Laser photoacoustic spectroscopy (LPAS) in the context of biomedicine

CRISTINA POPA, MIOARA PETRUS, ANA-MARIA BRATU
Laser Department
National Institute for Laser, Plasma and Radiation Physics, 409 Atomistilor St., PO Box MG-36
ROMANIA

Abstract: - Helicobacter pylori (HP) is a bacterium commonly found in the stomach, and its detection is crucial in clinical practice due to its association with peptic ulcers, chronic stomach inflammation, and stomach cancer. HP possesses an enzyme called urease, which naturally hydrolyzes urea into ammonium carbonate.

In this research study, the concentration of ammonia in the breath of individuals with HP was measured using the photoacoustic spectroscopy method. The goal was to compare the levels of ammonia in the breath of individuals with HP to those of healthy individuals. The results of the study revealed that the concentration of ammonia was elevated by 498 parts per billion (ppb) in individuals with HP compared to the breath of healthy individuals. This finding suggests a potential correlation between HP infection and increased levels of ammonia in breath samples. The measurement of ammonia levels using photoacoustic spectroscopy provides valuable insights into the metabolic processes and bacterial activity associated with HP infection. Further research is warranted to elucidate the mechanisms underlying this observed increase in ammonia concentration and its clinical implications for the diagnosis and management of HP-related conditions.

Key-Words: human respiration, photoacoustics, ammonia, helicobacter pylori, breath test

Received: July 19, 2023. Revised: October 28, 2023. Accepted: February 2, 2024. Published: March 14, 2024.

1 Introduction

The statement discusses the significance of Helicobacter pylori (HP) in the human stomach, which releases volatile organic compounds (VOCs) that can be detected in exhaled air from the lungs. Human respiration consists of various gases and compounds, including nitrogen, oxygen, carbon dioxide, water vapor, inert gases, as well as inorganic and organic compounds at different concentration levels (ppbV, ppmV, pptV). These compounds can offer valuable insights into human health, as they can originate from metabolic pathways or bacterial strains residing in the human digestive system [1-3].

The research underscores the importance of non-invasive, real-time monitoring tools in modern scientific endeavors. In this context, the study employs laser photoacoustic spectroscopy method to characterize the respiration of individuals with or without Helicobacter pylori infection, contrasting with other studies that used different techniques [4-8].

Laser photoacoustic spectroscopy offers several advantages over non-spectral operations, including improved safety, stability, and system lifespan due to the absence of chemical reactions and consumption of detection materials. Moreover, it provides indirect measurements without background signal interference, enhancing its applicability in medical, industrial, and environmental fields [9].

Using photoacoustic spectroscopy, the research assesses breath ammonia levels in individuals with HP infection and compares them with those of healthy individuals. This approach contributes to understanding the potential association between HP infection and breath biomarkers, paving the way for non-invasive diagnostic and monitoring strategies in clinical settings.

2 Problem Formulation

The research aimed to investigate the presence of ammonia gases in human respiration, specifically focusing on individuals with Helicobacter pylori (HP) compared to a control group. The study utilized the CO2 laser photoacoustic spectroscopy method, referencing CO2 laser frequencies documented in previous literature (references [9-20], see Figure 1).

To collect breath samples, all participants exhaled into 0.75-L bags coated with aluminum, designed to retain multiple breath samples [20]. Participants placed the piece in their mouth and exhaled naturally into the sampling kit. Once a sufficient breath sample was collected, participants ceased exhalation. The bag containing the collected breath was then transferred to the resonant measuring cell for analysis of ammonia gases using a gas flow controller.

The transfer of breath samples from the aluminum bag to the resonant cell occurred at a controlled flow rate of 36L/h, with gas pressure established using a Baratron pressure gauge. Typically, the sample gas required approximately 1 minute to transfer, resulting in a final pressure of around 800 mbar inside the detection cell, with an equivalent responsivity of 240 cmV/W.

The photoacoustic spectroscopy gas detection method employed for ammonia evaluation is summarized in Figure 1 and detailed in various research papers (references [15-20]). This method allows for sensitive detection and quantification of ammonia levels in breath samples, enabling comparisons between individuals with and without HP infection.

In summary, the study utilized sophisticated techniques to assess breath ammonia levels, providing valuable insights into the potential association between HP infection and respiratory biomarkers.

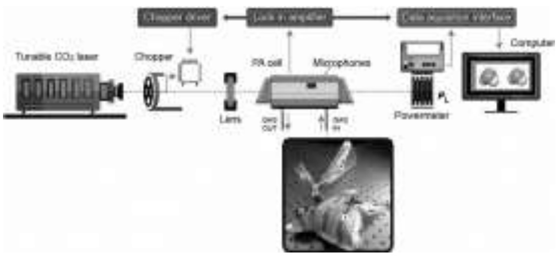

Fig. 1. photoacoustic spectroscopy gas detection experimental set-up.

The photoacoustic spectroscopy gas detection arrangement is comprised of several key components, including: CO_2 Laser, Lens, Chopper, Photoacoustic Resonant Cell, Powermeter, Lock-in Amplifier, Acquisition Panel, Data-Processing Computer.

Additionally, the experimental detection system includes a gas handling system, which plays a crucial role in controlling the flow and composition of the gases studied. This system ensures precise control over experimental conditions and facilitates the manipulation of gases within the photoacoustic resonant cell.

The gas handling system may perform various actions beyond gas control, as described in more detail in other articles referenced (references [9-20]). These actions could include sample preparation, calibration, and maintenance procedures to ensure the reliability and accuracy of the experimental setup.

3 Problem Solution

In the study, a total of 10 participants were recruited, all of whom underwent thorough screening criteria. Participants with Helicobacter pylori (HP) infection were specifically selected to be non- or ex-smokers, non-alcoholic, non-renal, non-diabetic, and free from chronic mental or physical health problems. Before breath analysis, participants were instructed to abstain from alcohol, coffee, food, beverages, onions, leeks, eggs, and garlic for at least 6 hours prior to or during breath sample collection. Detailed information regarding age, body weight, body height, timing and nature of the last meal and drink, recent exercise activity, medication use, and smoking status was collected from each participant.

It's noteworthy that all data published about the volunteers were obtained with their informed consent. Participants were fully briefed on the purpose and requirements of the experimental study before providing written consent to participate.

Figure 5 presents the average concentrations of breath ammonia (ABT) for individual participants, with and without HP infection. The median ammonia concentration for healthy participants was 819 parts per billion (ppb), whereas for HP participants, the concentration increased to 1317 ppb. This difference in ammonia levels between the two groups suggests a potential association between HP infection and altered breath biomarkers, highlighting the importance of further research in this area.

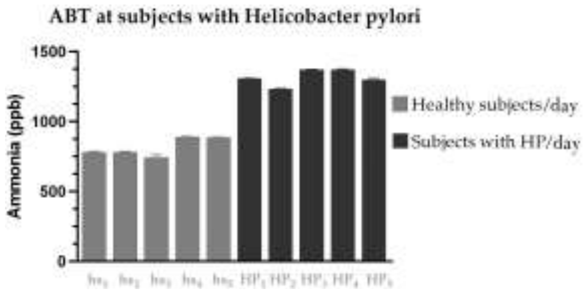

Fig.2. Ammonia breath test-ABT measured for participants with or without Helicobacter pylori -HP.

When analyzing figure 2, the average ammonia concentration/day for hs_1 (healthy subject) was

781 ppb, for hs_2: 780 ppb, for hs_3: 754 ppb, for hs_4: 891 ppb and for hs_5: 889 ppb while the average ammonia concentration/day for HP_1 (participant with HP) was 1308 ppb, for HP_2: 1233 ppb, for HP_3: 1373 ppb, for HP_4: 1370 ppb and for HP_5: 1300 ppb.

From the obtained results, the ammonia concentration from the HP participant's respiration was established to be in a higher concentration when the values were correlated to the control.

4 Conclusion

The study conducted determinations using CO_2 laser photoacoustic spectroscopy for breath samples collected from both healthy participants and those with Helicobacter pylori (HP) infection. Participants exhaled via the mouth each morning over a one-month period, allowing for the quantification of trace compounds and the determination of concentration distributions. It was observed that the level of ammonia was consistently higher in participants with HP when compared to healthy participants' breath.

The research employed a methodology aimed at ensuring optimal conditions for measuring real concentrations of ammonia in the breath of participants, whether they had HP or not. The CO_2 laser photoacoustic spectroscopy gas detection method was identified as a novel and potentially easier approach for assessing molecules that may shed light on the effects of HP on breath biomarkers. It's acknowledged that the composition of compounds in human breath can vary slightly based on factors such as oral hygiene, time of day, and individual differences in metabolism, resulting in a unique "breathprint" for each person. Further extensive studies involving a larger number of participants are warranted to provide clarity and validation to the preliminary findings of this research. Future research endeavors should explore additional molecules in breath samples from individuals with HP, using the current study as a reference point. This approach can contribute to a deeper understanding of the respiratory profile of individuals with HP infection and its implications for diagnosis and management.

Acknowledgement:
This work was supported by the Romanian Ministry of Research, Innovation and Digitalization under the project number PN-III-P1-1.1-TE-2021-0717.

References:

[1] Ulanowska A, Kowalkowski T, Hrynkiewicz K, Jackowski M, Buszewski B. Determination of volatile organic compounds in human breath for Helicobacter pylori detection by SPME-GC/MS. Biomed Chromatogr. 2011 Mar;25(3):391-7. doi: 10.1002/bmc.1460. PMID: 21321973.

[2] Allardyce RA, Langford VS, Hill AL and Murdoch DR. Detection of volatile metabolites produced by bacterial growth in blood culture media by selected ion flow tube mass spectrometry (SIFT-MS). Journal of Microbiological Methods 2006; 65: 361–365.

[3] Pawel Mochalski *et al* 2018 *J. Breath Res.* 12 046005 DOI 10.1088/1752-7163/aacbfb.

[4] Sagnik Das and Mrinal Pal 2020 *J. Electrochem. Soc.* 167 037562 DOI 10.1149/1945-7111/ab67a6.

[5] Testerman TL, Morris J. Beyond the stomach: an updated view of Helicobacter pylori pathogenesis, diagnosis, and treatment. World J Gastroenterol. 2014 Sep 28;20(36):12781-808. doi: 10.3748/wjg.v20.i36.12781. PMID: 25278678; PMCID: PMC4177463.

[6] Maaike K. van Gelder, Jacobus A.W. Jong, Laura Folkertsma, Yong Guo, Christian Blüchel, Marianne C. Verhaar, Mathieu Odijk, Cornelus F. Van Nostrum, Wim E. Hennink, Karin G.F. Gerritsen, Urea removal strategies for dialysate regeneration in a wearable artificial kidney, Biomaterials, Volume 234,2020.

[7] Dun CDR, Blac M, Cowell DC, Penaul C, Ratcliffe NM, Spence R, Teare C. Ammonia vapour in the mouth as a diagnostic marker for Helicobacter pylori infection: preliminary 'proof of principle' pharmacological investigations. Br J Biomed Sci. 2001;58(2):66-75. PMID: 11440209.

[8] Kearney, D.J., Hubbard, T. & Putnam, D. Breath Ammonia Measurement in *Helicobacter pylori* Infection. *Dig Dis Sci* 47, 2523–2530 (2002). https://doi.org/10.1023/A:1020568227868

[9] Popa, C.; Petrus, M.; Bratu, A.M. Effect of Wearing Surgical Face Masks on Gas Detection from Respiration Using Photoacoustic Spectroscopy. *Molecules* 2022, *27*, 3618. https://doi.org/10.3390/molecules27113618

[10] Popa, C.; Bratu, A.M.; Bacalum, M.; Prepelita, P. Application of the laser technology on the effect of Cd phytotoxicity in the detection of NH_3, C_2H_4, C_2H_5OH and CO_2 biomolecules at Triticumaestivum plantlets. Sustain. Chem. Pharm. 2020, 15, 100208.

[11] Popa, C.; Petrus, M.; Bratu, A.M. Ammonia and ethylene biomarkers in the respiration of the

people with schizophrenia using photoacoustic spectroscopy. J. Biomed. Opt. 2015, 20, 57006

[12] Popa, C. Ethylene Measurements from Sweet Fruits Flowers Using Photoacoustic Spectroscopy. Molecules 2019, 24, 1144

[13] Bratu, A.-M.; Petrus, M.; Popa, C. Identification of Absorption Spectrum for IED Precursors Using Laser Photoacoustic Spectroscopy. *Molecules* 2023, *28*, 6908. https://doi.org/10.3390/molecules28196908

[14] Ana-Maria Bratu, Mihaela Bojan, Stefan Banita, Mioara Petrus, Cristina Popa, NIR and THz spectroscopy: An experimental investigation toward nicotine-related devices 2023, https://doi.org/10.1002/jbio.202300120.

[15] Bratu A.M., Popa C., Matei C., Banita S., Dutu D.C.A., Dumitras D.C. Removal of interfering gases in breath biomarker measurements. J. Optoelectron. Adv. Mater. 2011;13:1045–1050.

[16] Cristina Popa, Ana Maria Bratu, Mioara Petrus, A comparative photoacoustic study of multi gases from human respiration: mouth breathing vs. nasal breathing, Microchemical Journal, 139, 2018, 196 202,doi.org/10.1016/j.microc.2018.02.030.

[17] https://webbook.nist.gov/cgi/cbook.cgi?ID=C7664417&Units=SI&Type=IR-SPEC&Index=1#IR-SPEC

[18] Popa, C., Dutu, D.C.A., Cernat, R. *et al.* Ethylene and ammonia traces measurements from the patients' breath with renal failure via LPAS method. *Appl. Phys. B* 105, 669–674 (2011). https://doi.org/10.1007/s00340-011-4716-8

[19] Dumitras, D.C.; Petrus, M.; Bratu, A.-M.; Popa, C. Applications of Near Infrared Photoacoustic Spectroscopy for Analysis of Human Respiration: A Review. *Molecules* 2020, *25*, 1728. https://doi.org/10.3390/molecules25071728

[20] Dumitras, D.C.; Banita, S.; Bratu, A.M.; Cernat, R.; Dutu, D.C.A.; Matei, C.; Patachia, M.; Petrus, M.; Popa, C. Ultrasensitive CO_2 laser photoacoustic system. *Infrared Phys. Tech. J.*2010, *53*, 308–314.

Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Author Contributions: Please, indicate the role and the contribution of each author:

Cristina Popa writing the manuscript draft, organized and executed the experiments of the current manuscript.

Mioara Petrus was responsible for the interpretation of the results.

Ana-Maria Bratu was responsible for the conclusions of the manuscript.