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Instrumental techniques used in the analysis of exhaled air

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ABSTRACT

Exhaled air composition changes depending on the health status of the patient, making it possible to use breath analysis for diagnosis and monitoring purposes. Despite the fact that it is not yet used in every day medical practice, it potential application could facilitate the diagnostics of various diseases such as metabolic disorders, respiratory tract and gastrointestinal diseases. Described in this paper are different application of exhaled air analysis. Additionally, currently available techniques of sample analysis are discussed, as well as their potential advantages and disadvantages.

***Keywords*:** Breath analysis, Diagnosis, Disease-state monitoring, Lung disease, Metabolic disorder, Volatile organic compound

1. INTRODUCTION

Breath analysis is gaining an increasing interest due to the possibility of its use in the assessment of patient's general health and the identification of pathophysiological processes in the body. Exhaled air analysis for diagnostic applications is non-invasive and can be performed even without the aid of advanced analytical techniques. Since ancient times physicians have known that its use can provide clues for correct diagnosis. For example, due to presence of acetone in exhaled air, breath of patients with untreated diabetes often resembles the smell of rotten apples. Musty, fishy odour may indicate abnormal liver function, renal failure is often accompanied by the smell of ammonia [1,2].

Exhaled air is a mixture of inorganic gases (e.g., NO, CO_2 and CO), volatile organic compounds (e.g., ethane, pentane, methanol and acetone) and typically non-volatile compounds (e.g., cytokines and prostaglandins) that form an aerosol [3,4]. Because breath components can be both exogenous and endogenous, their analysis can provide information about the pathways of absorption and the processes taking place within the organism [5,6]. Exhaled breath analysis combined with the use of statistical methods should be able to provide information about changes in the human body than could be used in both early diagnostics and prediction of possible effects of diagnosed diseases.

2. SAMPLE ANALYSIS

There are several technologies that can be applied in analysis of the exhaled air composition, come of which are listed in Table. Currently, the gold standard is gas chromatography [7]. Its use, most often coupled with mass spectrometry, enables the separation and identification of the compounds present in human breath. It exhibits high sensitivity and reproducibility. The application of GC and GC-MS allows for both qualitative and quantitative analysis of even complex mixtures, which is the reason for their employment in multiple clinical studies [8,9]. However, these methods are rather expensive and require time-consuming sample preparation. In addition, the use of gas chromatography in medical diagnostics would require trained personnel in order to perform tests and interpret the results [10].

In order to shorten the time of analysis, ion mobility spectrometry (IMS) can be used. This method uses the differences in mobility of ions in the electric field for their separation. Major advantages of IMS are the wide range of conditions in which the measuring instruments can be used and possibility of its modification so as to adapt to specific need [11]. Furthermore, IMS devices can be relatively small and portable, especially when re-circulation of carrier gas is introduced, and so can possibly be used in bed-side diagnostics.

Unlike GC, proton transfer reaction mass spectrometry (PTR-MS) can be applied to determinate composition of breath samples in real time. In this technique, chemical ionisation is induced by proton transfer reaction, usually, but not exclusively from hydronium ions. In addition, the main constituents of atmospheric air such as nitrogen, carbon dioxide and water vapor in the sample does not impede the measurement of trace amounts, as these compounds will not be ionised under standard operating conditions, which renders sample preparation unnecessary.

Use of PTR-MS makes it possible to observe rapid changes of the concentration of volatile compounds present in the exhaled air sample which can be used to monitor bodily processes and assess organ function in real time. Unfortunately, the use of this method does not allow the distinction between the isomers present in the sample and the chemical identification of unknown compounds [2,12,13] other than tentative identification based on ion mass and isotope ratios.

However, PTR-MS' disadvantages do not prevent its use in medical diagnostics, but merely points to the need to select the appropriate method of chemometric analysis if the holistic "fingerprinting" approach is to be taken.

Table 1. Advantages and disadvantages of some instrumental techniques used in breath analysis

Analytical method	Advantages	Disadvantages
GC-MS	Complete profile analysis is possible Qualitative and quantitative analysis	Sample preparation is needed No real-time measurement
IMS	Shorter time of analysis Possibility of adaptation High sensitivity	No complete profile analysis No real-time measurement
PTR-MS	Real-time analysis is possible High sensitivity No need for sample preparation	No qualitative analysis possible
SIFT-MS	Real-time analysis is possible High sensitivity No need for sample preparation	No complete profile analysis No qualitative analysis possible
E-Nose	Low cost Short tome of analysis No need for sample preparation	No qualitative analysis possible

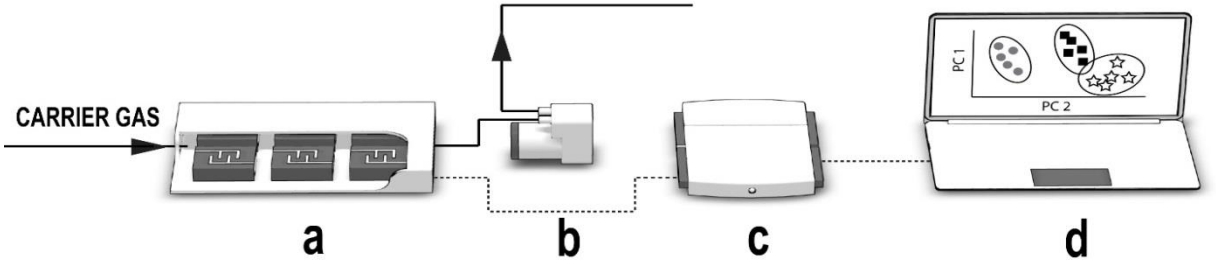

Figure 1. Basic elements of an e-nose: (a) sensors chamber; (b) vacuum pump; (c) analogue-digital converter; (d) data acquisition and processing

Another technique for real-time analysis of trace gases present in exhaled air is selected ion flow tube mass spectrometry (SIFT-MS). It is based on ionisation of molecules by precursor ions and is characterised by both a high sensitivity and a short response time, which allows simultaneous measurement of the concentration of several substances in real time. Moreover, the presence of water vapour, carbon dioxide and other highly concentrated gases does not disturb the analysis of compounds occurring in trace amounts. Unfortunately, the use

of this method as is the case with PTR-MS, does not allow the conclusive identification of the compounds present in the sample [3,14].

Another method of performing real-time measurements is the use of electronic noses. E-noses use a set of non-selective gas sensors and pattern-recognition techniques that allow them to compare breathprints, that is the volatile profile of the expiration [5,15]. This method does not provide qualitative information, but because of its low cost, high sensitivity, short-time of a single analysis and relatively small size, it may find its use in medical diagnostics directly at the patient's bed [16,17]. A schematic representation of the basic elements of an electronic nose is shown in Figure 1.

3. MEDICAL DIAGNOSIS

Breath analysis can find application as diagnostic method for a variety of diseases (Table), out of which respiratory tract diseases are gaining the biggest interest. This is not only because the affected organs are in direct contact with the exhaled air, but also because of the difficulty of correct diagnosis. Asthma, one of the most common chronic diseases, is usually diagnosed based on the evaluation of symptoms such as wheezing, cough or breathing problems. It is also advisable to perform spirometry, as part of a prophylaxis or to confirm a diagnosis. However, its use is not always possible since mild or progressive disease may not produce unequivocal results and because spirometry is not feasible for all patients due to the need for their cooperation which sometimes may be difficult to achieve e.g. in the case of small children. Nevertheless, recent studies suggest that it is possible use nitric oxide or nitrotysorin levels in asthma diagnostics [18]. Research indicate that with use of e-nose, it may be possible to distinguish healthy breath samples from samples from patients suffering from bronchial asthma with over 86% accuracy, which is superior to many currently used diagnostic methods. In addition, the use of breath exhalation techniques also makes it possible to distinguish asthmatics from those suffering from other respiratory diseases such as lung cancer [19]. Based on the obtained results, it seems that these techniques can be used to diagnose asthma.

Chronic Obstructive Pulmonary Disease (COPD) is an incurable disease most commonly caused by exposure to tobacco smoke. It is characterized by progressive restriction of airflow through the respiratory tract which can lead to death. Its proper treatment allows to slow down the progression of the disease and reduce its impact on the patient's life. However, due to the non-specificity of the COPD symptoms it is very difficult to diagnose and easily confused with other respiratory diseases. Previous studies have shown that it is possible to use exhaled air to distinguish chronic obstructive pulmonary disease from other diseases with an accuracy of up to 90% [20,21]. This indicates the possibility of using breath tests in diagnostics, in particular in screening, although further examination is needed.

Pulmonary tuberculosis (TB) is a potentially deadly infectious disease that usually attacks the lungs. It currently predominantly occurs in developing countries, although it is estimated that more than one third of the population is or was exposed to tuberculosis mycobacteria. Since TB can develop asymptomatically, it is not possible to diagnose it based on symptoms. The most commonly used diagnostic test is tuberculin skin test. However, some medications, immune deficiencies, or other illnesses may produce false positive or false negative results [22].

It would prove beneficial to develop a cheap, accurate diagnostic method that could help to identify tuberculosis and, consequently, limit its spread. Numerous studies indicate the potential effectiveness of exhaled air analysis, out of which the studies investigating potential application of e-nose seem to be the most promising in regard to the development of inexpensive screening tests. Zetola *et al.* [23] found electronic nose capable of distinguishing between infected patients and healthy controls with sensitivity of 94,1% and specificity of 90,0%. Moreover, they observed time-dependant changes in the composition of breath samples which may indicate the possibility of application exhaled breath analysis in treatment progress control.

Table 2. Potential volatile markers of selected diseases

Disease	Technique	Marker	Ref.
Asthma	E-nose GC	NO, nitrotysorin, Penthane, ethane	[24], [25]
COPD	GC E-nose	Alkanes, NO, CO, H_2O_2 Breathprint	[20], [26]
Pneumonia	E-nose	Breathprint	[16]
Tuberculosis	E-nose	Bacterial metabolites	[27]
Lung Cancer	E-nose PTR-MS IMS GC	Alkanes NO	[9], [12], [17], [25], [28]
Renal Failure	PTR-MS SIFT-MS E-nose	Ammonia Amines	[1], [29]
Diabetes	GC E-nose IMS	Acetone	[25], [28], [29]
H. pylori infection	SIFT-MS GC	CO_2	[1], [4]
Sarcoidosis	IMS E-nose	Breathprint	[16], [30]
Hepatic pathologies	E-nose GC	Breathprint Acetaldehyde	[29], [35]
Carbohydrate malabsorbtion	GC PTR-MS	H_2	[25], [31]

Lung cancer is one of the leading causes of death. Initially, it can develop without any symptoms, and those that appear in advanced stages of illness are usually not characteristic, making accurate diagnosis difficult. Therefore, lung cancer is diagnosed primarily on the basis of histopathological evaluation of tumour segments. Because prognosis depends largely on how early the diagnosis was made, it would seem necessary to develop a method to diagnose and evaluate the progression of the disease quickly and without any additional complications associated with the subsequent extraction of tissues [10]. Although none of the studies of cancer identification based on the presence of a single compound in the exhaled air sample have yielded satisfactory results, many studies indicate that group analysis or holistic breath analysis can discriminate between lung cancer patients and healthy controls or patients affected by other respiratory diseases [5,14]. First measurement were performed in the late 1980's, when Gordon et al [32] were able to distinguish sick patients from healthy controls based on the analysis of several volatile organic compounds found in the air exhaled by lung cancer patients. It is also worth noting that the possibility of discrimination between patients suffering from various types of cancer such as lung, prostate or rectal cancer is being investigated and the results appear to be promising despite the fact that the tumours from the outside of the respiratory system usually do not affect its performance [33].

Breath analysis is of great interest not only because of its potential use in diagnostics of respiratory diseases. Diabetes is a group of diseases in which the blood glucose level in the patient is elevated. This may be a primary, insufficient insulin production (type 1 diabetes), insulin resistance (type 2 diabetes), or hormonal changes due to pregnancy (gestational diabetes). Because the primary symptom of diabetes is elevated level of blood glucose, the diagnosis is usually based on blood tests. Blood glucose meters are also used by patients to monitor changes in glucose levels during the day, but that form of diabetic self-management is associated with discomfort, which, especially in children, can reduce the patient's cooperation [34]. In addition, blood is an infectious bodily fluid, which makes it not ideal diagnostic material, especially in regions of the world suffering from AIDS epidemic. However, various studies indicate that the breath analysis allows for distinguishing the diabetic patients from healthy subjects with sensitivity of 90% and specificity of 92% [35].

Another illness in which blood tests are used in both diagnostics and organ function's assessment is renal failure. However, uremic breath reflects the accumulation of volatile metabolites caused by acute or chronic kidney impairment, which means it may be possible to replace blood creatinine determination with exhaled air analysis [36]. The results so far have shown the ability to distinguish healthy people from patients with renal failure and to discriminate between patients with chronic and acute forms of the disease. In addition, it is possible to diagnose patients before and after dialysis on the basis of exhaled air analysis, which suggests that it may be used in the future to assess the effectiveness of the process [37].

4. CONCLUSION

Breath testing can be a new diagnostic method for various diseases, even in their early stages. Moreover, it may become a technique used to evaluate the course of the processes taking place in the body. Hence, a quick, non-invasive analysis of exhaled air samples could be routinely performed in a clinical environment. However, in spite of its potentially wide range of uses, exhaled air analysis still has its disadvantages. Definition of compounds or

groups of compounds that could act as markers, as well as creation of the universal method of analysis and sampling are needed. Further research in the field should aim improve breath tests for the evaluation, treatment and monitoring of patients with various disorders.

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