Optimization of capillary electrophoretic separation electrolytes for simultaneous C^4D and LIF detection of biologically important ions.

Júlia Lačná[1,4], Norio Teshima[3], Hiroya Murakami[3], František Foret[1,2], Jan Přikryl[2] and Petr Kubáň[1,2]

[1]*Bioanalytical Instrumentation, CEITEC Masaryk University, Veveří 97, 602 00, Brno, Czech Republic*

[2]*Department of Bioanalytical Instrumentation, Institute of Analytical Chemistry of the Czech Academy of Sciences, Veveří 97, 602 00, Brno, Czech Republic*

[3]*Aichi Institute of Technology, Department of Applied Chemistry, Toyota 4700392, Japan*

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

Contact information: Júlia Lačná, juliahod@gmail.com

Capillary electrophoresis (CE) offers a unique separation platform for the analysis of biological fluids due to its high separation efficiency, short analysis times and low sample volume requirement. The complexity of biological samples (such as exhaled breath condensate, saliva), however does not allow complete analysis of compounds of interest, for instance markers of nitrosative and oxidative stress, using a single detection mode. Nitrite and nitrate, present in biological fluids as metabolites of nitrosative stress promoter NO_x, are typically at micromolar concentrations, while glutathione (GSH) is present at low nM concentrations. Simultaneous analysis of complete biomarker pattern is therefore not possible using single detection approach.

In this work we present an attempt on developing an optimized background electrolyte for simultaneous separation and detection of small ionic species including nitrosative stress markers nitrite and nitrate, inorganic cationic species and GSH as an oxidative stress marker using dual - capacitively-coupled contactless conductivity detector (C^4D) and laser induced fluorescence (LIF) - detection mode. By applying the double opposite end injection, we achieve efficient separation of small anionic and cationic analyte groups, including the fluorescently labelled GSH with low limit of detections. Optimized background electrolyte (BGE) containing 30mM HEPES, 15mM TRIS, 2 mM 18-crown-6 offered an efficient separation of complete biomarker pattern in a single run. Using only one CE system decreases the time for analysis, consumption of samples and chemicals and the instrumental complexity. This work offers new insights into selection and design of BGEs for simultaneous separations by CE, especially in cases when C^4D is combined with LIF.