SPECTROSCOPIC STUDIES OF ETHYLENE AND AMMONIA AS BIOMARKERS AT PATIENTS WITH DIFFERENT MEDICAL DISORDERS

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Analiza aerului expirat permite observarea proceselor biochimice in organism prin intermediul unei investigatii neinvazive. Concentratia de etilena si ammoniac din aerul exalat este crescuta in bolile inflamatorii. Vom descrie masuratori precise de coeficienti de absorbtie ai etilenei si amoniacului la lungimile de unda ale laserului cu CO_2 prin utilizarea unei celule fotoacusitce in configuratie externa si vom compara rezultatele noastre cu alte valori raportate in literatura de specialitate. Setul de valori al coeficientilor de absorbtie, pentru toate lungimile de unda laser, si pentru un gaz special sau vapor se numeste spectrul de absorbtie sau "amprenta"; coeficientii de absorbtie sunt entitati absolute, unice doar la frecventa laserului si a speciilor, care ofera specificul de performanta al instrumentului in ceea ce priveste limita de detectie si respingere a interferentei.

Analysis of exhaled air enables the observation of biochemical processes in the body through a noninvasive investigation. Exhaled concentration of ethylene and ammonia is increased in inflammatory diseases. We describe accurate measurements of the ethylene and ammonia absorption coefficients at the CO_2 laser wavelengths by using a photoacoustic cell in an external configuration, and we compare our results with other values reported in the literature. The set of values of the absorption coefficients for all laser wavelengths and a particular gas or vapor is called the absorption spectrum or "fingerprint"; the absorption coefficients are absolute entities, unique only to the laser frequency and species, which provide the specifics of instrument performance in terms of detection limit and interference rejection.

Keywords: Absorption coefficients, ethylene, ammonia, breath analysis, laser photoacoustic spectroscopy.

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1. Introduction

Laser photoacosutic spectroscopy (LPAS) has emerged over the last decade as a very powerful investigation technique, capable of measuring trace gas concentrations at sub-ppbV level (1ppb = 10^{-9}atm). The technique operates on the principle that the amount of light absorbed by a sample is related to the concentration of the target species in the sample. Light of known intensity is directed through a gas sample cell and the amount of light absorbed by the sample is measured as a sound intensity by a detector, usually a sensitive microphone.

$$V = \alpha C S_M P_L c \quad (1)$$

where: V (V) is the photoacoustic signal; α (cm^{-1} atm^{-1}), the gas absorption coefficient at a given wavelength; C (Pa cm W^{-1}), the cell constant; S_M (V Pa^{-1}), the microphone responsivity; P_L (W), the cw laser power; and c (atm), the trace gas concentration (usually given in units of per cent, ppmV, ppbV or pptV). Equation (1) shows that the photoacoustic signal is linearly dependent on laser power. Thus, sensitive measurements benefit from using as much laser power as is reasonably available. Moreover, the signal is directly dependent on the number of molecules in the optical path (trace gas concentration), which means that this technique is truly a "zero-baseline" approach, since no signal will be generated if the target molecules are not present [1, 2]. In this study we focused on the application of LPAS method for the measurement of ethylene and ammonia absorption coefficients.

2. Exhaled human biomarkers and their diagnostic importance

Biomarkers are present in all parts of the body including body fluids and tissues. The bulk matrix of breath is a mixture of nitrogen, oxygen, CO_2 (exhaled in a fraction equal to about four percent of volume), H_2O, and others gases (Table 1).

Table 1

Composition of inhaled and exhaled air

Component	Inhaled air [%]	Exhaled air [%]
nitrogen	78.0	78.0
oxygen	21.0	15.0
carbon dioxide	0.04	4.0
water vapour	0.96	3.0

The residual volatile organic compounds (VOC_S) may be endogenous (produced in the body) or exogenous (assimilated as contaminants from the environment). The risk of exposure to VOC_S is defined as "an event that occurs when there is contact at a boundary between a human and the environment with a contaminant of specific concentration for an interval of time" according to the

National Academy of Sciences (NAS) [3]. The way for compounds to enter the body is through: ingestion, inhalation, or dermal contact. The chemicals can either be absorbed into the systemic blood supply or they can pass through the body not absorbed and be excreted directly in the feces. In general, nonpersistent chemicals with short biological half-lives in blood are eliminated in the urine, or if they are volatile, are eliminated in the exhaled breath air [3].

The endogenous compounds found in human breath, such as inorganic gases (e.g., NO and CO), VOC_s (e.g., isoprene, ethane, pentane, acetone), and other nonvolatile substances such as isoprostanes, peroxynitrite, or cytokines, can be determined in expired human breath. Tests for endogenous and exogenous biomarkers can provide valuable information concerning a possible disease state or they can indicate recent exposure to drugs or environmental pollutants [3-5].

The most prominent disease marker in exhaled human breath is nitric oxide (NO). It was found that, the average concentration of NO in the breath of healthy humans is 10 to 50 ppb; it is regarded primarily as a noxious gaseous component of air pollution. Intense basic research and clinical investigation have shown that NO is produced by a variety of human tissues. NO is now known to be a central mediator in biological systems and a biomarker for lung cancer, pulmonary hypertension, upper respiratory infection, inflammatory processes in stomach, cancer of digestive organs, acute sepsis, asthma, bronchieactasis and rhinitis [6-9].

Ethane (C_2H_6) is a biomarker for oxidative stress and destruction caused by free radicals. C_2H_6 (the average concentration in the breath of healthy humans is 0 to 10 ppb) is also an indicator of vitamin E deficiency, cystic fibrosis, ubiquinol status at peroxidation of lipid, smokers and nonsmokers [10].

Ammonia (NH_3 - the average concentration in the breath of healthy humans is 0 to 1 ppm), a product of urease hydrolysis of urea to ammonia and carbamate, is one of the key steps in the nitrogen cycle. It serves as a biomarker for lung cancer, asthma, acute and chronic radiation diseases, metabolism of monoamines in lungs, kidney insufficiency (nephritises, hypertonic disease, atherosclerosis of kidney arteries, toxicosis and nephropatia of pregnants, toxic kidney damage), liver insufficiency at jaundices, hepatitises, liver cirrhosis and toxic hepatitis [8, 11]. A colourless gas with a characteristic pungent odour, NH_3 is vibrationally excited to the ν_2 state, usually by means of the *saR*(5, *K*) transitions at $\lambda = 9.22$ μm. These levels can be excited by the 9R(30) line of the CO_2 laser, where the absorption coefficient α has a value of 57.12 $cm^{-1}atm^{-1}$.

Ethylene (C_2H_4) also known as ethene, is a biomarker for acute myocardial infarction, inflammatory processes (chronic asthma, peritonitis), ultraviolet radiation damage of human skin and lipid peroxidation in the lung epithelium. Exhaled concentrations may also be correlated with disease status of non-lung inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease [12]. Ethylene is vibrationally excited to the ν_7 state, at $\lambda = 10.53$ μm by the 10P(14) line of the CO_2 laser, where the absorption coefficient α is 30.4 $cm^{-1}atm^{-1}$.

The correlation between a biomarker and a distinctive disease is often many-fold. In some cases, a breath species is a biomarker that is indicative of about more than one disease or metabolic-disorder; in other cases, one particular disease or metabolic disorder can be characterized by more than one chemical species.

3. Analysis of exhaled breath

What is the chain of processes involved in a given biomarker? The analysis of exhaled breath to detect or assess a given biomarker with the aim of performing a diagnosis or study a body function is called breath test and can be represented in the general form as follows: production of the biomarker during a particular biochemical reaction or a complex metabolic process; diffusion of biomarker through tissues and input into haematic flow; possible intermediate accumulation (buffering); possible trapping of biomarker by utilization and assimilation systems or natural chemical transformation; transport to the lungs; transmembrane diffusion to the air space of lungs; diffusion of biomarker and their mixing with inhaled air in the alveolar space of lungs; release of biomarker in the breathing air; collection of a breath sample and assessment of the biomarker in the breath sample [13].

Breath analysis offers many unique benefits: safe, rapid, simple to perform, non-invasive and frequently repeatable sampling; potential for real-time analyses. Given that human breath contains up to of 200 chemicals, the potential for developing new applications is high. Much of the current knowledge on breath analysis in respiratory medicine derives from years of experience gained in occupational settings, where breath analysis has been used to assess exposure to VOC_S.

4. Measurement of ethylene and ammonia absorption coefficients using LPAS

LPAS technique is successfully applied in gas absorption measurements because it offers a high sensitivity that makes possible to measure absorptions coefficients on the order of 10^{-8} cm^{-1}. The laser-based photoacoustic detector is able to distinguish between different gases by making use of their wavelength-dependent fingerprint absorption characteriscs.

A schematic diagram of the setup is given in Fig. 1. The experimental setup consists of a line-tunable CO_2 laser emitting radiation in the 9.2 to 10.8 μm region on 57 different vibrational-rotational lines and a photoacoustic cell (PA), where the gas is detected. The requirements for gases to be detected with this sensitive laser is that they possess a high absorption strength and a characteristic absorption pattern in the wavelength range of the CO_2 laser (10.53 μm for ethylene and 9.22 μm for ammonia).

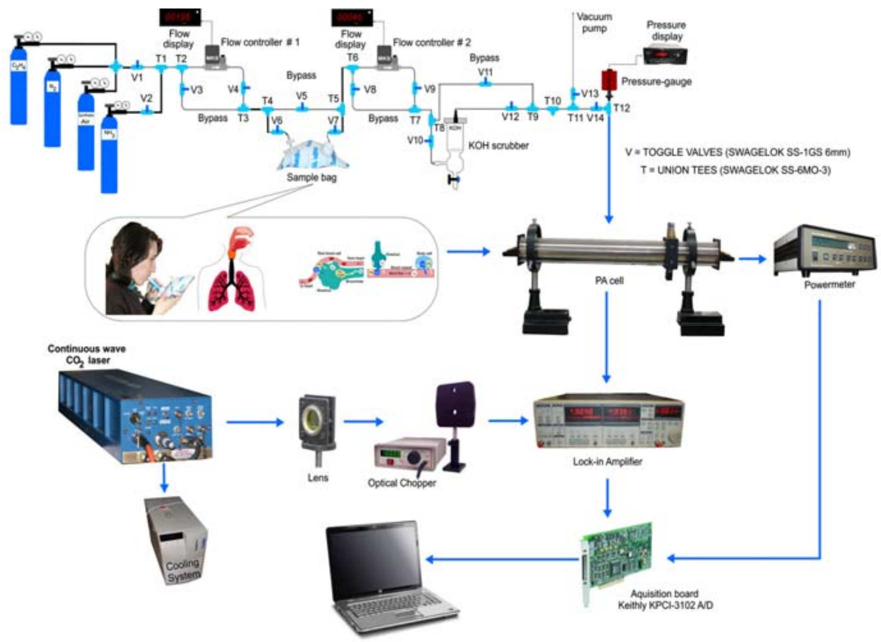

Fig. 1 General scheme of the LPAS system

Inside the PA, traces of ethylene and ammonia can absorb the laser radiation and the absorbed energy is released into heat, which creates an increase in pressure inside a closed volume. By modulating the laser beam with a mechanical chopper-model DigiRad C-980 (operated at the appropriate resonant frequency of the PA cell -564 Hz), pressure waves are generated and detected with four sensitive miniature microphones (Knowles electret EK-3033 or EK- 23024 miniature microphones connected in series) mounted in the cell wall. There electric signal is fed into a dual-phase, digital lock-in amplifier (Stanford Research Systems model SR 830) and its filtered output signal is introduced in the data acquisition interface. All experimental data are processed in real time and stored by a computer [14]. More details about the PA cell and the experimental protocol are found in other publications [1, 14, 15].

An important element in these measurements is the gas handling system due to its role in ensuring gas purity in the PA cell (the upper part of Fig.1). It can be used to pump out the cell, to introduce the sample gas in the PA cell at a controlled flow rate, and monitor the total and partial pressures of gas mixtures. Also, the gas handling system can perform several functions without necessitating any disconnections.

To improve the measurement of ethylene and ammonia absorption coefficients, a special procedure was followed. Prior to each run, the gas mixtures was flowed at 100 sccm (standard cubic centimeters per minute) for several minutes to stabilize the boundary layer on the cell walls, since a certain amount of adsorption would occur and possibly influence background signals; after this conditioning period, the cell was closed off and used in measurement. For every gas fill with 10 ppm ammonia or 0.96 ppm ethylene respectively, buffered in pure nitrogen, the responsivity of the cell was determined supposing an absorption coefficient of 57.12 $cm^{-1}atm^{-1}$ at 9R(30) laser transition for ammonia and 30.4 $cm^{-1}atm^{-1}$ at 10P(14) laser transition for ethylene. After measurements at all laser lines, the cell responsivity was checked again, to eliminate any possibility of gas desorption during the measurement. The absorbtion coefficients values at each laser line were obtained from Eq. (1) by using the measured PA signal and laser power and by knowing precisely the gas concentration and the responsivity of the PA cell. An average over several independent measurements at each line was used to improve the overall accuracy of the results [1, 14].

To measure the absorption coefficients of ethylene and ammonia, the software user interface allows recording the laser power, the PA signal and the calculated absorption coefficients on different panels**.** The multiple measurements in time of the absorption coefficients can be also displayed (Fig. 2).

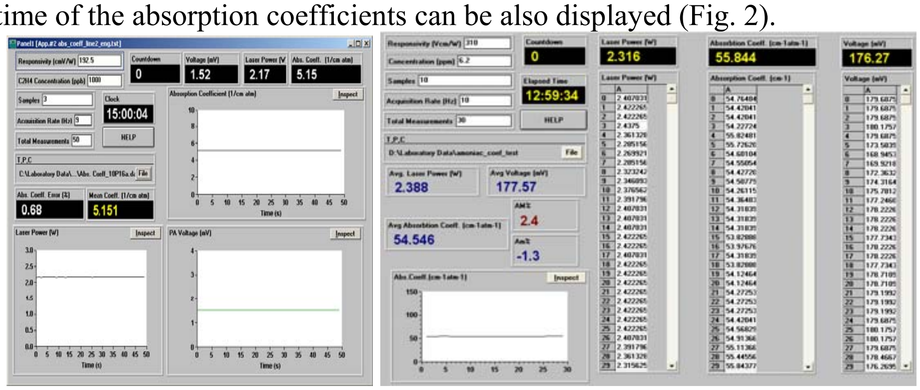

Fig. 2. Software user interface to measure the absorption coefficients of ethylene and ammonia

The experimental results are a spectral representation unique to the ammonia and ethylene molecules. A strong absorption for ammonia is obtained at the 9R(30) laser line with the absorption coefficient α = 57.12 $cm^{-1}atm^{-1}$ (error ± 1.2%). Ammonia has weaker absorption coefficients at other CO_2 laser transitions; some other significant values for the absorption coefficient were found for 9R and 9P bands: 9R(16) - α = 11.29 $cm^{-1}atm^{-1}$ (error ± 1.4%), 9P(20) - α = 2.10 $cm^{-1}atm^{-1}$, (error ± 2%) and 9P(34) - α = 3.99 $cm^{-1}atm^{-1}$ (error ± 0.62%). In the 10R band the measurements gave: 10R(14) - α = 6.17 $cm^{-1}atm^{-1}$ (error ± 1.5%), 10R(8) - α = 20.08 $cm^{-1}atm^{-1}$ (error ± 1.3%), 10R(6) - α = 26.2 $cm^{-1}atm^{-1}$ (error ± 1.7%), and for the 10P band: 10P(32) - α = 12.45 $cm^{-1}atm^{-1}$, (error ± 2.9%), 10P(34) - α = 14.07 $cm^{-1}atm^{-1}$ (error ± 0.48%) and 10P(36) - α = 7.39 $cm^{-1}atm^{-1}$ (error ± 0.83%).

For ethylene a strong absorption is obtained at the 10P(14) laser line (absorption coefficient of 30.4 $cm^{-1}atm^{-1}$ at 949.479 cm^{-1} with error ± 2.1%). C_2H_4 has weaker absorption coefficients at the 10P(12) and 10P(16) CO_2 laser transitions (4.36 $cm^{-1}atm^{-1}$ with error ± 1.5% and 5.10 $cm^{-1}atm^{-1}$ with error ± 1.9%). Other fundamental values for the absorbption coefficient of ethylene were found at 10R band: 10R(24) - α = 4.51 $cm^{-1}atm^{-1}$, (error ± 2.6 %), 10R(22) - α = 2.67 $cm^{-1}atm^{-1}$, (error ± 1.5%), 10R(28) - α = 2.10 $cm^{-1}atm^{-1}$, (error ± 2 %) and 10R(6) - α = 1.86 $cm^{-1}atm^{-1}$, (error ± 4.1 %).

Compared to the other measurements reported previously in the literature [16, 17], our values indicate a general good agreement.

The present work was carried out using a methodology which gave the best possible control over the ethylene and ammonia partial pressure and background signals. The background levels and calibration of the PA cell were checked before and after every experimental run. The present study is considered reliable, particularly in view of the careful attention that was paid to controlling the gas composition and noise signals.

LPAS have been demonstrated to play an important role in the future of exhaled breath analysis [1-6, 8, 10-15]. The key attributes of that technique are sensitivity, selectivity, fast response, ease-of-use, size and low operating cost which make laser photoacoustic spectroscopy a competitive technology for a number of exhaled bio-markers including ethylene and ammonia.

5. Conclusions and future work

The present work was carried out by applying a methodology which assured the better conditions to measure ethylene and ammonia absorption coefficients, due to its relative simplicity, ruggedness and overall sensitivity.

We have presented accurate measurement of absorption coefficients for ethylene and ammonia at 9 µm and 10 µm bands of the CO_2 laser by means of the photoaocustic technique. The results obtained for the absorption coefficients of ethylene and ammonia in comparison with the data reported in literature have demonstrated the suitability of our experimental PA system for trace gas detection. After these measurements, the photoacoustic technique will allow us to examin the presence of trace amounts of ethylene and ammonia in the exhaled air from volunteers with damage caused by free radicals and volunteers with renal failure, by using the CO_2 laser lines where the ethylene [10P(14)] and ammonia [9R(30)] absorption coefficients have the maximum values.

Noninvasive medical diagnosis using breath analysis method is a topic of great interest because of its ability to distinguish more than 200 compounds in human breath. Many of these compounds, if measured accurately at very low concentrations levels, typically in the range of few ppbV, can be used to identify particular medical conditions.

With the relevant characteristics of high sensitivity and specificity, laser photoacoustic spectroscopy holds a great potential for medical diagnostics.

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