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Direct analysis of amino acids by reactive paper spray mass spectrometry for newborn screening

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Palavras Chave: Newborn screening, Amino acids, Microfluidic paper device, Ambient ionization, Online derivatization, Diagnostics.

Highlights (up to 200 characters with spaces)

Increase ionization of polar compounds using reactive paper spray;
Microfluidic paper device to collect, store and analyze plasma samples;
Rapid diagnostic of aminoacidopathies for newborn screening.

Abstract

Newborn screening is considered one of the most significant advances in preventive medicine and one of the largest public health campaigns. The early diagnosis of diseases, even for the rare ones, can reduce or eliminate irreversible sequels that could occur if they were not detected prematurely.[1] A group of rare diseases analyzed in newborn screening is the aminoacidopathies, which are metabolic disorders caused by a specific deficiency in determined enzymes or transporters, such as phenylketonuria, leucinosis (maple syrup urine disease), and tyrosinemia type II.[2] The classic analysis for newborn screening involves the utilization of dried blood spots followed by chromatographic techniques with mass spectrometry detection analysis, however, dried plasma spot (DPS) is a simpler and easier-to-use sampling method that reduces the risk of contamination for the operator, eliminates the hematocrit interference, and facilitates transportation and storage.[3] In this way, we proposed to use reactive paper spray (PS) mass spectrometry as a tool to increase the ionization efficiency of high polar molecules as amino acids and integrate the advantages of a paper platform (3D-μPAD), such as low cost, availability, flexibility, hydrophilicity, light weight, biocompatibility, and power-free fluid transport with the reliability of mass spectrometric.

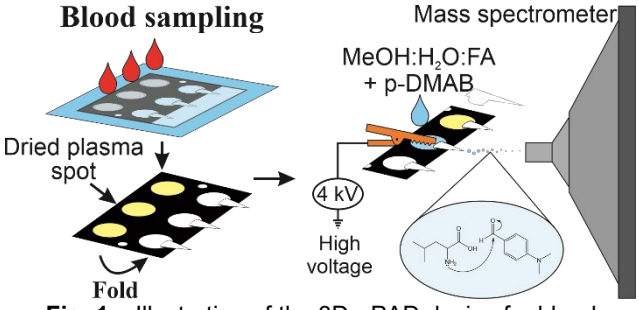

Fig. 1 – Illustration of the 3D-μPAD device for blood collection and DPS analysis by reactive PS-MS.

The proposed 3D-μPAD device fabricated using lamination, paper, toner, and plasma separation membrane can be employed to sample blood, separate the red cells to form DPS, and store the plasma sample for analysis and/or shipping. The 3D-μPAD device reproducibility and homogeneity to separate the red cells to form DPS was tested using fingertip blood (~20 μL) and analyzed by an offline spectrophotometric ninhydrin assay. No statistical difference ($p < 0,05$) between the DPS formed in the device's detection layers demonstrates a homogenous distribution by the plasma (CV < 10%), showing that it can be used for blood microsampling to form DPS.

After sampling, the detection layer of the proposed device was removed, folded, and positioned in front of the mass spectrometer inlet using an alligator clip. An aliquot of *p*-dimethylaminobenzaldehyde (*p*-DMAB) solution in MeOH:H_2O:formic acid 80:19:1 was added to the DPS to extract the analyte and generate the spray to promote the determination via reactive PS-MS. The online derivatization among the *p*-DMAB and the amino acids was possible because the microdroplet spray environment accelerated the derivatization reaction. Analytical curves were constructed from 2.5 to 100 mg L^{-1} for valine (267 → 150), leucine (281 → 150), isoleucine (281 → 150), phenylalanine (315 → 150), and tyrosine (331 → 150) via the loss of the specific amino acid using a multiple reaction monitoring in a TSQ Quantum Access mass spectrometer (Thermo Fisher/ triple quadrupole). Limits of detection between 0.57 and 0.95 mg L^{-1} and limits of quantification between 1.47 and 1.91 mg L^{-1} for the selected amino acids were obtained. The 3D-μPAD was successfully integrated with mass spectrometry analysis using reactive paper spray ionization and the proposed methodology can be used to determine amino acids with no sample preparation in plasma samples for aminoacidopathy screening.

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