Improving the Analytical Performance and Versatility of Paper Spray Mass Spectrometry via Paper Microfluidics

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Supplemental Information

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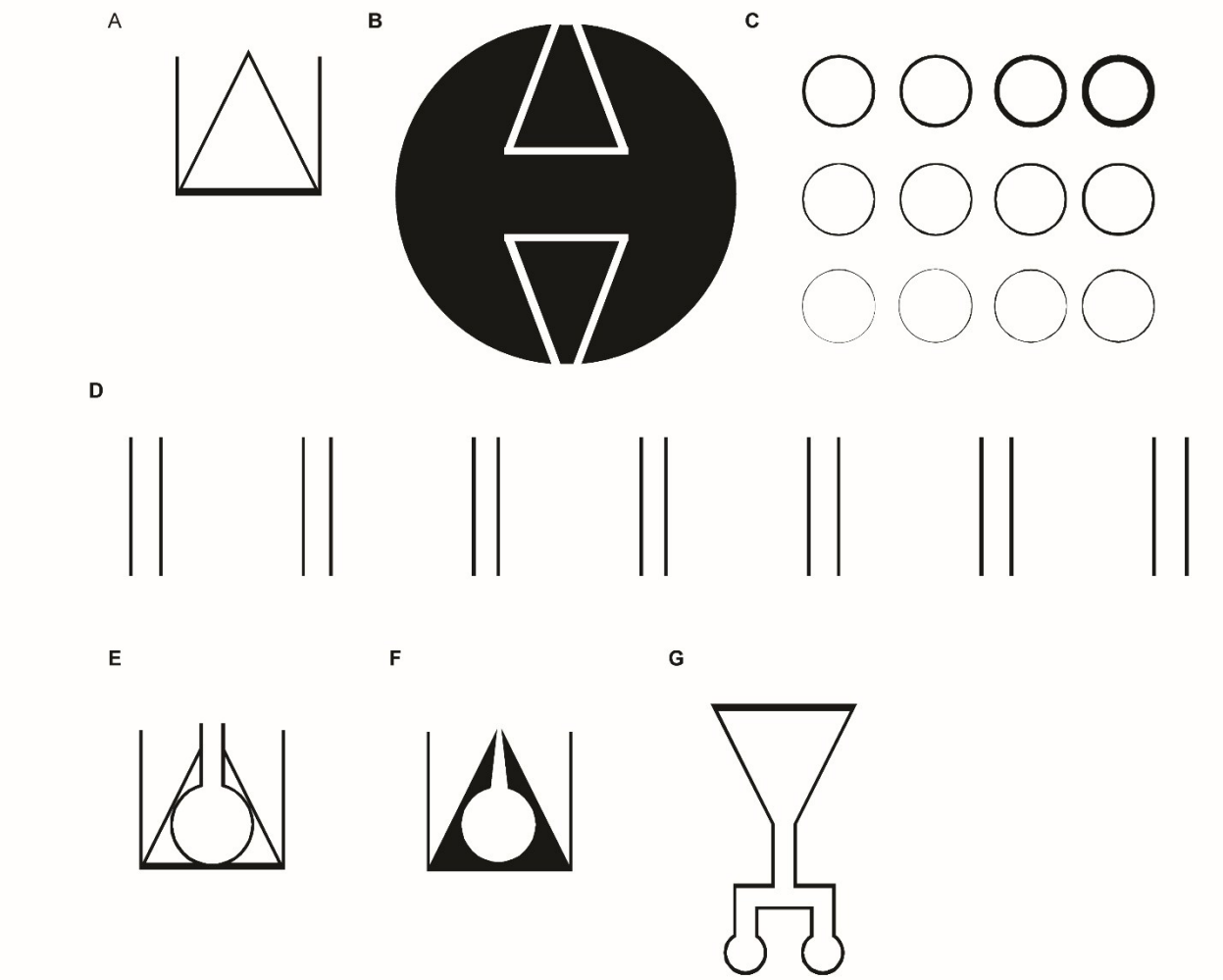
Supplemental Fabrication Information.

No adjustments were made to the printer's factory settings were made during these experiments. Designs intended for use with photoresist were sent to DTPRESS Commercial

Printing (Greensboro, NC) to be printed as transparent film photomasks. Photoresist barrier fabrication took place in a clean room located in the Biomedical Engineering Department (NC State University, Raleigh, NC).The UV-lamp used to develop photoresist barriers was set at 9.7mW/cm^3 and designs were exposed for 100 seconds.



Supplemental Figure 1: All designs used during the experiment. Designs were created in Adobe Illustrator and exported as PDF's for use. **(A)** Simple wax barrier design. **(B)** Photoresist mask design. Printed on transparency by DTPRESS Commercial Printing (Greensboro, NC). **(C)** 12 wax-printed wells varying from 1mm to .05mm in width. Latter wells might appear none existent in print due common printer limitations. **(D)** Seven sets of equivalent wax parallel lines used to

determine wall swelling from organics. Only 6 were used during the experiment. **(E)** Constricted wax design. **(F)** Increasingly restricted wax design. **(G)** Wax mixing design.

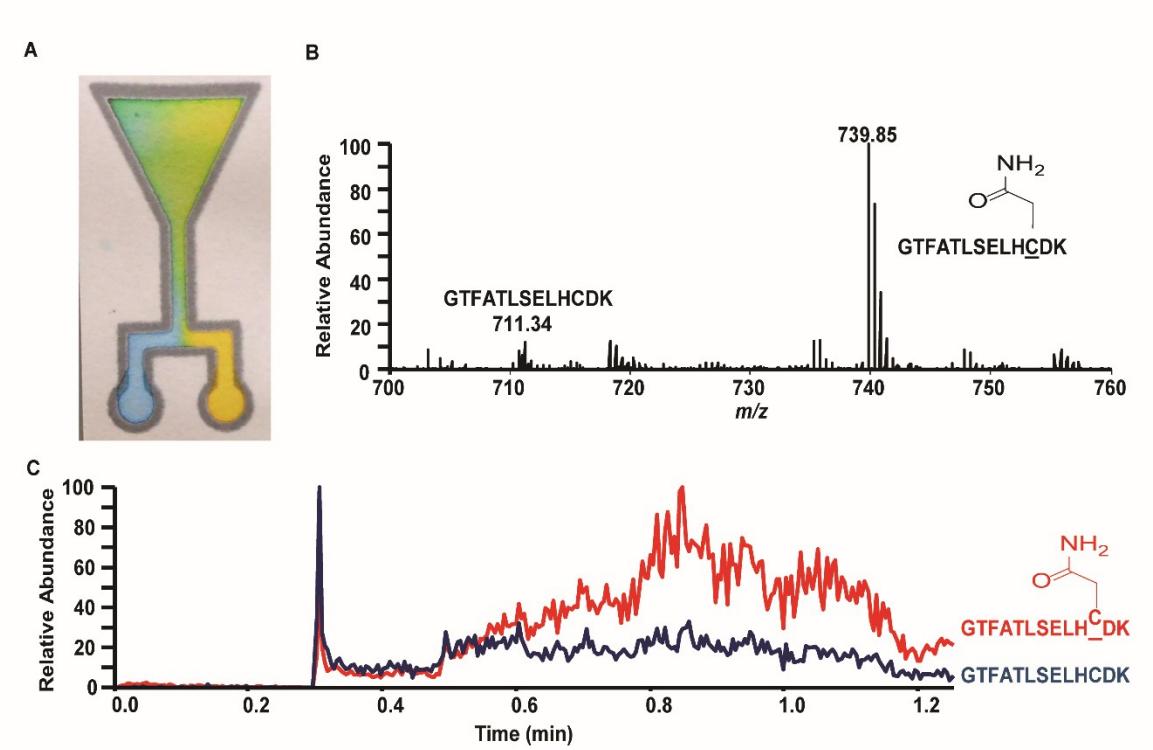


Figure 2: **(A)** Optical representation of the mixing of two solutions in a wax printer design with food coloring. **(B)** PSI-MS spectra of a hemoglobin peptide with iodoacetamide and their respective peaks labeled. **(C)** Extracted ion chromatograms of both unadducted hemoglobin peptide (Blue) and carbamidomethylated hemoglobin peptide (Red).

It should be noted that channel length and final reservoir design for the mixing device, **Supplemental Figure 2A**, was optimized for rapid analysis (<2 mins). By using hydrophobic barriers, the capillary flow can be simplified into one dimension and can be expressed by the Washburn equation, where L is the distance of the traveled by the solvent front, t is time, D is the pore diameter, γ is the surface tension and μ is the viscosity.[1]

Equation 1: $$L^2 = \gamma D t / 4\mu$$

By assuming γ, D, and μ are constant, Washburn-like flow can therefore be effectively reduced to $L \sim \sqrt{t}$. This shows that the capillary flow decreases over time and therefore long channel lengths

result in impractical mixing times. Capillary flow in triangular shapes with various cut angles have been previously studied[2]. A 60^{o} entrance was used in the final design to ensure quick filling of the final reservoir. Herein an example of mixing is demonstrated via the alkylation of the hemoglobin peptide that contains a free cysteine (GTFATLSELHCDK, *m/z* 711). This peptide is of interest in the field of adductomics due to the high abundance of hemoglobin in blood and the reactivity of the free cysteine which essentially acts as a "sink" to reactive species.[3] The peptide was allowed to passively mix with the alkylating reagent to achieve desired peptide adduct. After mixing and mass analysis, the alkylated peptide (GTFATLSELHCDK, *m/z* 739.85) was the most prominent peak. As seen in **Supplemental Figure 2B**, the unadducted peptide was detected but with a significantly weaker signal. Extracted ion chromatograms for both the unadducted and adducted are included in **Supplemental Figure 2C** to compare relative abundances of each ion. The adducted peptide was detected at 4 times greater intensity than the unadducted peptide.

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