# SPECTROSCOPIC STUDY OF AMMONIA AT SUBJECTS WITH KIDNEY FAILURE: A CASE CONTROL STUDY

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The present study was aimed to assess the ammonia biomarker status in dialyzed patients before and after treatment. The investigations regarded patients who had visited the Nephrology Department and were diagnosed of kidney failure. The ammonia traces were measured before and after dialysis (in comparison to the healthy controls), with the help of photoacoustic spectroscopy technique. Clinical usual tests for urea were performed and compared to the ammonia biomarker levels from breath. We have found out that the composition of exhaled breath in patients with kidney failure contains ammonia and gives important information for determining efficiency of dialysis treatment.
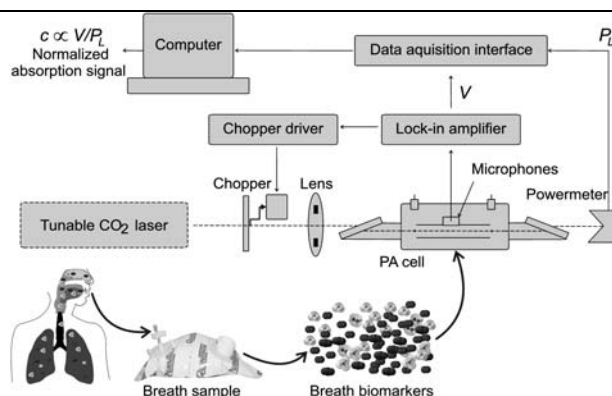
INTRODUCTION

Healthy kidneys clean the blood by removing excess fluid, minerals, and wastes. They also produce hormones that keep the bones strong and the blood healthy. When the kidneys fail, harmful wastes build up in the body, the blood pressure may rise, the body may retain excess fluid and a decrease in the number of red blood cells can occur. When this happens, treatment is needed to replace the function of the failed kidneys.[1]

Haemodialysis is a method for extracorporeal removing of the waste products such as creatinine and urea, as well as water from the blood when the kidneys are in kidney failure. Haemodialysis is one of three renal replacement therapies (the other two being renal transplant and peritoneal dialysis).

The concept that blood, urine, and other body fluids and tissues can be collected and analyzed to yield information for diagnosis of disease status or to monitor disease progression and/or therapy is the foundation of modern medicine. The use of breath as a collectable sample has not received yet comparable clinical use.

With each breath we exhale, thousands of molecules are expelled in our breath and each one of us has a breathprint that can provide a lot of parameters able to characterize our state of health. While this may be news to some, it should not be to people in medicine.

Human bodies use ammonia in a number of ways, including for the maintenance of the normal pH balance necessary to sustain life. Ammonia is processed in the liver, kidneys and skeletal muscles. Typically, ammonia and ammonium ions

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are converted into urea in the liver through the urea cycle. [2, 3]

The urea cycle comprises five enzymes: carbamoylphosphate synthetase I, ornithine *trans*carbamylase, argininosuccinate synthetase, argininosuccinatelyase and arginase. For efficient functioning of the pathway in vivo, however, further proteins are required, such as liver glutaminase, mitochondrial carbonic anhydrase V, N-acetylglutamate synthetase, the mitochondrial ornithine and citrulline antiporters and citrin, the mitochondrial aspartate/glutamate antiporter. [2-5]

The liver is quantitatively the major organ involved in urea synthesis and it is doubtful whether other cell types, such as enterocytes, can produce significant amounts of urea. However, at least some urea cycle enzymes are found in extrahepatic tissues, where they are involved in providing arginine for nitric oxide synthesis. The initial reaction of the urea cycle is the formation of carbamoyl phosphate from ammonia and bicarbonate, a reaction catalysed by carbamoylphosphate synthetase I, which requires N-acetylglutamate as an allosteric cofactor. Condensation of carbamoyl phosphate with ornithine yields citrulline (by ornithine *trans*carbamylase); this in turn condenses with aspartate to give argininosuccinate (by argininosuccinate synthetase), a reaction that requires the cleavage of two further high-energy phosphate bonds. Argininosuccinate is hydrolysed to fumarate and arginine (by argininosuccinase). Arginine is cleaved by arginase to give urea and ornithine. Ornithine *trans*carbamylase, like carbamoylphosphate synthetase I, is also a major mitochondrial protein; the remaining enzymes are in the cytoplasm of hepatocytes. This necessitates the entry of ornithine into mitochondria and the exit of citrulline, which is brought about by the ornithine/citrulline transporters the mitochondrial ornithine and citrulline antiporters. This series of reactions, returning to ornithine, is known as the urea cycle. It takes part not only in the removal of potentially toxic ammonia, but also in the irreversible removal of bicarbonate. Although the arginase reaction is the major fate of arginine in liver, arginine can also be used for nitric oxide synthesis or undergo decarboxylation to form agmatine. Agmatinase converts arginine to putrescine and urea and the urea is then transported through the blood-stream to be excreted into urine by the kidneys. [4,5] The reversibility of the process requires an equilibrium concentration of ammonia related to the blood urea nitrogen loading of the blood. As small molecules, ammonia and ammonium ions can penetrate the blood-lung barrier, and appear in exhaled breath. In the case of kidney dysfunction, urea is unable to be excreted, causing an excessive build up of ammonia in the blood. People with kidney failure have a marked odor of ammonia ("fishy") on their breath, which can be an indicator of this disease. [2-5]

Laser photoacoustic spectroscopy – LPAS is a sensitive technique for detection and monitoring of trace gases at very low concentrations. The CO_2 laser is of special interest, as it ensures high output power in a wavelength region where more than 200 molecular gases of environmental concern for atmospheric, industrial, medical, military, and scientific spheres exhibit strong absorption bands. This laser can be only stepwise tuned when operated in cw, and is an ideal source to push the sensitivity of photoacoustic gas detection into the concentration range of part-per-billion-by volume (ppbV) or even lower. Instruments based on LPAS method have nearly attained the theoretical noise equivalent absorption detectivity of $10^{-10} cm^{-1}$ in controlled laboratory conditions. This high sensitivity cannot be achieved in real detection conditions due to the coherent photoacoustical background signal and interfering background absorption of normal atmospheric constituents.

RESULTS AND DISCUSSION

Ammonia biomarker was measured using the laser photoacoustic spectroscopy (LPAS) system and subjects were recruited from patients receiving dialysis treatment at the renal dialysis clinics at the IHS Fundeni, Bucharest.

Experimental determinations in order to detect traces of ammonia and also to measure the urea level were performed for a healthy volunteer and for 13 patients with kidney failure.

The schematic diagram of the LPAS system is illustrated in Fig. 1, and consists of following components.

1. Line-tunable CO_2 laser

We used a homebuilt, line-tunable and frequency stabilized CO_2 laser. This laser has a maximum power of 6.5 W on the 10P(20) line and a tunability on 62 vibrational-rotational lines in all four spectral bands. [6-8] For ammonia, the measurements were made on the 9R(30) line, where the absorption coefficient is maximum.
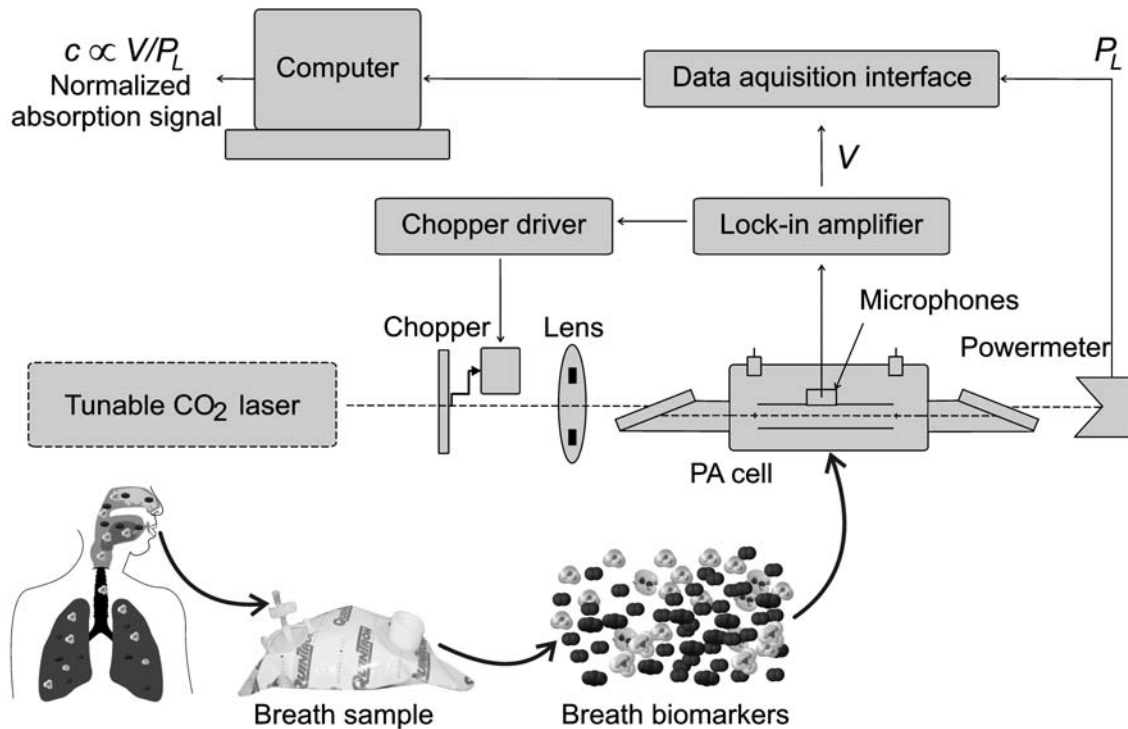
Fig. 1 – LPAS system.

2. Mechanical chopper

Our light beam was modulated by a high quality, low vibration noise and variable speed (4-4000 Hz) mechanical chopper model DigiRad C-980 and C-995 (30 aperture blade), operated at the appropriate resonant frequency of the cell (564 Hz). The rotation of the chopper produces a radiant signal that fluctuates periodically between zero and some maximum intensity. In general it is desirable to chop the signal as close to its source as possible because only the noise that arises after chopping is removed by the process. The laser beam diameter is typically 6.2 mm at the point of insertion of the chopper blade and is nearly equal to the width of the chopper aperture. An approximately square waveform is produced with a modulation depth of 100% and a duty cycle of 50%, so that the average power measured by the powermeter at the exit of the PA cell is half the cw power value. [6-11]

3. Lock-in amplifier

Lock-in amplifiers are used to detect and measure very small AC signals. They can recover signals in the presence of an overwhelming noisy background. Accurate measurements may be made even when the small signal is obscured by noise sources many thousands of times larger. Lock-in amplifiers use a technique known as phase-sensitive detection to single out the component of the signal at a specific reference frequency and phase. Noise signals, at the specific frequency – 564 Hz, in our case, are rejected and do not affect the measurement.

We used a dual-phase, digital lock-in amplifier Stanford Research Systems model SR 830 with the following characteristics: full scale sensitivity, 2 nV – 1 V; input noise, 6 nV (rms)/ $\sqrt{Hz}$ at 1 kHz; dynamic reserve, greater than 100 dB; frequency range, 1 mHz –102 kHz; time constants, 10 μs – 30 s, or up to 30000 s. [11]

4. Photoacoustic cell

PA cell is known as the "heart" of photoacoustic spectroscopy and in our case has a total volume of approximately 1.0 dm^3. It is made of stainless steel and Teflon to reduce the outgassing problems and consists of an acoustic resonator, windows, gas inlets and outlets, and microphones. It also contains an acoustic filter to suppress the window noise. The ZnSe windows are positioned at Brewster angle to their mounts. The resonant conditions are obtained as longitudinal standing waves in an open tube (excited in its first

longitudinal mode). To achieve a larger signal, we chose a long absorption path length (300 mm) and an inner diameter of the pipe of $2r = 7$ mm. The fundamental longitudinal wave, therefore, has a nominal wavelength $\lambda_s = 2L = 600$ mm (and a resonance frequency of 564 Hz). The two buffer volumes placed near the Brewster windows have a length $L_{buf} = 75$ mm and a diameter $2r_{buf} = 57$ mm. 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 to prevent bypassing of gas in the flow system; the other 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. The acoustic waves generated in the PA cell are detected by four Knowles electret EK-3033 (or EK-23024) miniature microphones in series (sensitivity 20 mV/Pa each) mounted flush with the wall. They are situated at the loops of the standing wave pattern, at an angle of 90° to one another. The electrical output from these microphones is summed and the signal is selectively amplified by the lock-in amplifier (tuned to the chopper frequency). [10-14]

5. Data analysis interface

We utilized a modular software architecture (Keithley TestPoint software) aimed at controlling the experiments, collecting data, and preprocessing information. It helps to automate the process of collecting and processing the experimental results. The software transfers powermeter readings, normalizes data, and automatically stores files. It allows the user to record parameters such as the PA cell responsivity (a constant used to normalize raw data), gas absorption coefficient, number of averaged samples at every measurement point, sample acquisition rate, and the total number of measurement points. This software interfaces the lock-in amplifier, the chopper, the laser powermeter and the gas flowmeter. It allows the user to set or read input data and instantaneous values for the PA voltage, average laser power after chopper, and trace gas concentration. [11-15]

6. Spectroscopic study of ammonia

To assess the level of ammonia molecules in dialysed patients, we analyzed the exhaled breath by using the LPAS technique, because is emerging as a standard method for measuring extremely low absorptions independent of the path length and offer a degree of parameter control that cannot be attained by other methods. In this method, the resulting signal, processed by the phase sensitive detector, is directly proportional to the absorption coefficient and the laser power. The sensitivity of the technique is such that absorptions of $<10^{-7}$ cm^{-1} can be measured over path lengths of a few tens of centimeters. The small volume of the chamber makes it possible to accurately control the gas parameters, and the system can be operated with static fills or in continuous gas flow mode. [11-14]

The experimental values of ammonia absorption coefficients, for all laser wavelengths, is called the optoacoustic absorption spectrum or signature and is unique for the laser frequency and ammonia molecules.
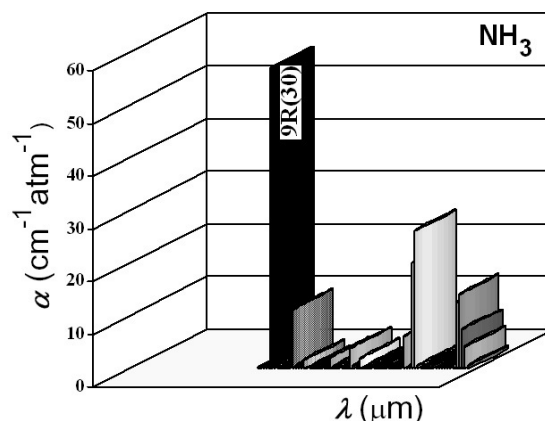

Fig. 2 – Absorption coefficients of ammonia at laser wavelengths by photoacoustic spectroscopy technique using a homebuilt CO_2 laser with a maximum power of 6.5 W and a tunability on 62 lines.

A strong absorption is obtained at the 9R(30) laser line with the absorption coefficient $\alpha = 57.12$ cm^{-1}atm^{-1}. As it can be seen from Fig. 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) - $\alpha = 11.29$ cm^{-1}atm^{-1}, 9P(20) - $\alpha = 2.10$ cm^{-1}atm^{-1}, and 9P(34) - $\alpha = 3.99$ cm^{-1}atm^{-1}. In the 10R band the measurements gave: 10R(14) - $\alpha = 6.17$ cm^{-1}atm^{-1}, 10R(8) - $\alpha = 20.08$ cm^{-1}atm^{-1}, 10R(6) - $\alpha = 26.2$ cm^{-1}atm^{-1}, and for the 10P band: 10P(32) - $\alpha = 12.45$ cm^{-1}atm^{-1}, 10P(34) - $\alpha = 14.07$ cm^{-1}atm^{-1} and 10P(36) - $\alpha = 7.39$ cm^{-1}atm^{-1}.

The high sensitivity of the LPAS method will allow us to investigate the presence of trace amounts of ammonia in the exhaled air that are linked to kidney malfunction, by using the CO_2

laser line where the ammonia absorption coefficient [9R(30)] has the highest value. The present work was carried out using a methodology which assured the best conditions to measure ammonia concentrations, due to its relative simplicity, ruggedness and overall sensitivity.

7. Breath ammonia concentration vs. blood urea concentration at patients with kidney failure

Analysis of pre-dialysis urea level and post-dialysis urea level were made at MedCenter, Bucharest and the results are presented in Fig. 3.

The exhaled air samples were collected before, and after the dialysis procedure stopped. We have analyzed ammonia exhaled from patients receiving haemodialysis for treatment of kidney failure.

All the data and results published were the subject to the patient's consent.

To get a breath air sample, we used aluminized multi-patient collection bags (750 mL aluminum-coated bags), composed of a disposable mouthpiece and a tee-mouthpiece assembly (it includes a plastic tee and a removable one-way flutter valve).

After an approximately normal inspiration (avoiding filling the lungs at maximum), the subject places the mouthpiece in his/her 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 alveolar sample. The first portion of the expired air goes out, after which the valve is opened the tee-piece, the remaining expired air being redirected into the collection bag. When a suitable sample is collected, the patient stops exhaling and removes the mouthpiece and then the sample gas is transferred into the photoacoustic cell (PA cell) and can be analyzed immediately or later.
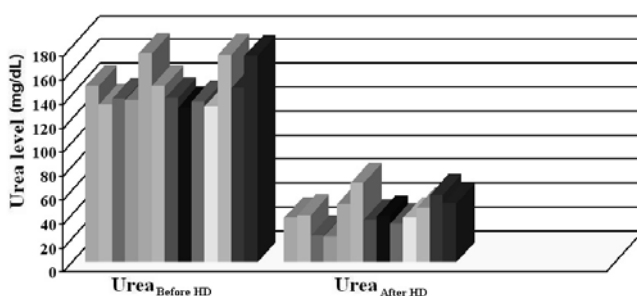
Experimental measurements of breath ammonia concentrations for the patients with renal failure and for the healthy subject were performed and the results are presented in Fig. 4. The control value for breath ammonia was 0.25 ppm (healthy subject).

In Fig. 4, we observed a reduction of ammonia concentration in exhaled breath at patients under haemodialysis treatment, which means that ammonia detection in human breath using LPAS system can be used for determining the exact time necessary for the desired state of haemodialysis for a patient with kidney failure at every session and, in the same time, could serve as a broad noninvasive screen for incipient kidney disease.

We can see also a remarkable positive correlation between urea data from Fig. 3 and the breath ammonia concentration from Fig. 4.

CONCLUSIONS

Analysis of ammonia can be used to detect disease, monitor disease progression, or monitor therapy in case of kidney failure. The ammonia test is noninvasive, easily repeated, and does not have the discomfort or embarrassment associate with blood and urine tests.

In the present study, we have presented absorption measurement of ammonia and we found out that ammonia can be used for selecting the optimum hemodialysis duration for a desired state (our data show that haemodialysed patients present a reduction of the ammonia concentration, correlated to the level of urea results).

These measurements were possible because of the high sensitivity of our CO_2 LPAS system, sensitivity that was obtained through successively improvements in optics, laser source and electronics (faster response, low noise equipment).
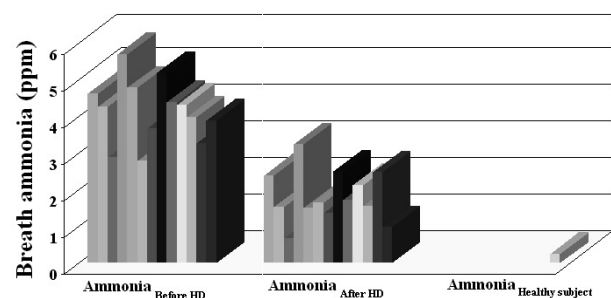

Fig. 3 – Urea data measured for 13 patients with kidney failure made at MedCenter with VITROS 51 apparatus.

Fig. 4 – Ammonia concentration for 13 patients with kidney failure before and after the treatment using LPAS.

REFERENCES

1. P. Stephen and B. James, *NEJM*, **1998**, 338, 1428.
2. L.R. Narasimhan, W. Goodman, C. Kumar N. Patel, *PNAS*, **2001**, 98, 4617.
3. M. R. McCurdy, Y. Bakhirkin, G. Wysocki, R. Lewicki, F. K. Tittel, *J. of Breath Res*., **2007**, 1, 12.
4. H. Dieter , Ammonia, urea production and pH regulation, **2011**, 2.3.7, 181.
5. K. Brigden, and R. Stringer, Greenpeace Research Laboratories, Department of Biological Sciences, University of Exeter, Exeter, UK., *Stop Poll.*, **2000**, 1.
6. D. C. Dumitras, D. C. Dutu, C. Matei, A. Magureanu, M. Petrus, C. Popa, *JOAM*, **2007**, 9, 3655.
7. D.C. Dumitras, D.C. Dutu, C. Matei, A.M. Magureanu, M. Petrus, C. Popa, M. Patachia, *Rom.Rep. Phys.*, **2008**, 60, 593.
8. D.C. Dumitras, D.C. Dutu, C. Matei, R. Cernat, S. Banita, M. Patachia, A. M. Bratu, M. Petrus, C. Popa, *Las. Phys*., **2011**, 21, 796.
9. D.C. Dumitras, S. Banita, A.M. Bratu, R. Cernat, D.C.A. Dutu, C. Matei, M. Patachia, M. Petrus, C. Popa, *Infrared Physics & Technology Journal*, **2010**, 53, 308.
10. D.C. Dumitras, D.C. Dutu, C. Matei, A. M. Magureanu, M. Petrus, and C. Popa, *U. P. B. Sci. Bull*., *Series A 69*, **2007**, 45.
11. M. Bratu, C. Popa, C. Matei, S. Banita, D. C. A. Dutu, and D. C. Dumitras, *JOAM*, **2011**, 13, 1045.
12. C. Popa, A. M. Bratu, Ramona Cernat, Doru C. A. Dutu, S. Banita and Dan C. Dumitras, *U. P. B. Sci. Bull.*, *Series A*, 73, **2011**, 167.
13. C. Popa, A. M. Bratu, C. Matei, R. Cernat, A. Popescu, and D.C. Dumitras, *Las. Phys*., **2011**, 2, 1336.
14. C. Popa, D. C. A. Dutu, Ramona Cernat, C. Matei, A. M. Bratu, S. Banita and Dan C. Dumitras, *App. Phys. B*, **2011**, 105, 669.