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Breathe Analysis for Medical Diagnostics –A Review

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Abstract:
It is essential to develop a new diagnostic and detection technology for the increasing number of clinical challenges in coming years. Though there are investigations such as EEG, ECG, bronchoscopy and needle biopsy to detect cancers, but they have associated risks and substantial costs and hence they are not suitable for early screening. This paper is an effort to review on the breath analysis, which is a new diagnostics frontier based on the detection of disease-related volatile organic compounds (VOCs). Breath Analysis, involves testing of exhaled breath samples, is a rapid, simple, portable, relatively inexpensive and non-invasive approach of analyzing malignant or benign diseases. It also aims to study the potential of various biomarkers to diagnose a disease and to evaluate various techniques in breath analysis. Finally the importance of nanomaterial based sensors is reviewed stating its advantages compared to other techniques. We conclude with a brief discussion on the diagnosis of diseases like diabetes, lung disorder and kidney disorder using nanomaterial based sensors and the expected outcomes and limitations.

Keywords: *Breath analyzers, Biomarkers, Chemiresistive sensors, Exhaled breath, Metabolic disorders, Nanoparticles, VOCs.*

1. Introduction

Breath Analysis[i][ii] is a health monitoring system where in various exhaled breath gases are detected and monitored for early diagnosis of diseases as shown in Fig 1. It is one of the simplest non-invasive, point of care and early diagnostic techniques available. Ancient Greece physicians knew that the specific odor of exhaled breath could be associated with certain diseases. It is only today that breath analysis is beginning to find a wide range of practical applications.

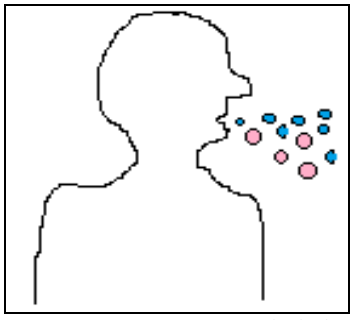

Figure 1: VOCs present in Exhaled Breath

From Fig 1, Exhaled Breath (EB) contains around 200-300 volatile organic compounds (VOCs) called biomarkers and the past studies have demonstrated that there is a strong correlation between exhaled breath components and specific diseases, thus offering strong potential for clinical diagnostic application using exhaled breath sensors. Formulating relevant conclusions from breath analysis requires accurate and repeatable measurements at very low concentrations which is a challenging task.
Modern breath analysis started in the year 1971 when Nobel Prize winner Linus Pauling separated the components of breath by gas chromatography (Pauling et al., 1971) which indicated that human breath is a complex gas comprising of more than 200 volatile organic compounds in Pico molar concentrations. Breath analysis offers unique advantage as it is rapid, simple, and non-invasive and often can be repeated easily.

Breath samples can be used in two important fields:

(i) Clinical diagnosis to analyse volatile compounds generated in the organism through exhaled breath (endogenous compounds)
(ii) Exposure analysis in order to have fast and accurate information regarding the levels of potentially noxious inhaled VOCs from environment, reaching the blood stream (exogenous compounds).

With the rapid advent in the area of nano technology, fabrication of sensors using various nanomaterial and their combinations has proved to be one of the great achievement. Though there are various techniques which were used for analyzing biomarkers and thereby to detect the related metabolic disorders, nanoparticle based sensors offers many advantages over the traditional methods which uses Gas Chromatography/mass spectrometer.

This paper also review some of the nanomaterial based chemiresistive sensors used to detect diseases like Diabetes, Lung cancer, Kidney cancer etc.

2. Biomarkers- Exhaled Breath Components

Breath analysis is one of the promising areas, focused on the identification of specific biomarkers [iii] in exhaled breath. Biomarkers are mixture of volatile organic compounds (VOCs) in the gaseous phase present in the exhaled breath. They are also called breath markers, as National Institute of Health states that "a biomarker is an indicator of any normal biological, pathogenic or pharmacological responses that take place in the human body". McCulloch [iv] have also demonstrated that dogs could be trained to detect lung cancer and breast cancer in subjects with various stages of disease with almost 100% accuracy, merely by smelling the subject's breath. These observations suggest that there are biomarkers in exhaled breath that are potentially useful for diagnosing disease. These biomarkers are present in various parts of the human body such as, the cells of the tumor growth, fluids in the body which include blood, urine, cerebrospinal fluid, sputum, saliva and breath. Thus their discovery, will lead to a non-invasive, early detection of a few types of cancer [v].

These volatile compounds may be generated in the body (endogenous) or may be absorbed as contaminants from the environment (exogenous). The composition of VOCs in breath varies widely from person to person, both qualitatively and quantitatively.

Breath contains a mixture of nitrogen (N), oxygen (O_2), Carbon-di-oxide (CO_2), Water (H_2O), and inert gases in bulk and contains traces of 1000 volatile compounds (VOCs) in fractions with concentrations in the range of parts per million (ppm) to parts per trillion (ppt) by volume. These VOCs, which is the main target in the breath analysis comes from the deeper breath called as alveolar air.

Sr. No	Diseases	Promising Breath Marker
1.	Asthma[vii]	Increased NO, CO, H_2O_2, nitrite/ nitrate, isoprostanes.
2.	Chronic	Stable NO.
3.	Bronchitis[viii]	NO, H_2O_2, Pentane, Isoprene.
4.	COPD[viii]	Increased NO.
5.	Chronic Cough[viii]	NO
6.	Influenza[viii]	NO, Decreased Isoprene, Decreased acetone,
	Lung Cancer[ix]	Decreased methanol, Benzene, Heptanol, Methyl, Toluene.
7.	Liver Cirrhosis[x] [xi]	2-butanone, 2 or 3 pentanone, C8-ketone, C9-ketone, monoterpene, heptadienol, methanol, 2,4-heptadienol.
8.	CKD[xii] [xiii]	TMA, Acetone, Isoprene, Pentane, Increased Ammonia.
9.	Diabetes[xiv]	Increased acetone, CO, altered levels of
10.	Tuberculosis[xv] [xvi]	Isoprene, methyl nitrate.
		NO, Naphthalene, Heptane, CO_2

Table 1: Breath markers for various diseases [xvii]

Abbreviation: NO –Nitrogen Monoxide, CO-Carbon Monoxide, H_2O_2–Hydrogen peroxide, COPD- Chronic Obstructive Pulmonary Diseases, CKD- Chronic Kidney Diseases, TMA- Trimethylamine.

Advances in analytical instrumentation [vi] have allowed researchers and clinicians to measure trace concentrations of the biomarkers for the detection of normal and abnormal biological function, which is critical but helps in early detection. Some of the infectious and non –infectious disease diagnostics using specific biomarkers are listed in Table I.

3. Techniques Used in Breath Analysis

The non-invasive approach of breath analysis technique is more advantageous than the commonly used diagnostic techniques like tomography, endoscopy etc. The various techniques involved in breath testing can be broadly classified into two main groups.

3.1. The Method That Utilizes Tools Coupled with Gas Chromatography

Tools coupled with Gas chromatography include the following techniques:

(i) Gas chromatography/mass spectroscopy (GC/MS) [xviii] [vi]: GC provides good separation for the large number of VOCs when coupled with mass spectrometry (MS) can be used to identify the VOCs based on their retention time and characteristic fragmentation patterns contained in the mass spectrum. Various detection methods employed in GC to identify compounds in

human breath are flame ionization detection (FID), mass spectrometry (MS), and ion mobility spectrometry (IMS). The main disadvantage of GC or GC –MS is that pre-concentration is necessary and therefore data cannot be obtained in real–time. Pre – concentration is commonly achieved through sorbent traps, coated fibers (solid phase micro extraction) or cryofocusing.

(ii) Proton transfer reaction-mass spectrometry (PTR-MS) [xix]:
It was developed by Hansel, Jordan, Lindinger, et al.[xix] for online measurements of complex mixtures of trace gas compounds in air with concentrations as low as one part per billion (ppbv). Here the samples must be ionized, which is done by proton transfer of gaseous sample inside a drift tube. This proton source is normally protonated water, H_3O^+.The technique is particularly advantageous for breath analysis as large volumetric contributions from N_2, O_2, CO_2 and water as they do not interfere with measurement. It does not require pre-concentration and separation procedure as in GC/MS. This instrument has very high sensitivity; down to ppt (parts per trillion), frequent and rapid measurements are possible. PTR-MS is, therefore, a promising technique for VOC analysis in breath gas particularly suited for online and multiple measurements. However, the PTR-MS characterizes the substances solely according to their mass-to-charge ratio; chemical identification is thus not possible and must be provided by other techniques. For increased accuracy PTR-MS is usually coupled with GC to provide additional separation.

(iii) Selected-ion flow-tube mass spectroscopy (SIFT-MS) [xx] [xxi]:
It is a new analytical technique similar to PTR-MS for real-time analysis of several traces of gases. It was developed by N.G Adams and D. Smith in 1976[xxii] for the study of ion reactions under certain thermal conditions. It pre- selects the three reagent ion (H_3O+, NO+, O_2) for chemical ionization of the gases in a reaction tube and allows the reaction between proton donor and analyte to occur under low pressure field conditions. It allows identification of VOCs of ppb levels, from the selected sample. Compared to PTR-MS, SIFT-MS is less sensitive.

3.2. Nanomaterial Based Gas Sensors

In order to tailor the need of patients, the research has made to focus on the development of portable, compact and point of care tools. The nano scale size of nanomaterial provides large surface–to–volume ratio and unique chemical, optical and electrical properties thus increasing the sensitivity and lowering the response and recovery times. Their sensitivity and detection level goes upto to pico, femto, atto or even zepto scales (10^{-12} to 10^{-21}).Hence Nanomaterials[xxiv] are used to sense traces of VOCs present in the exhaled breath to detect various diseases associated with the biomarkers in the body as depicted in the Fig 2.

Nanomaterials include nanoparticles, nanowires, nanotubes etc. The composition of sensor[xxv]classifies the type of sensors and the output depends on the type of sensor being used (E.g. conductivity, mass, vibration and color).Apart from Breath analyzers, nanomaterial also found in the application area like Electronic Nose or E-Nose [xxvi]which has the capability to detect the odor of various gases.

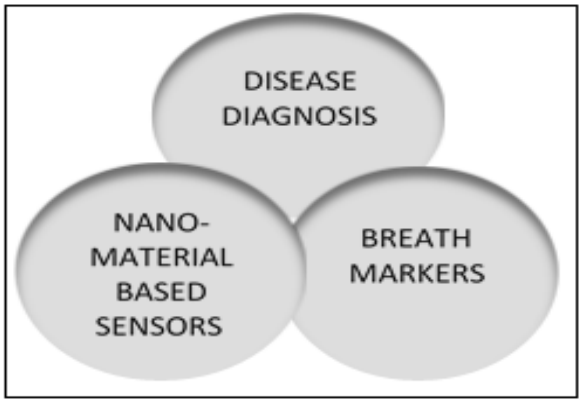

Figure 2: The Relevance

Thus various nanomaterial based gas sensors can be classified as:

(i) Electrochemical sensor [xxvii]:

Analytical Method	Mode of operation	Principle	Limit of Detection (LOD)	Sensitivity
GC- MS	Pre-concentration	Analyses by separating compounds by MS using chromatic column.	Ppt-Ppb	Very High
PTR-MS	Direct/Real time	Based on ionized molecules of target analyte by reaction with H_3O^+ by MS.	Ppt	High
SIFT-MS	Direct/Real time	Analyses by the ions produced by reaction between analyte and precursor ions.	Ppb	High
Nano-material based sensors	Real time	Measurement of resistivity/ conductivity by varying thickness of the depletion layer of SMOs.	Ppb	Medium

Table 2: comparison of characteristics of breath research technique [23]

Abbreviation: MS - Mass spectroscopy, H_3O^+- Protonated water, SMO – Semiconducting metal oxide.Ppt – Parts per trillion, Ppb- Parts per billion, Ppm- Parts per million, GC-Gas chromatography, SIFT- Selected- ion flow- tube, PTR- Proton transfer reaction.

These sensors make use of charge transfer from an electrolyte sample (solid/liquid/polymer) to an electrode or vice-versa. It is based on the detection of electro active species involved in chemical recognition processes. There are further classified as Amperometric, potentiometric and conduct metric sensors where current, resistance or conductance can be made proportional to the target gas concentration. These sensors have ambient temperature operation, low power consumption and are very sensitive to diverse VOCs. But they are bulky and have a limited sensitivity towards low molecular weight gases.

(ii) Semiconducting metal oxide (SMO) based Chemi-resistive sensor [xxviii][xxix]:
Breath analysis using Electric sensors based on SMO are one of the portable, cost effective and point of care tools. The major responsibility of a sensor is to trace multiple analyte in low concentration, in the non-standard changing environment. SMO based sensing materials include SnO_2,TiO_2, ZnO, In_2O_3, and $WO_3$of which the latter in crystalline form has been intensively studied as a detector for acetone, a known biomarker of diabetes. Current sensors adapt the γ-phase due to its stability at room temperature to detect NO_x, NH_3, H_2S and O_3whereas it adapts ε-phase due to its stability at temperatures below 230 K to detect WO_3.

(iii) Calorimetric sensors[xxx]:
These sensor devices use calorimetric analysis as a transduction principle and operate by measuring the temperature difference of a reaction on the sensor surface.

(iv) Magnetic sensors [xxxi] :
They are based on the change of paramagnetic properties of the gas being analyzed. They are represented by certain types of oxygen monitors as they have high susceptibility comparatively.

4. Comparison of Techniques on Various Parameters

Because of the low concentrations of VOCs in the range ppb (parts per billion) to ppt (parts per trillion) in exhaled breath, sensitive and highly accurate GCs and mass spectrometers have been utilized in last decades. GCs and mass spectrometers have limited application in a clinical setting because of their expense, difficulty of use due to its preconcentrating steps, bulkiness and the need for highly experienced analysts to operate them and interpret the results[xxi].At this point of time, a real time analysis techniques such as PTR-MS and SIFT_MS are widely used. Though they are able to detect the VOCs in the range Ppt and Ppb, respectively, they are bulky also requires trained operators. They have low sensitivity and specificity as compared to GC-MS. Also IMS technique require high vacuum condition which is expensive, and failing to which lowers the sensitivity of the technique.

An emerging breath sensing technique which is a real time, portable and relatively inexpensive is the nanomaterial based sensors. These nanomaterial based sensors, semiconductor- based sensor arrays or chemiresistive type sensors are the one which has the capability to detect VOCs in ppm (parts per million) range which is less compared to other methods. To improve the sensitivity of these sensors extensive research is carried out on various nanoparticles and polymers [32] or their combinations based sensors which can be used to detect the target VOCs. Table II gives a description of the characteristics of the available breath research techniques [xxiii].

5. Application of Breath Analysis in Detecting Various Diseases

5.1. Diabetes

Acetone is the main breath marker for diabetes. Higher acetone concentration ranges from 1.7 ppm to 3.7 ppm could be detected in breath for those who are diabetic, while the breath of healthy human is typically contains less than 0.8 ppm [xxxiii]. Jonathan C. Claussen[xxxiv] et.al; have developed sensors using scalable nanofabrication methods, based on Multi-layered Graphene Petal

Nanosheets (MGPNs), Platinum (Pt) nanoparticles and a bio recognition element (glucose oxidase). By changing the size, density and morphology of electrodeposited Pt nanoparticles on the MGPNs performance of biosensors can be altered. The observation showed an enhanced glucose sensitivity of 0.3 μM detection limit, 0.01–50 mM linear sensing range, a long stable shelf-life (> 1 month) and a high selectivity over electro active, interfering species commonly found in human serum samples. Jungwoo Shin et.al[xxxv] have developed hierarchical SnO_2 fibers synthesized by controlling the microphase separation between tin precursors and polymers, dimension of which ranges from 10nm to 500nm in length along fiber direction. The inner SnO_2 fiber layer is uniformly coated with Pt nanoparticles which acts like a catalyst which enhances the acetone response, which is the biomarker used for diagnosis of diabetes. It was further observed that the same sensor can be used to detect lung cancer, if the acetone level in the breath decreases.

5.2. Lung Disorder

Lung disorders are mainly identified by low acetone levels and high Nitric Oxide levels in the exhaled breath. Acute lung cancer is concerned with concentration of benzene in EB (Exhaled Breath). M. P. Fernandes et.al; [xxxvi], have achieved fourteen different chemiresistive Monolayer-Capped Gold Nanoparticles (MCNP) films deposited on micrometric electrical transducers and combined onto a sensor array. Exposing this array to VOCs, causes change in the electrical resistance of the film occurs due to the absorption of the breath onto the sensor array. As a result, these chemiresistors can identify the pattern of each breath biomarker and therefore quantitative data can be obtained. These data are then analyzed using Principal Component Analysis (PCA) algorithm. Anton Amann, et.al [xxv] describes the ability of a new gas sensor using calorimetric sensor array to detect patterns of VOCs. They have used 36 spots composed of different chemically sensitive compounds. The color of these spots changes thus detecting the unique pattern of VOCs from EB samples of patients with lung cancer.

5.3. Kidney Disorder

Kidney disorders are mainly identified by extremely high ammonia content in human exhaled breath [xxxix]. The ammonia odor in the mouth of kidney failure patients is associated with high levels of blood urea nitrogen. Excess of urea will be deposited into ammonia under the effect of urea enzymes in the gastrointestinal tract. Morad K. Nakhleh, et.al, [xxxviii] describes Organic- layer capped Gold Nano Particle (GNP) based biosensors of diameter 3-4nm, used to analyze breath samples in an acute kidney injury model. The exposure resulted in a fully reversible change in resistance of the sensor which was recorded. It was observed that this technique gives early and better results than GC/MS method and other blood/ urine culture based technique.

6. Limitations of Breath Analysis

Due to technical problems of sampling and analysis and a lack of normalization and standardization, huge variations exist between results of different studies [xxxvii]. This is among the main reasons why breath analysis could not yet been introduced into clinical practice. Though the advances in analytical instrumentation have allowed researchers and clinicians to measure trace concentrations of the biomarkers for the detection of normal and abnormal biological function, but fabricating a sensor to detect and analyze low concentration of VOCs in the exhaled breath to identify the metabolic disorder is very critical.

7. Conclusion

Breath analysis is useful for clinical diagnostics, real time therapy monitoring and control of metabolic disorders. Breath is a rich mixture containing volatile organic compounds at concentration levels in ppt or ppb. Its analytical results are qualitative and quantitative as compared to analysis of blood and urine. Compared to diagnostic techniques like tomography, endoscopy etc., breath analysis is a rapid, non-invasive, point of care technique used for early detection of diseases at any age of life. VOCs can potentially be used as disease-specific biomarkers for non-invasive early detection and monitoring of the disease. Though the GC analyzing method is highly sensitive and selective for diagnosis, it is expensive, bulky, involves a complex analysis, and is inconvenient for portable use. Hence compared to chromatography, nanomaterial based electrochemical sensors have advantages which include a high accuracy in discriminating between malignant and benign disease. Also they are highly insensitivity to humidity.

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