**Capillary electrophoresis with laser induced fluorescence as a useful tool for biomarker analysis**

Júlia Lačná1,2, František Foret1,3, Petr Kubáň1,2,3

*1 Department of Bioanalytical instrumentation, CEITEC MU, Veveří 97, 602 00 Brno, Czech Republic*

*2 Department of Chemistry, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic,*

*3 Department of Bioanalytical Instrumentation, Institute of Analytical Chemistry, Czech Academy of Sciences, v.v.i., Veveří 97, 602 00, Brno, Czech Republic*

For accurate and reliable clinical diagnosis, suitable analytical methods for the determination of biological markers are necessary. Non-invasive samples, such as exhaled breath condensate (EBC), are more comfortable for patients, but more challenging for the analysts due to low sample volumes and low concentrations of biomarkers. Sensitive analytical methods are required. Capillary electrophoresis (CE) with laser induced fluorescence (LIF) detection can increase the detection sensitivity to nM levels and improve selectivity.

Sensitive CE methods with LIF detection for the determination of malondialdehyde (MDA) and glutathione (GSH) in various biological fluids were developed. The developed methods offer unprecedented detection sensitivity, as low as 1nM. The methods proved to be applicable not only to EBC but also to other biological fluids, such as blood plasma and saliva. Presented electrophoretic methods were for the first time used in the determination of glutathione and malondialdehyde in EBC. [1,2]

Highly efficient separations, short analysis times, and small sample consumption are the main assets of the CE-LIF technique, especially for the analysis of non-invasive samples. In combination with another strong detection method as contactless conductivity (C4D) – CE‑C4D–LIF provided an efficient separation and quantitation of ionic content and GSH at concentrations differing by more than three orders of magnitude in a single run. We have optimized the composition of background electrolyte for the simultaneous analysis of biologically important ions using dual C4D–LIF detection. Sensitive detection of anions, cations, and organic acids with micromolar LODs using C4D and simultaneously glutathione with nanomolar LODs using LIF was achieved in a single run. The developed electrolyte may be useful in analyses of biological samples containing analytes with differing concentrations of several orders of magnitude that is not possible with a single detection mode. [3]

CE-C4D technique was also used in another long-term project, aimed at biomarker analysis for diagnosis of gastroesophageal reflux disease (GERD). Up to 20-40% of the population in the western world suffers from GERD with typical symptoms including heartburn and regurgitation. The aim of this study was to compare the composition of EBC in a group of patients with proven GERD and a group of healthy controls. The ionic profile of EBC samples (anions, cations, organic acids) was also measured by CE-C4D and several compounds were identified as potential markers that could be used to distinguish the GERD patients and healthy controls. The developed approach can provide a fast and non-invasive preselection of patients with reflux. [4]

[1] Hodakova, J., Preisler, J., Foret, F., Kuban, P., Sensitive determination of glutathione in biological samples by capillary electrophoresis with green (515 nm) laser induced fluorescence detection, *Journal of Chromatography A 2015*, 1391, 102–108.

[2] Lacna, J., Foret, F., Kuban, P., Sensitive determination of malondialdehyde in exhaled breath condensate and biological fluids by capillary electrophoresis with laser induced fluorescence detection, *Talanta 2017*, 169, 85–90.

[3] Lacna, J., Prikryl, J., Teshima, N., Murakami, H., Esaka, Y., Foret, F., Kuban, P., Optimization of background electrolyte composition for simultaneous contactless conductivity and fluorescence detection in capillary electrophoresis of biological samples, *Electrophoresis 2019*, 40, 18-19.

[4] Lacna, J., Durc, P., Gregus, M., Skrickova, J., Doubkova, M., Pokojova, E., Kindlova, D., Dolina, J., Konecny, S., Foret, F., Kuban, P., Capillary electrophoretic analysis of ionic content in exhaled breath condensate and pH monitoring as a non-invasive method in gastroesophageal reflux disease diagnostics, *Journal of Chromatography B 2019*, 1134, 1-7.