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UNCOVERING THE FORMATION OF COLOR GRADIENTS ON MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICES BY MASS SPECTROMETRY IMAGING

Soraia V. de Freitas (PG)[a]*, Fabrício R. de Souza (PG)[a], Jorge C. R. Neto (PG)[a,b], Géssica A. Vasconcelos (PG)[a], Patrícia V. Abdelnur (PQ)[a,b], Boniek G. Vaz (PQ)[a], Charles S. Henry (PQ)[c] and Wendell K. T. Coltro (PQ)[a,d]

[a]Universidade Federal de Goiás, Instituto de Química, 74690-900, Goiânia/GO, Brazil.
[b]Embrapa Agroenergia, Empresa Brasileira de Pesquisa Agropecuária, 70770-901, Brasília/DF, Brazil.
[c]Colorado State University, 80523, Fort Collins/CO, USA.
[d]Instituto Nacional de Ciência e Tecnologia de Bioanalítica, 13084-971, Campinas/SP, Brazil.
*e-mail: soraiavasfreitas@gmail.com

Colorimetric detection has been widely used with microfluidic paper-based devices (μPADs) for different types of studies.[1,2] Even colorimetric detection is an attractive detection tool, the formation of color gradients significantly impacts assay sensitivity and reproducibility with μPADs. This study describes the use of mass spectrometry imaging with matrix-assisted laser desorption/ionization (MALDI) and desorption electrospray ionization (DESI) to understand the color gradient generation commonly seen in μPADs. The glucose enzymatic assay using potassium iodide (KI) as a chromogenic agent was selected to investigate the color gradient generated across a detection spot. Colorimetric measurements revealed that the relative standard deviation for the recorded pixel intensities ranged between 34 and 40%, compromising the analytical reliability. Then, mass spectrometry imaging using MALDI and DESI was applied to understand the non-uniform color distribution on the detection zone. MALDI experiments were first explored to monitor the spatial distribution of the glucose oxidase and horseradish peroxidase mixture, before and after lateral flow assay with and without KI. MALDI(+)-TOF data revealed uniform enzyme distribution on the detection spots. On the other hand, after the complete assay DESI(-) measurements revealed a heterogeneous shape indicating the presence of iodide at the zone edge. Furthermore, DESI(-) showed the presence of triiodide ions (I_3^-), generated from the interaction between molecular iodine and the iodide spotted on the detection zones. The I_3^- is transported by lateral flow towards the zone edge, generating the color gradient. Mass spectrometry imaging has been used for the first time to prove that color gradient forms as result of the mobility small molecules and not the enzyme distribution on μPADs surface.

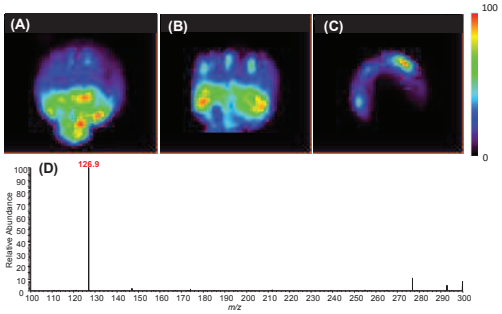

Figure 1. DESI imaging showing the spatial distribution of I- ((m/z 126,90389) in the detection zone containing (A) KI before lateral flow assay, (B) KI and (C) KI plus GOx/HRP after lateral flow for glucose detection. Graph (D) shows the MS spectrum for the mass range monitored in the paper surface

[1] Morbioli, G. G.; Mazzu-Nascimento, T.; Stockton, A. M.; Carrilho, E. Technical Aspects and Challenges of Colorimetric Detection with Microfluidic Paper-Based Analytical Devices (MPADs) - A Review. *Anal. Chim. Acta* **2017**, *970*, 1–22.
[2] Li, X.; Ballerini, D. R.; Shen, W. A Perspective on Paper-Based Microfluidics: Current Status and Future Trends. *Biomicrofluidics* **2012**, *6* (1).

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