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QUALITATIVE AND QUANTITATIVE DETERMINATION OF HUMAN BIOMARKER BY LASER PHOTOACOUSTIC SPECTROSCOPY METHOD

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Abstract:

Biomarkers can be defined as a characteristic that can objectively measure different biological sample and that can be evaluated as an exposure marker of normal or pathogenic biological processes or of responses to a certain intervention. The biological sample are blood, red blood cells, plasma, serum, urine, nails, saliva, faeces and sample of different tissues. The analysis of breath air has major advantages because it is a non-invasive method, represents minimal risk to personnel collecting the samples and can be often sampled. Breath air samples from the human subjects were collected using aluminized bags from QuinTron and analyzed using the laser photoacoustic spectroscopy (LPAS) technique. LPAS is used to detect traces of ethylene in breath air resulting from lipid peroxidation in lung epithelium following the radiotherapy and also traces of ammonia from patients subjected to hemodialysis for treatment of renal failure. The levels of ethylene and ammonia in exhaled air, from patients with cancer and renal failure, respectively, were measured and compared with breath air contents from healthy humans. Human gas biomarkers were measured at subppb (parts per billion) concentration sensitivities. It has been demonstrated that LPAS technique will play an important role in the future of exhaled breath air analysis.

The key attributes of this technique are sensitivity, selectivity, fast and real time response, as well as its simplicity.

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INTRODUCTION:

Biomarkers can be defined as a characteristic that can objectively measure different biological sample and that can be evaluated as an exposure marker of normal or pathogenic biological processes or of responses to a certain intervention. The biological samples are blood, red blood cells, plasma, serum, urine, nails, saliva, faeces and sample of different tissues. Advances in omics are opening up new possibilities for obtaining new biomarkers of various kinds, using genomics, epigenomics, transcriptomics, lipidomics, proteomics and metabolomics.

Biomarker in Drug Development Process:

Biomarkers play a critical role in each phase of the drug development process from drug discovery, preclinical and clinical trai l phases (phase 1-IV). The stage of the drug development process is impacted by biomarker. Biomarkers can help to predict who might respond better to a drug from a safety and efficacy perspective, monitor the safety of the therapy to determine whether treatment is having desired effect on the body. Biomarkers based strategy allow targeted approach to be adopted by the pharma companies to enable the time and cost savings in clinical trails. This would give higher probability of success rate by having molecule with desired efficacy and safety.

Characteristics OfBiomarker:

Biomarkers help not only in disease diagnosis but also in tracking progression, regression and outcome after intervention. Biomarkers helps in the identification of a disease and could be bio-moleacules like carbohydrates, proteins, lipids to genes, DNA, RNA, platelets, enzymes, hormones or a metabolite, a change in biological structure.

Ideal biomarkers:

- Specific for a particular disease and able to differentiation between different physiological states
- Safe and easy to measure.
- Rapid so as to enable faster diagnosis.
- Cheap and cost efficient to follow up.
- Able to give accurate results.
- Consistent between different ethnic groups and genders

The biomarkers can be implemented if sampling has to be non-invasive technique, minimum volume of blood, or other biological sample type, collection and transportation. Also need to develop the right validation, analytical methodology and regulatory requirement, bio-banking facility to keep the sample safe, track and retrieve the sample.

Classification of biomarkers:

a) According to genetics and molecular biology method, biomarker can be classification as Type 0, Type 1 and Type 2 biomarkers.

Type 0 biomarkers (natural history biomarkers):

They help measure the natural history of a disease and correlate over time with known clinical indicators.

Type 1 biomarkers (drug activity biomarkers):

These indicate the effect of drug intervention.

They may be futher divided into

1. Efficacy biomarkers –indicating the therapeutic effect of a drug.
2. Mechanism biomarkers –giving information about the mechanism of action of a drug
3. Toxicity biomarkers –indicating the toxicological effect of drug.

Type 2 biomarkers (surrogate markers):

They serve as a substitute for a clinical outcome of a disease and also help predict the effect of a therapeutic intervention.

b)classification can be prognostic biomarker, predictive biomarkers and pharmacodynamic biomarkers

c) classification of biomarkers can be biomarkers of exposure and biomarkers of disease and biomarkers as drug related or disease related.

Photoacoustic spectroscopy:

Photoacoustic spectroscopy is a class of photo thermal techniques, in which an impinging light beam is absorbed altering the “thermal state” of the sample. This “thermal state” can manifest itself as a change in temperature, density, or in other measurable properties of the sample. If the incoming light is modulated, the absorbing sample warms and cools in a cycle. If the cycle is so fast that the sample does not have time to expand and contract, in response to the modulated light, a sudden change in pressure is taking place. This pressure “wave” can lead to the production of a sound wave (i.e., a photoacoustic effect). The sound waves can be detected by a sensitive microphone, a piezoelectric device, or by optical methods.

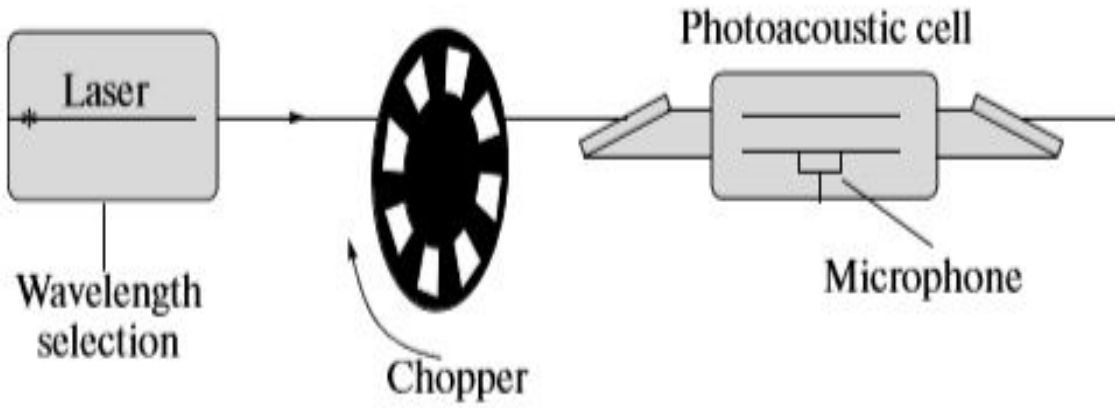


Fig. 1. General scheme of laser photoacoustic detection.

Photoacoustic spectroscopy is a new analytical tool that provides a simple nondestructive technique for obtaining information about the electronic absorption spectrum of samples such as powders, semisolids, gels, and liquids. It can also be applied to samples which cannot be examined by conventional optical methods. Numerous applications of this technique in the field of inorganic and organic semiconductors, biology, and catalysis have been described. Among the advantages of photoacoustic spectroscopy, the signal is almost insensitive to light scattering by the sample and information can be obtained about non-radiative deactivation processes.

Blood, urine and other body fluids and tissues can be sampled and analyzed to produce clinical information for disease diagnosis or therapy monitoring is the basis of modern clinical diagnosis and medical practice. The analysis of breath air has major advantages because it is a noninvasive method, represents minimal risk to personnel collecting the samples and can be often sampled. **Breath air samples** from the human subjects were collected using aluminized bags from QuinTron and analyzed using the laser photoacoustic spectroscopy (LPAS) technique.

These measurements were made to detect ethylene and ammonia from the exhaled breath of a patient

Breath Sample Collection:

with mammary cancer, a patient with renal disease and two control healthy volunteers.

LPAS is a powerful spectroscopic technology for quantitative and qualitative trace gas analysis in exhaled human breath air. This ultrasensitive technique measures extremely low concentrations and offers a degree of confidence that cannot be attained by other methods.

Exhaled breath tests:

The breath air is a mixture of nitrogen, oxygen, carbon dioxide, water, inert gases, and traces of volatile organic compounds (VOCs). The matrix elements in breath air vary widely from person to person, both qualitatively and quantitatively, particularly for VOCs. More than 1000 trace VOCs have been distinguished in human breath air, at concentrations from ppmv (parts per million by volume) to pptv (parts per trillion by volume) levels. Among these VOCsisoprene, acetone, ethane, and methanol, which are products of core metabolic processes. In addition to these VOCs, exhaled NO, H2, NH3, and CO are related to health condition and can reflect a potential disease of the individual or a recent exposure to a drug or an environmental pollutant.

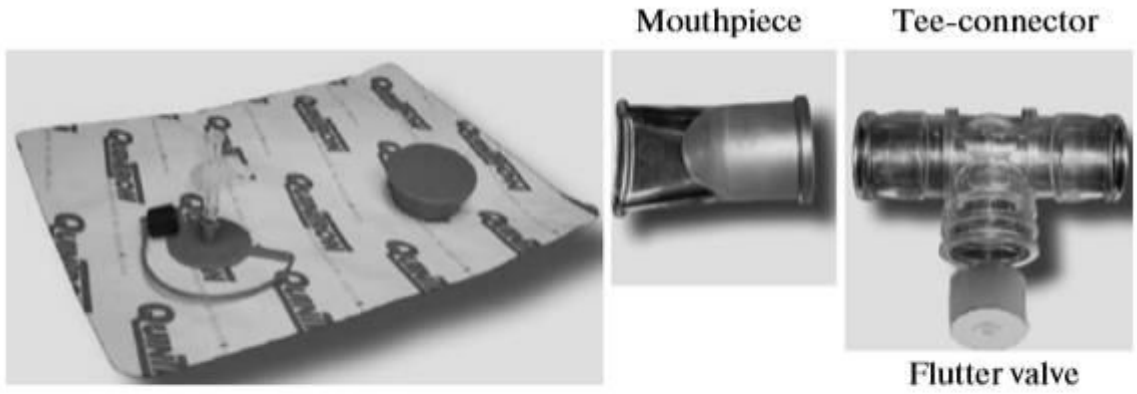


Fig. 2. Breath sample collection system.

To get an efficient breath air sample, we used aluminized multipatient collection bags (750mL aluminum coated bags QuinTron), composed of a disposable mouthpiece and a tee-mouthpiece assembly (it includes a plastic tee and a removable one way flutter valve). Multipatient collection bags are designed to collect multiple samples from patients and hold a sample for maximum 6 h.

After the alveolar air sample is collected, the sample gas is transferred into the photoacoustic cell (PA cell) and can be analyzed immediately or later.

Breath Traps:

There are large amounts of water vapors and CO2 in human breath air. The concentration of water vapors in the collecting bags may reduce volatile compounds that are soluble in water, resulting in falsely depressed concentrations of some analytes.isopropyl alcohol/solid CO2 cooling system to remove water vapors. However, many compounds were lost when water vapors were removed by a water trap.

Generally, there are three approaches to achieve pre concentration:

1.chemical
2.cryogenic
3.Adsorptive methods.

1.Chemical trapping:

In this usually uses traditional "wet chemistry" where breath air is bubbled through a reagent solution that captures a specific compound, such as ethanol or acetone. The method is simple and direct, and the trapped derivative, often colored, can be easily measured. The disadvantages of this approach are its poor sensitivity and the physical effort required to the donor.

2. cryogenic trapping:

In this biomarkers are captured by freezing. The breath travels through a tube immersed in such a cooling fluids as liquid nitrogen, which is at –196°C. Unfortunately, a cold trap alsofreezes the water vapors and carbon dioxide in the breath air and may rapidly become plugged by ice.

3.Adsorptive trapping:

It has become the most convenient and most widely used method today. It captures biomarkers by binding them to different agents such as activatedcarbon and absorptive resins, followed by a thermal desorption and final analysis.

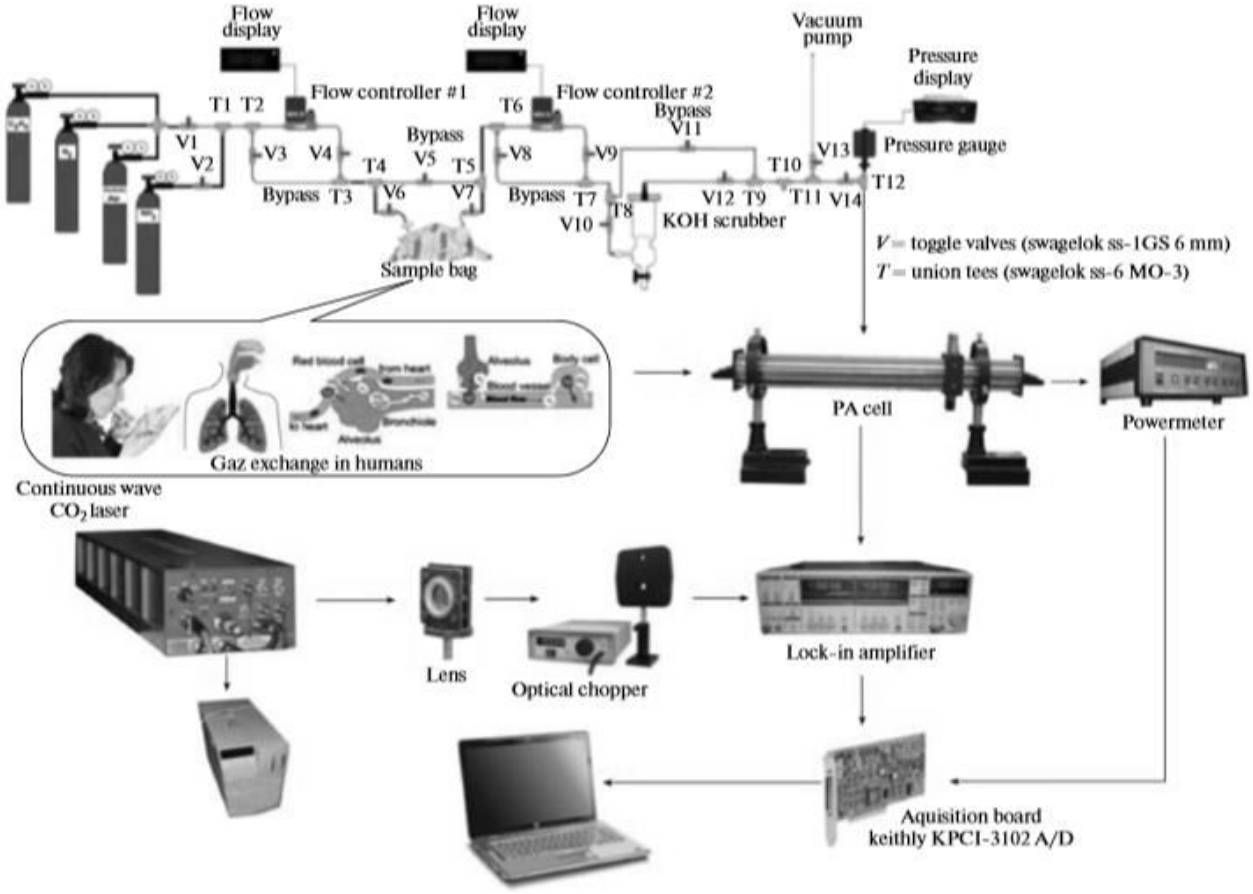
Photoacoustic Cell Design:

The PA cell is made of stainless steel and Teflon, in order to reduce the outgassing problems and consists of an acoustic resonator (pipe), windows, gas inlets and outlets, and microphones. ZnSe windows, at the Brewster angle, are glued with epoxy to their respective mounts. The resonant condition is obtained for longitudinal standing waves, produced in an open tube (resonator) placed coaxially inside a larger chamber.

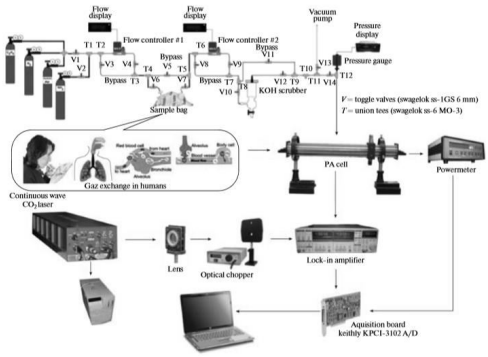
We use an open end tube type of resonator, excited in its first longitudinal mode. To achieve a larger signal, the pipe was designed with a long absorption path length (L = 300 mm) and a smallerinner diameter of 2r = 7 mm. Therefore the fundamental longitudinal wave has a nominal wavelength $\lambda s = 2L = 600$ mm (and a resonance frequency, f0 = 564 Hz). The inner wall of the stainless steel resonator tube is highly polished. It is centered inside the outer stainless steel tube with Teflon spacers.

A massive spacer is positioned at one end in order to prevent bypassing of gas in the flow system; the other one is partially open to avoid the formation of closed volumes. Gas is admitted and exhausted through two ports located near the ends of the resonator tube. The perturbation of the acoustic resonator amplitude by the gas flow noise is thus minimized. Inside the PA cell, the traces of ethylene and ammonia absorb the laser radiations then the absorbed energy is released

into heat, which creates a sudden pressure increase inside the closed volume. pressure waves are generated and detected with four sensitive miniature microphones mounted in the cell wall Their electric signal is fed into a dualphase, digital lockin amplifier and the filtered output signal is delivered to the data acquisition interface.



Laser photoacoustic spectroscopy



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Volatile compounds can be produced in the body, transported via the am, and exhaled through the lungs. They t physiological or pathological biochemical such as lipid peroxidation, liver disease or ıre.

urements were made to detect ethylene and from the exhaled breath of a patient with cancer, a patient with renal disease and two control healthy volunteers. Breath test is noninvasive, easily to repeat, and does not have the

Exhaled breath air analysis is an attractive and promising novel approach for noninvasive detection of human biomarkers associated with different

discomfort or embarrassment associated with blood and urine tests.

Breath air is a much less complicated mixture than serum or urine and it is amenable to complete analysis of all present compounds. No workup of breath sample is required, in contrast to many analyses performed on serum or urine samples. Breath air analysis provides directinformation on respiratory function that is not obtainable by other means and can dynamically monitor the decay in realtime of biomarkers in the human organism.

The rapid development of LPAS method and its use for gas analysis shows that this technique is promising for studying the composition of exhaled air for developing new diagnostic methods in medicine.

CONCLUSIONS:

Exhaled breath air analysis is an attractive and promising novel approach for noninvasive detection of human biomarkers associated with different diseases. Volatile compounds can be produced anywhere in the body, transported via the bloodstream, and exhaled through the lungs. They can reflect physiological or pathological biochemical processes such as lipid peroxidation, liver disease or renal failure. LPAS is a powerful spectroscopic technology for quantitative and qualitative trace gas analysis in exhaled human breath air. This ultrasensitive technique measures extremely low concentrations and offers a degree of confidence that cannot be attained by other methods

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