

## BREATH AMMONIA DETECTION IN PATIENTS WITH SCHIZOPHRENIA USING LASER PHOTOACOUSTIC SPECTROSCOPY

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*Abstract.* Over the years schizophrenia become a challenging disorder in the understanding of its causes, course and treatment. The processes for the causes of neuropsychiatric disturbances in schizophrenia are still not completely known. Investigating metabolic changes in schizophrenia is important to capture valuable insights about mechanisms, progression, and diagnostic biomarkers. Priority of investigation in any patient with psychiatric presentation is recommended to be ammonia. We used CO<sub>2</sub> laser photoacoustic spectroscopy method to analyze breath ammonia levels in 15 patients with schizophrenia that not withdrawn from their usual medication and 13 control subjects without schizophrenia. Breath ammonia concentrations were evaluated and compared between study group and the group with schizophrenia. The interesting observation is that the breath ammonia levels of patients with schizophrenia are clearly increased compared with healthy subjects. The measurements of breath ammonia presented here demonstrate the quality of breath analyses that can be achieved using CO<sub>2</sub> laser photoacoustic spectroscopy method. Breath ammonia analysis might offer a new approach to the detection of schizophrenia and better understanding of the metabolic basis of this disorder.

*Key words:* laser photoacoustic spectroscopy, breath ammonia analysis, schizophrenia.

### 1. INTRODUCTION

Schizophrenia is a complex brain disorder manifested through hallucinations, delusions, paranoia and thought dysfunction [1]. The precise cause of schizophrenia remains poorly understood. In patients with schizophrenia have been reported changes in key brain functions, such as perception, emotions, and behavior, and that may indicate that the brain is the biological site of schizophrenia [2]. In many people schizophrenia is manifested by the lose touch with reality and do not know which thoughts and experiences are true and real and which are not. It usually begins in late adolescence or early adulthood and the cause and course of this disorder is unique for each person [3]. Some people with schizophrenia

experience only a few brief episodes. For others, it is a chronic condition. Although there is no cure, schizophrenia is a treatable disorder. There are effective treatments for schizophrenia and people affected by it can lead a productive life and be integrated in society [5]. Treatment includes special medication for schizophrenia like antipsychotics and ensuring that the patient has adequate social support. Medication works by reducing the psychotic symptoms. Understanding any relation between schizophrenia, antipsychotic treatment and changes in metabolic variables in people with schizophrenia is important in the incidence of these events.

Dysfunctions of ammonia metabolism are associated with severe neurological impairment [6]. Ammonia is an important part in the human body being a major byproduct of systematic and cerebral nitrogen metabolism [7]. It is a source of nitrogen supply and helps in the synthesis of amino acids that are considered to be the building blocks of protein in the body. As a byproduct of protein metabolism large portion of ammonia is generated by the gastrointestinal tract. Bacteria present in the gastrointestinal tract digest protein into polypeptides, amino acids and ammonia. Ammonia is converted into urea by the liver, and finally excreted by the body in the form of urine through kidneys. In human body urea is produced in the liver by a complex cyclical series of reactions known as urea cycle. Ammonia is generated in the liver from glutamate and in the kidney by deamidation of glutamine. The glutamine derived from brain, muscle and other tissues act as energy sources and release ammonia for urea synthesis. In too high concentrations it becomes toxic to the human body [8, 9]. Thus, for a healthy person, blood ammonia is tightly regulated *via* the urea cycle, with excess ammonia being converted to urea and excreted through urine.

Ammonia is toxic to the central nervous system when reacts with  $\alpha$ -ketoglutarate to form glutamate. The metabolite  $\alpha$ -ketoglutarate impairs the function of the citric acid cycle in neurons, depriving them energy production. Because glutamate is a potent neurotransmitter, any significant increase in the concentration of glutamate could have abnormal effects in synaptic transmission [6, 10, 11]. The neurotransmitter glutamate system presents a strong involvement in the pathogenesis of schizophrenia [12]. The brain shows changes in the levels of glutamate and in the function and expression of its transporters and receptors [13].

Abnormal body concentrations of ammonia can now be studied by breath analysis. This analysis offers a unique and non-invasive method for compounds that circulate in the blood, rapidly diffuse across the pulmonary alveolar membrane, and appear in the exhaled breath to be detected [14, 15].

The level of ammonia in human breath has been measured as being between 50 and 2000 parts-per-billion (ppbv) (where 1 ppbv of ammonia in human breath is approximately  $0.67 \mu\text{g m}^{-3}$ ) and is dependent on a range of factors including the health status of the patient, the route of sampling (nasal or oral), contribution from oral bacteria, as well as diet, pharmaceutical use and levels of metabolic activity [10, 16].

In the view of above mentioned, the detection and quantification of trace gases is of great interest in human breath analysis for medical diagnosis. These applications require trace gas sensors characterized by high sensitivity and selectivity. Today, a widely recognized technique to measure trace gases at parts-per million or parts-per-billion level is the laser photoacoustic spectroscopy (LPAS) [17]. The favorable properties of LPAS are essentially determined by the characteristics of the laser. The kind and number of detectable substances is related to the spectral overlapping of the laser emission with the absorption bands of the trace gas molecules. The CO<sub>2</sub> laser is of special interest, as it ensures high output power in a wavelength region (9–11 μm) where more than 250 molecular gases/vapors exhibit strong absorption bands. Nevertheless, it is an ideal source to push the sensitivity of photoacoustic (PA) gas detection into the concentration range of ppbV or even lower.

We report here a study where CO<sub>2</sub> laser photoacoustic spectroscopy method is used to analyze ammonia exhalations from individuals in a healthy physiological state and ammonia exhalations from a pathological state (from schizophrenic patients).

## 2. METHOD

Breath samples were investigated at the Optics and Lasers in Life Sciences, Environment and Manufacturing Laboratory from National Institute for Laser, Plasma and Radiation Physics.

A group of 13 subjects without any history of psychiatric disorder or other diseases and non-smokers, were selected as a control group (10 males and 3 females, age range from 25 to 33) and included in the study.

Schizophrenic subjects were recruited from C.S.C.C.H.S. Center, Calarasi, Romania by invitation having given written informed consent to participate in this research under a protocol approved by the institutional review boards of both institutions. All subjects had a clinical diagnosis of schizophrenia ( $n = 15$ ) as evaluated in the Complex Evaluation Service of the C.S.C.C.H.S. Center. Patients were not withdrawn from their usual medication from the purpose of this study. Schizophrenic patients were under medication therapies with an antipsychotic and anxiolytic treatment: Levomepromazine (Methotrimeprazine). Breath collections were performed between 08.00 h–12.00 h. The patients were 6 males and 9 females, age range from 20 to 23 years, non-smokers and with no known acute or chronic medical illness.

Breath samples were collected in special sample bags. To collect a clean breath air sample, we used aluminized multi-patient collection bags from QuinTron (750 mL aluminum-coated bags), designed to collect multiple samples from patients and hold a sample for maximum 6 hours. The sample bags are composed of a disposable mouthpiece, a tee-mouthpiece assembly (it includes a plastic tee

and a removable one-way flutter valve), and a discard bag. After an approximately normal inspiration, the subject places the mouthpiece in his mouth, forming a tight seal around it with the lips. A normal expiration is then made through the mouth, in order to empty the lungs of as much air as required to provide the breath sample. For the mouth exhaled breath sample, the first portion of the expired air is directed into the discard bag (with the role in collection of the “dead-space” air: the first portion of an expired breath), while the alveolar air is diverted to the collection bag. When an adequate sample is collected, the subject stops exhaling and removes the mouthpiece.

The breath samples are then analyzed using CO<sub>2</sub> laser photoacoustic spectroscopy. This method is very sensitive and selective and well known in the field of trace gas detection (ppbv level). The system mainly consists of a CO<sub>2</sub> laser and a PA cell where the gas is detected (Fig. 1). The requirement for gases to be detected with this sensitive laser instrument is that they should possess high absorption strength and a characteristic absorption pattern in the wavelength range of the CO<sub>2</sub> laser. The system has been described before and only a short presentation is given here [17, 18, 19].

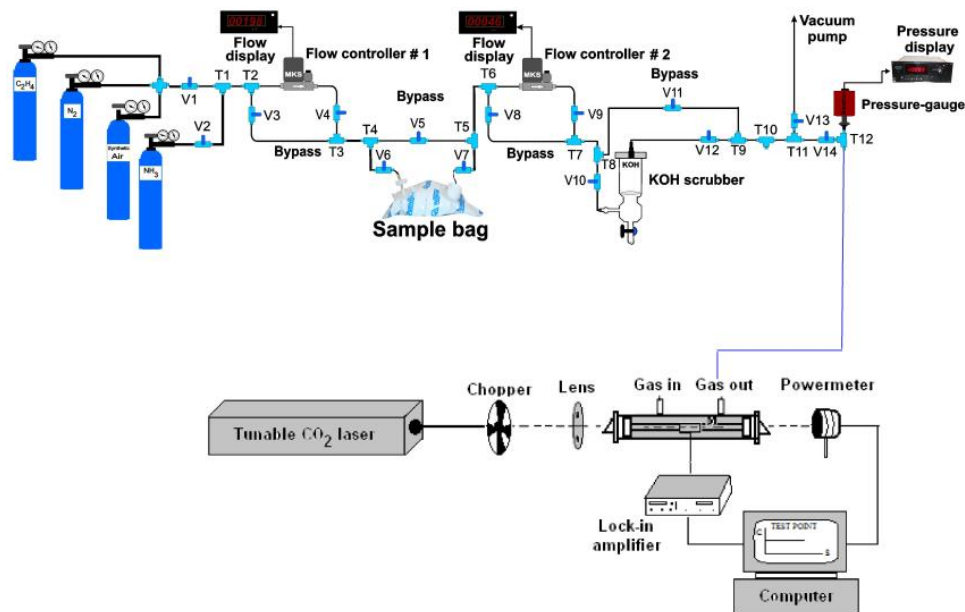


Fig. 1 – Schematic of the CO<sub>2</sub> laser-photoacoustic spectroscopy instrument.

The continuous, tunable CO<sub>2</sub>-laser beam is modulated by a mechanical chopper (DigiRad C-980 or C-995), focused by a ZnSe lens ( $f = 400$  mm), and introduced in the PA cell where is locally absorbed by IR active molecules. An

optimum acoustically resonant PA cell is used with a responsivity of 320 cmV/W. Inside the cell, the pressure waves, generated when the laser radiation is absorbed by the trace gas molecules, are measured with sensitive miniature microphones (Knowles electret – EK-23024). After the PA cell, the power of the laser beam is measured by a laser powermeter (Rk-5700 from Laser Probe Inc). Its digital output is introduced in the data acquisition interface module together with the output from a lock-in amplifier (Stanford Research Systems model SR 830), which filters and amplifies the signal from microphones. The gas concentration is proportional to the ratio between signal and laser power. All experimental data are processed and stored by a computer.

The transfer of the breath sample to the PA cell is performed using a vacuum/gas handling system that ensures the PA cell purity. The vacuum system provides evacuation of the entire gas handling system, including the PA cell, either totally or in different sections. The gas or gas mixture is introduced in the system at controlled flow rates within a broad range (10–1000 sccm-standard cubic centimeters per minute). The process permits rinsing the PA cell or the gas handling system with pure nitrogen, calibrating the PA spectrometer with a certified gas mixture, or the admission of the sample and carrier gas on different paths. A number of pressure gauges allow gas pressure reading inside the PA cell, as well as in different segments of the system.

The sample bags were connected to the system and the stored breath samples are transferred from bags into the measuring cell by the gas flow controller #2 (MKS 1179A) at a controlled flow rate 600 sccm. Before entering the PA cell, the gas mixture passes through a potassium hydroxide (KOH) scrubber, which retains most of the interfering carbon dioxide and water vapors. The KOH granules were typically Merck KOH pellets for analysis, ovals with approximate dimensions of  $10 \times 7 \times 2$  mm<sup>3</sup>. In a previous study we determined experimentally that in the process of CO<sub>2</sub> removal from the breath air samples, a quantity of minimum 120 cm<sup>3</sup> KOH pellets should be used for a sampling bag of 750 mL and a new fill of KOH scrubber must be introduced after each measurement [20].

For cell response calibration, a certified mixture of 10 ppmV ammonia in pure nitrogen (Linde Gas) was used. For calibration we examined this reference mixture at a total pressure of approximately 1013 mbar and a temperature of 23°C. The absorption coefficients of ammonia at different CO<sub>2</sub> laser wavelengths were precisely measured and published previously [21]. A strong absorption was obtained at the 9R(30) laser line with the absorption coefficient 57 cm<sup>-1</sup>atm<sup>-1</sup>.

The laser-based photoacoustic detector is able to distinguish between different gases by making use of their wavelength-dependent fingerprint absorption characteristics. Over the years CO<sub>2</sub> laser photoacoustic spectroscopy has been successfully used considering the wide gamut of its application areas [22, 23, 24, 25, 26].

### 3. RESULTS AND DISCUSSIONS

Breath samples were collected from 15 patients diagnosed as suffering from schizophrenia, and from 13 healthy controls. Samples were obtained without any adverse effects and were assayed for breath ammonia.

Subjects were asked to exhale fully into the collection bags. To analyze breath samples from the bags, firstly we make sure that the PA cell is clean by flushing the system with pure nitrogen at atmospheric pressure for 30 minutes. After that, we evacuate the extra gas by the vacuum handling system and we release the samples into the system at a controlled flow rate of 600 sccm. The breath samples pass through the KOH trap and then are introduced in the PA cell.

For determining the concentration of ammonia, the CO<sub>2</sub> laser was kept tuned at 9R(30) line where ammonia exhibits a strong absorption of 57 cm<sup>-1</sup>atm<sup>-1</sup>.

In this study, ammonia concentrations were measured from breath samples of schizophrenia patients and the results were compared with healthy controls using CO<sub>2</sub> laser photoacoustic spectroscopy.

Figure 2 shows the average concentrations of breath ammonia for patients with schizophrenia compared to the ammonia concentrations of a healthy group control.

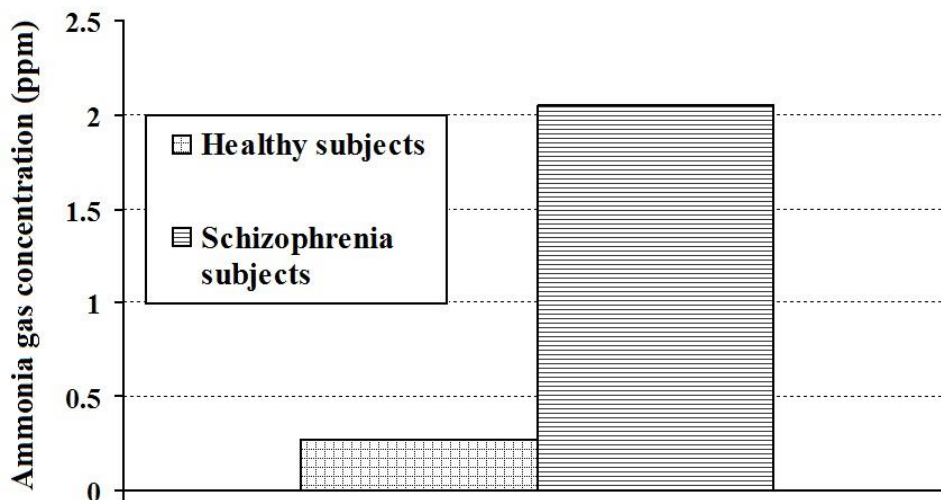


Fig. 2 – Breath average level of ammonia for 13 healthy subjects and for 15 schizophrenic subjects.

It should be pointed out that the mean ammonia level of schizophrenic patients is higher (2.02 ppm) compared to the mean ammonia level of healthy subjects (0.29 ppm). Other possible confounding variables, such as age or sex showed no statistically significant differences between the two groups.

The mechanisms by which ammonia contributes to the manifestations of schizophrenic people still remain poorly defined. There are several causes that may lead to the increased ammonia in the patients with schizophrenia.

First of all, high concentrations of ammonia may indicate that the body is not effectively metabolizing and eliminating ammonia. In healthy humans ammonia is a regular metabolite in all tissues and organs, including the brain. Generally the liver produces enzymes that change ammonia into a form called urea, which the body can remove in the urine. Ammonia abnormalities appear if this process is disturbed and ammonia levels begin to rise. The major ammonia detoxifying mechanism involves the conversion of ammonia and glutamate to glutamine *via* the enzyme glutamine synthetase. The synthesis of glutamine provides protection also against ammonia toxicity in the brain and maintains homeostasis of the excitatory neurotransmitter glutamate [27]. The neurotransmitter glutamate and the reduced function of the N-methyl-D-aspartate glutamate receptor has been shown to have an important role in schizophrenia [28].

On the other hand, proteins are also potential targets of reactive oxygen species (ROS), and their structure and function can be affected by modification [29, 30]. Oxidative attack on proteins results in site-specific amino acid modifications, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis. Thus it destroys the structure, functions of essential proteins and enzymes and whole cell metabolism is blocked. Ammonia originates in the catabolism of amino acids that are primarily produced by the degradation of proteins. We can say that ammonia also is a consequence of existent oxidative stress in people with schizophrenia. Oxidative stress means that, at the cellular level, there is an excess of oxidants that overcome the body's antioxidant capabilities to deal with them.

All of the patients with schizophrenia were under specific treatment. The relation between level of ammonia in the exhaled breath and schizophrenia could be explained by the treatments that can lead to a deficiency of amino acids, required to detoxify toxins in the liver. Along with their useful effects, most medicines can cause unwanted side-effects although not everyone experiences them. Some medicines at schizophrenic patients, seem that, mildly reduced kidney function with an insufficient detoxification pathway and therefore a very small accumulation of ammonia in the breath [31, 32].

It is presently unclear the main origin of the increased ammonia concentrations in people with schizophrenia. Nevertheless, such an increase in breath ammonia concentration is not desired having been implicated in a variety of liver and kidney disorders and even in brain abnormalities. Further research is needed to examine the cause of the breath changes observed.

#### 4. CONCLUSIONS

A new understanding in the biological basis of schizophrenia could lead to new treatments for people with this disorder. We conducted a quantitative research on breath ammonia in the people with schizophrenia. We hope that our findings already lead to new information about schizophrenia, and new technologies to investigate it.

In the present work the CO<sub>2</sub> laser-photoacoustic spectroscopy technique was applying to measure ammonia from exhaled breath of schizophrenia patients vs. healthy subjects, due to its high sensitivity. The purpose of this study was to determine if ammonia biomarker from the breath of schizophrenia patients have different levels compared with a healthy control group.

The higher level of ammonia in the exhaled breath of schizophrenic patients may indicate that the body is not effectively eliminating ammonia as a byproduct of the metabolism of protein, as a consequence of oxidative stress in body, or as deficiency of amino acids after medication, required to detoxify toxins in the liver. Ammonia also has a influence on many neurotransmitters that are out of balance in neurological disorders. Considering all these aspects the role of ammonia in the brain changes of patients with schizophrenia is not yet completely identified.

The most important route for ammonia is the formation of urea in the liver, then the urea is transported to the blood from the liver to the kidneys. As small molecules, ammonia can penetrate the blood-lung barrier, and appear in exhaled breath of schizophrenic patients.

We conclude that CO<sub>2</sub> laser photoacoustic spectroscopy analyses of breath ammonia in alveolar air appeared to distinguish patients with schizophrenia from non-schizophrenic controls.

Our data shows an increase in ammonia concentration in people with schizophrenia and suggests new markers that may contribute to a better understanding of this disease. Both the feasibility and the importance of monitoring exhaled ammonia from different subjects have been shown.

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