

# *Portable sensor for the assessment of food impact on breath volatile organic compounds*

## A White Paper

### Introduction / Background

Food metabolism involves numerous processes that involve many structurally different metabolites. Some of these metabolites are volatile and tend to pass from the blood into the air in the lungs, reaching the exhaled breath.<sup>1</sup> The fact that breath sampling is simple and non-invasive, and that breath composition reflects the current state of the organism, a lot of scientists engage themselves in trying to seize and exploit all that potential which indisputably lays in the breath analysis. Diet and physical activity are inevitable parameters whose impact would be of interest to be monitored in this way.<sup>2</sup> So far, this area in breath analysis has been less studied in relation to attempts to identify certain diseases from volatile organic compounds (VOCs), but there are certainly studies that have started research in this area.<sup>3-9</sup>

To meet requirements for the sensor, this research aims to develop VOC sensor based on membrane inlet mass spectrometry (MIMS)<sup>10</sup>. Some of the previous applications of this analytical technique are bioreactor monitoring, environmental monitoring, air analysis, monitoring of metabolic processes, determination of gases dissolved in water. Recently it was used for human VOCs monitoring<sup>12</sup>, which substantiates our idea to utilize this simple technology in the breath VOCs analysis.

### Abstract / Business Case

Nutritional balance is among the major concerns of modern people, as it is known that diet directly impacts the overall state of the human body. To choose the optimal diet for everyone, a personalized approach is needed. Usually, such approach can be time and money consuming. Moreover, it is almost impossible to enable widespread application with conventional approaches and relying just on medical systems. On the other hand, we are living in the society where mobile devices and wearable sensors are a common thing and we are collecting large amount of information every day. The combination of the suitable sensor and user-friendly mobile application could be joined to provide non-invasive, fast, and affordable screening tool for monitoring users' nutritional status for gathering relevant information about their diet and lifestyle. Extraction of the significant conclusions from those could be used in ongoing improvement strategy. The purpose of this study is to validate a sensor for food impact assessment by analyzing the levels of volatile organic compounds (VOCs) in human breath. It aims to establish a prototype for future on-site technique for nutritional status clarification.



## Problem Statement / Introduction

To provide personalized treatment for PROTEIN app users, a suitable diagnostic sensor for breath analysis could be coupled with the mobile app if needed. Furthermore, the development of the bioanalytical method for breath analysis using selected sensor is the next step. Sensor and method verification on small cohort of users was performed in the initial stage. Large-scale validation of the sensor and the method on different user groups were conducted in the final stage.

## Proposed Solution(s)

1. Sensor development includes building a sensor which could meet the following criteria: to be portable, easy to handle, reliable and affordable. Also, it must be capable of generating results in a format suitable for the app, and to be integrated in the PROTEIN platform.
2. VOC sensor verification includes examination of sensor's functionality parameters.
3. VOC sensor bioanalytical method development is required.
4. VOC sensor large-scale validation requires application of the new sensor and the method for analyses of numerous samples collected from different user groups.

### *Introduction of Solution*

1. The VOC sensor is based on a simple analytical technique – membrane-inlet mass spectrometry (MIMS). Its principle lies in the process of pervaporation, where analytes in the gas phase migrate through a specific semi-permeable membrane.<sup>10</sup> Technical requirements for construction of this sensor type are simple, and the whole instrument can be integrated in a suitcase to enable portability.
2. VOC sensor functionality can be confirmed by evaluation of the resolution, response time (rise time and fall time) and linear dynamic range for selected breath VOCs (e.g., acetone, isoprene, n-pentane, ethanol).
3. VOC sensor method development covers:
  - optimization of protocol for sampling for food impact assessment (selection of relevant breath VOCs which reflect food impact, determination of the sampling time before and after the meal, sample volume, etc.)
  - construction of suitable calibration curves and quantification method for the extracting the breath VOCs concentration levels in breath samples.
  - analysis of samples collected from 50 adult healthy participants from Serbia, with participation information sheets and informed consents,
  - determination of the sensitivity and repeatability of the method using the results from these 50 participants,
  - evaluation of the food impact on the selected breath VOCs using different statistical tests.
4. The VOC sensor large-scale validation – This is done via pilot study by recruiting more than 150 participants from several European countries (Greece, Portugal, United Kingdom,



Germany, and Belgium), with informed consents and ethical approvals. Participants are people with various profiles and lifestyles: athletes, people who are overweight, people with obesity, diabetes, and cardiovascular disease, people who have poor dietary habits. Each participant will provide a sample before and after the meal. Also, information about participants' lifestyle will be collected via a short questionnaire. Results obtained will be statistically processed and food impact will be assessed.

### ***Application of Solution***

VOC sensor was successfully constructed and its functionality for breath VOCs analysis has been proven. Bioanalytical method for food impact assessment was developed and applied to 174 participants from different user groups. Results are generated in .csv format suitable for the PROTEIN app. Therefore, VOC sensor can be integrated in the PROTEIN platform and application and can serve as one of the sensors for personalized nutrition.

### **Future Direction / Long-Term Focus**

*As the proposed VOC sensor does not require laboratory conditions, its application could be widespread due to fast analysis, portability, and affordability. It may be considered to become on-site screening diagnostic device in local health centres and laboratories.*

### **Results / Conclusion**

VOC sensor is constructed by using a sheet membrane sample probe, vacuum system, (turbo pump, diaphragm pump) electron-impact (EI) ion source, single quadrupole mass analyzer, electron multiplier detector and electronic control unit. All the components are integrated in a suitcase with dimensions of 616x220x433 mm (LxHxW) and the whole system weighs 23 kg. This makes it suitable for field work.

VOC sensor functionality is confirmed by obtained unit resolution across the requested analytical range ( $m/z$  1-200) for VOCs determination. Duration of the scan across the entire range is about 1 min. Response times (rise times and fall times) for constructed sheet membrane probe are examined, and maximal signal rise time was 50 s and maximal fall time was 51 s. Along with short scan time, duration of the whole analysis by VOC sensor is approximately 3 min.

Moreover, calibration curves for selected VOCs (e.g., acetone, isoprene, ethanol, n-pentane) are constructed in required concentration ranges with satisfying linearity coefficients  $R^2 > 0.98$  for all analytes. Limits of detection for selected analytes are examined as well and are found to be in 5-25 ppb range. Repeatability of the method is presented as RSD value between three consecutive measurements of the same sample, and it was maximally 13%.

VOC sensor method was optimized on samples collected from 50 healthy participants in Serbia, and it was established that samples will be collected from each participant at 2 time points



- first sample before standardized meal (after 12h food restraint) and second sample 120 min after the meal. Samples are collected in 1L single-use Tedlar® bags and analyzed using the VOC sensor.

Large-scale sensor validation included recruitment of the participants and collection of the samples and relevant data from them at different institutions across the Europe with informed consents and adequate ethical approvals. Samples are transferred to Serbia for VOC sensor analysis. After the analysis, quantified ppb values of key breath VOC compounds are shared with all the project partners. Summary of the quantified ppb values during the VOC sensor pilot study, along with the information about the participants recruitment is presented in the Table 1.

Table 1. Mean ppb concentration levels obtained for acetone, isoprene, ethanol, and n-pentane for 174 participants in samples collected before the meal (BM) and after the meal (AM)

VOC sensor study				Mean (ppb)							
Participants recruitment				ACETONE		ETHANOL		ISOPRENE		n-PENTANE	
User group	Country	Number		BM	AM	BM	AM	BM	AM	BM	AM
People who are overweight	United Kingdom	15	35	640	556	339	308	153	137	23	19
	Greece	20									
People with obesity	Greece	22		737	751	301	318	235	233	20	21
People with diabetes type II	Germany	6		2785	361	514	494	286	224	28	13
People with CVD	Belgium	21		499	544	474	493	250	388	11	12
People with poor diet	United Kingdom	46		208	266	354	403	142	153	22	19
Athletes	Greece	24	44	626	565	407	386	215	200	17	15
	Portugal	20									

To evaluate the food impact on selected breath VOC levels, the *comparison factors* for each participant were calculated. These factors were ratios between VOC level after the meal and before the meal and they show if the VOC level decreased or increased upon meal. Food impact assessment is obtained using One-way ANOVA test on the set of the data collected via VOC questionnaire and the calculated comparison factors (Table 2).

Table 2. Summary of One-way ANOVA statistical tests results for several categorical parameters against comparison factors obtained experimentally. Highlighted p-values imply statistically significant difference between breath VOCs changes for distinct categories since p-value is below the threshold ( $\alpha=0.05$ ).

One way-ANOVA		p-value			
Categorical parameter		Acetone	Ethanol	Isoprene	n-Pentane
Participant category	OW vs. OB vs. DM vs. CVD vs. PD vs. AT	0.296	0.528	7.79E-07	0.961
Gender	Male vs. Female	0.325	0.104	0.136	0.315
Age	18-40 vs. 41-60 vs. >61	0.578	0.053	0.019	0.020
Living environment	Rural vs. Urban	0.586	0.295	4.04E-04	0.094
Working environment	Rural vs. Urban	0.778	0.783	0.004	0.267
Physical activity	Low vs. Moderate vs. High	0.311	0.279	0.513	0.207
Alcohol drinking habit	No vs. <2 drinks/wee vs. ; 2-8 drinks/week vs. >8 drinks/week	0.775	0.812	0.376	0.533
Cigarettes consumption habit	5-20 cig./day vs. <5 cig./day vs. >20 cig./day	0.943	0.896	0.874	0.743

Statistically significant difference was observed in change of isoprene levels upon meal consumption between different participants groups, people of different age, and people in different living and working environments. Also, significant difference was noticed in change of n-pentane levels upon meal consumption between people of different age.



**Appendix A – Authors**

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**Appendix B – References**

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