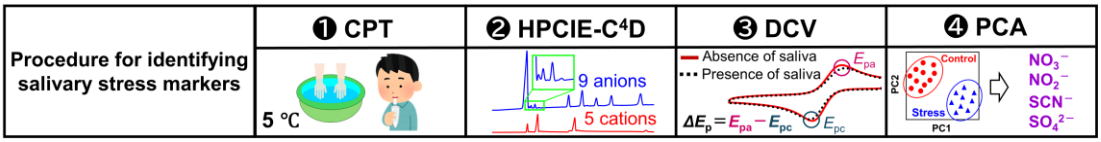
Identifying salivary stress markers using capillary ion electrophoresis target analysis with electrochemical correction

([1]*Graduate School of Integrated Arts and Sciences, Kochi University*, [2]*Graduate School of Medical Sciences, Nagoya City University*, [3]*Graduate School of Medicine, Gunma University*, [4]*Faculty of Health Care, Teikyo Heisei University*)
○Hironori Myochin[1], Tadaharu Ueda[1], Romanas Chaleckis[2], Noriyasu Ohshima[3], Takashi Izumi[4], Tatsuya Hisajima[4], Masanobu Mori[1]
Keywords: Stress; Saliva; Ion; Capillary electrophoresis; Cyclic voltammetry

Salivary analysis has been widely recognized as a non-invasive approach in the bioanalytical fields. Salivary ions, which play key roles in the transport, biosynthesis, and metabolism of biologically active substances, offer valuable insights into an individual health status as potential biomarkers [1]. However, the relationship between salivary ions and health status, especially stress, remains poorly understood. In this study, we developed an analysis method for identifying stress markers focusing on salivary ions.

High-performance capillary ion electrophoresis with a capacitively coupled contactless conductivity detector **(HPCIE-C^{4}D)** using a chemically coating capillary was effective for the simultaneous analysis of 14 targeted salivary ions [2]. The capillary, which consists of two different ionic polymers and a quaternary ammonium spacer, can suppress adsorption of glycoproteins. The ion concentrations measured using HPCIE-C^{4}D were corrected by the ratio in redox potential (ΔE_p), obtained from direct current cyclic voltammograms **(DCV)** of ferricyanide in the absence and in the presence of saliva. This correction solved the overestimations caused by glycoproteins related to the viscosity of saliva, enabling more accurate statistical analysis.

We applied these ion analytical methods to saliva samples obtained from a Cold Pressor Test **(CPT)**, which is an acute stress test, is conducted by immersing both hands in cold water (5 ℃) or lukewarm water (25 ℃, a control). Principal component analysis **(PCA)** was performed on the corrected data to identify stress-related ions. The results suggested that ions were related to the metabolism of vasodilators induced by acute stress due to cold water stimulation. Specifically, these are nitrate and nitrite ions, which are metabolites of nitric oxide [3], and thiocyanate and sulfate ions, which are metabolites of hydrogen sulfide [4]. In other words, this method is applicable to the diagnosis of acute stress using salivary ions.



References: [1] G. Giacomello, et al., *J. Pharm. Biomed. Anal.*, **191** (2020) 113604. [2] H. Myochin, et al., *J. Chromatogr. A*, **1720** (2024) 464769. [3] X. Chai, et al., *Front. Cardiovasc. Med.*, **11** (2024) 1337281. [4] B. Lv, et al., *J. Adv. Res.*, **27** (2021) 85.