 15 peaks for the lacrimal fluid. The LAV software were used for determination of the intensity of the peaks and there position, determined by the corresponding retention time (t_r) and drift time (t_d). The characteristic parameter of IMS spectra is referred to as ion mobility, K. It represent as a quotient of ion velocity and electric field on the tube, and can be calculated using the measured drift time, td, of an ion through a drift length, l_d, under electric field, E:

$$K = \frac{l_d}{Et_d}$$

However, this ion mobility value can be normalized to standard gas density, 2.687×1019 molecules/cm, corresponding to T_0=273 degree Kelvin and P_0=101325 Pascal, and reported as the reduced ion mobility, K_0 (Bensch and Leonhardt , 2002):

$$K_0 = K\left(\frac{P_d}{P_0}\right)\left(\frac{T_0}{T_d}\right)$$

In the Table 1 are represented the calculated values of reduced ion mobility, as well as the experimentally determined respective retention time, drift time and intensities for most relevant peaks from lacrimal fluids analysis of diabetic and healthy (control) group.

Table 1: Characteristic parameters for some relevant peaks obtained from the lacrimal fluid.

Peak	t_r (s)	t_d (ms)	K_0 $cm^2V^{-1}s^{-1}$	Intensity (control group)	Intensity (diabetes group)
L6	49,98	8,67	1,26	0,24	0,24
L7	55,20	8,39	1,31	0,29	0,24
L9	106,73	8,48	1,29	0,33	0,54
L11	151,88	13,07	0,84	0,13	0,11
L13	427,39	11,47	0,95	0,10	0,11
L17	428,76	14,88	0,74	0,08	0,08

It was observed that, from the qualitative point of view, all spectra of lacrimal fluids contain the same

characteristic peaks from the diabetic and healthy persons. However, some of them represent a different intensity: the peaks L7 and L11 have a lower intensity for the diabetes patients, while the intensity of the L9 is much higher.

These first results show that ion mobility spectrometry can provides an analysis of lacrimal fluids by detecting and visualization of volatile organic compounds.

4 CONCLUSIONS

A first feasibility study with ion mobility spectrometry was performed to find characteristic peaks of volatile organic compounds in lacrimal fluid matrix.

Obtained preliminary results indicate that MCC-IMS technology is suitable for express analysis of lacrimal fluid, enabling the detection and recognition of analytes from this non-invasive matrix which are relevant in underlying metabolic processes or diseases.

Further studies with greater numbers of patients are necessary. Additionally it will be interested to analyse under the same experimental conditions a glucose solution (1M) and human. A possibility to observe coincident peaks of the glucose and insulin with the analysis of the tear can clarify better if the glucose level can be monitored through the tear analysis by IMS.

The different intensities of detected peaks of insulin and glucose in both control and diabetic group can indicate a way for probable diagnostic or disease monitoring

Future experiments shall be performed in order to allow identification and quantitative analysis of the compounds present in the lacrimal fluid so that this method can have clinical application.

The additional testing of exhaled breath by GC-MS may to relate peaks to corresponding and chemically identified volatile organic compounds.

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