LIFE SCIENCES

POSTER SUMMARY

Microfluidic CE-MS: A Single System for Monitoring Small Molecule and Protein Biomarkers from Blood Samples

OVERVIEW

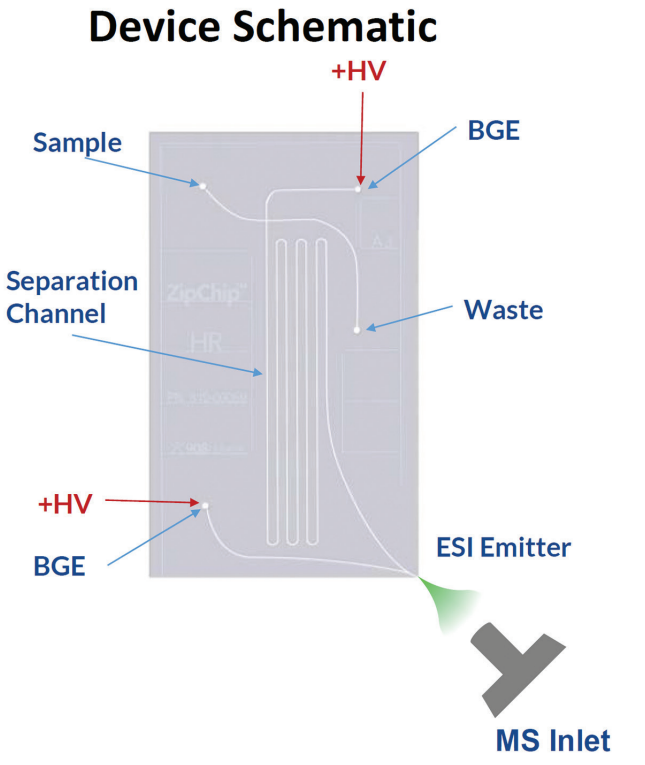
Whole blood or purified blood cells are often analyzed in an effort to identify specific compounds that may be indicative of disease states. The compounds that are measured can range from small molecule metabolites to larger protein biomarkers.1,2 This necessitates the need for multiple pieces of instrumentation as there are few techniques capable of effectively analyzing both small and large molecules rapidly and simply. In this work we present a microfluidic CE-MS system capable of analyzing large and small molecule biomarkers without the need to significantly alter the instrumentation. The ZipChip® microfluidic CE-MS interface coupled to Thermo mass spectrometers was used to analyze small molecule and protein based biomarkers from a variety of blood samples.

METHODS

Sample Preparation and Analysis. Although liquid blood samples could be used, samples for small molecule assays were derived from dried blood spots (DBS). The DBS were created by blotting samples of whole blood onto filter paper and allowing the spots to dry. To simulate samples indicative of disease states, phenylalanine or octanoylcarnitine were spiked into blood samples prior to blotting on the filter paper. 5 mm punches of the DBS were extracted with 250 µL of a MeOH/water/ammonium acetate solution doped with stable isotope internal standards (Cambridge Isotope Laboratories, Inc.). DBS punches were incubated in the extraction solution for 30 min at room T and then filtered with a 0.22 µm Nylon filter to remove particulates. Protein biomarkers were measured from whole blood or isolated erythrocytes by lysing the cells with an ACN/ water/formic acid solution. Samples were filtered with a 0.22 µm Nylon filter to remove particulates.

Instrumentation. The ZipChip® Device was used for all analyses (908 Devices Inc.) ZipChips utilize microfluidic technology to harness the inherent speed and efficiency of zone electrophoresis separations. The microfluidic device design, as seen below, incorporates an injection cross, serpentine separation channel, and an integrated ESI emitter where electrospray is generated directly off the corner of the device. Highly uniform and stable surface coatings suppress the electroosmotic flow and yield highly efficient separations.

Microfluidic chips with a 10 cm separation channel (HS, 908 Devices Inc.) and the Metabolite Assay Kit (908 Devices Inc.) were used for small molecule assays. An on-chip injection volume of 5 nL was performed and the separation was run at a field strength of 1000 V/cm. MS analysis was performed using a Thermo Exactive Plus EMR Orbitrap MS, and data was processed using Skyline (University of Washington). Chips with a 22 cm separation channel (HR, 908 Devices Inc.) and the Peptide Assay Kit (908 Devices Inc.) were used to analyze hemoglobin subunits. An on-chip injection volume of 1 nL was performed and the separations were run at a field strength of 500 V/cm. MS analysis was performed using a Thermo LTQ-XL MS and data was processed using Thermo BioPharmaFinder 1.0 SP1.



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SMALL MOLECULE ANALYSIS

Microfluidic CE-MS is well suited for analyzing small polar molecules since the separation mechanism is based on charge and hydrodynamic radius rather than retention on a stationary phase. Figure 1 shows the separation of a standard mixture of amino acids and acylcarnitines. These molecules are the key biomarkers for many inborn errors of metabolism. Separation of the molecules is rapid (~2 minutes) and patient samples can be tested for multiple disorders with a single run.

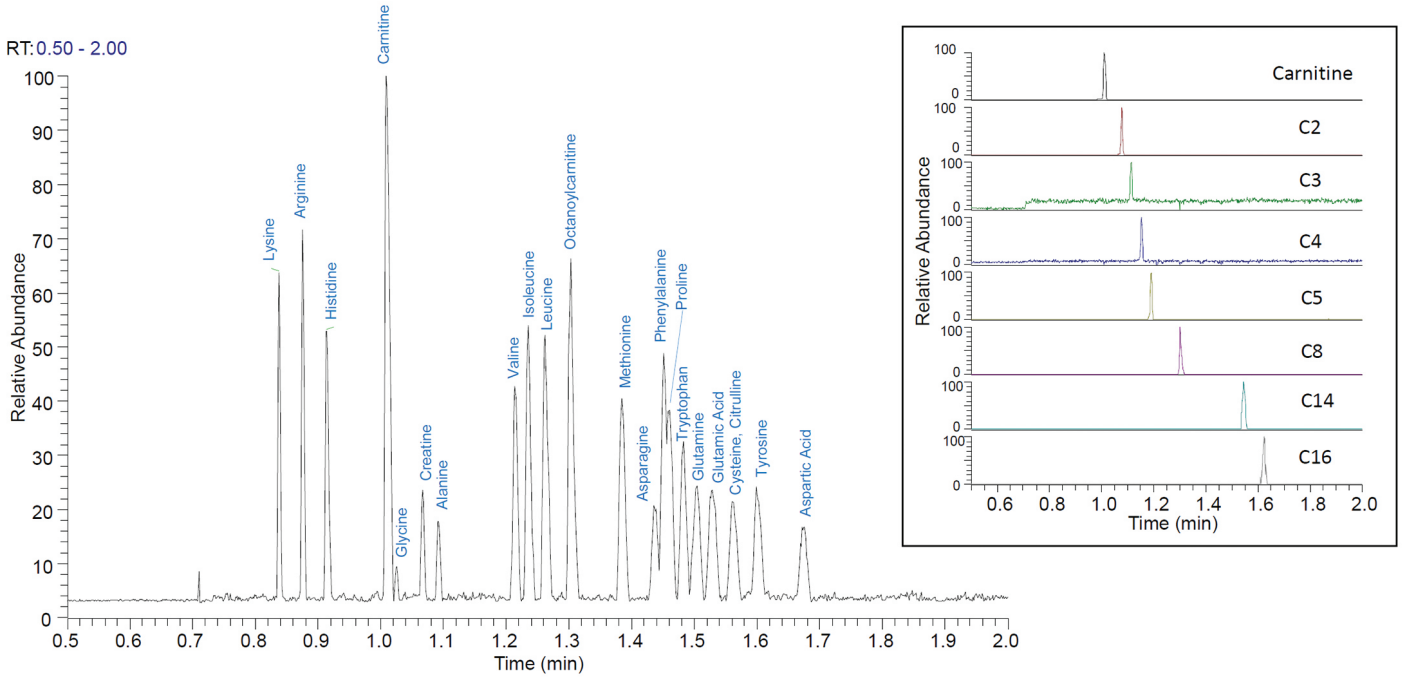

Figure 1. Microfluidic CE-MS separation of a standard mixture of amino acids and acylcarnitines. Peaks are labeled with the associated analyte(s). The inset shows extracted ion electropherograms for acylcarnitine species which are present at a lower concentration than the amino acids.

Figure 2 shows the microfluidic CE-MS analysis of dried blood spot extractions. A variety of small molecules are present in the DBS extractions, including amino acids and other metabolites. The tables in Figure 3 show the peak area ratios relative to the internal standard for DBS spiked with phenylalanine and octanoylcarnitine. These two biomarkers are indicative of the disorders phenylketonuria (PKU) and medium chain acyl CoA dehydrogenase deficiency (MCAD). The change in concentration of the targeted small molecules can be detected over a wide range of biologically relevant concentrations indicating that this technique could be useful for rapidly screening biological samples for a variety of potential disease markers.

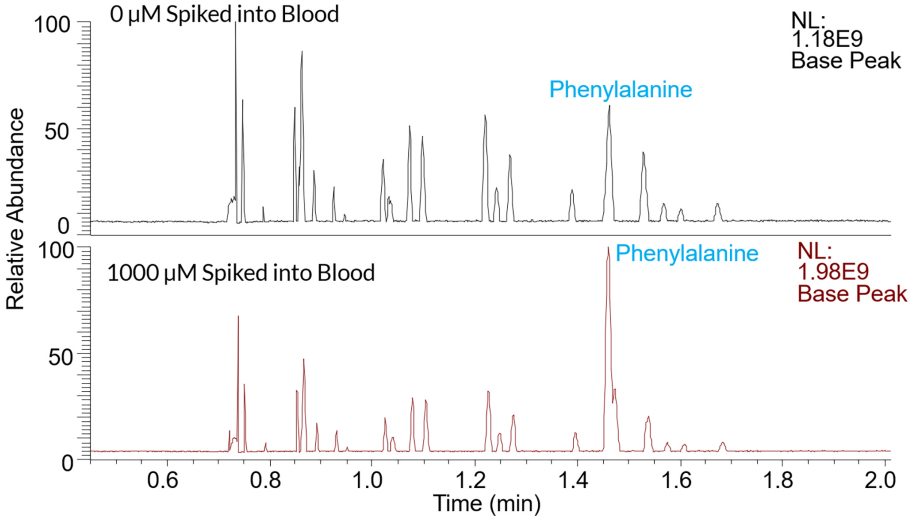

Figure 2. Microfluidic CE-MS analysis of dried blood spot extractions. The top trace shows the analysis of blood without phenylalanine spiked in. The bottom trace shows analysis of blood with 1000 µM phenylalanine spiked in.

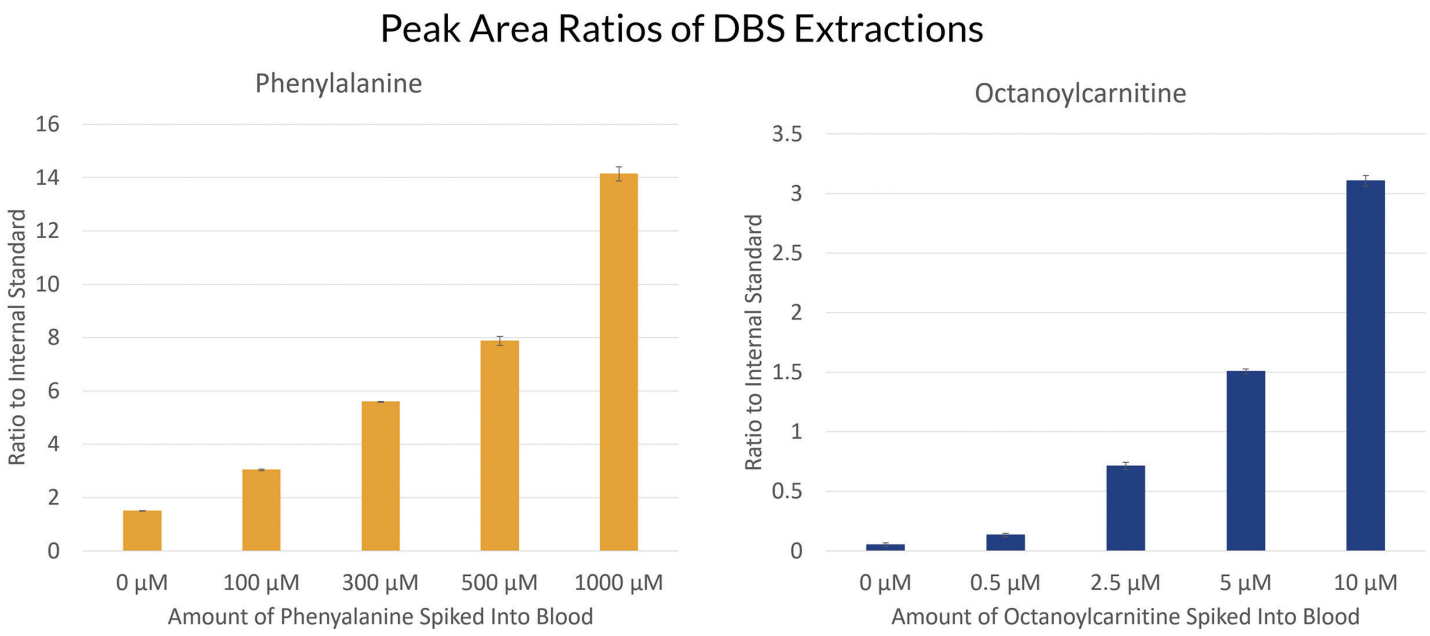

Figure 3. Peak area ratios of targeted small molecules to their internal standards. The increase in concentration due to spiking the blood samples is easily detected using the microfluidic CE-MS system.

HEMOGLOBINOPATHIES

Microfluidic CE-MS has previously been demonstrated for characterizing glycated hemoglobin as a means for assessing HbA1c levels in diabetic patients.3 Figure 4 shows an analysis of whole blood where the alpha and beta subunits are resolved as well as other minor subunits. In addition to glycated β-Hb, other types of relevant hemoglobin variants can be characterized using a similar method.

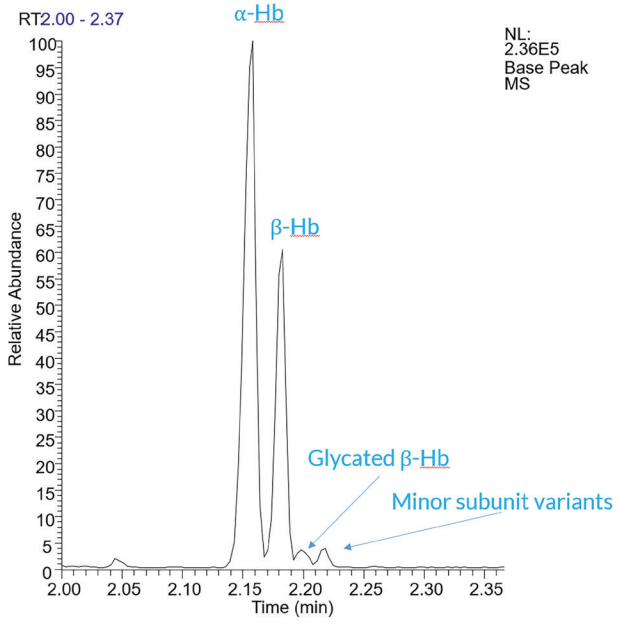

Figure 4. Microfluidic CE-MS analysis of a 500x dilution of whole blood lysate. The hemoglobin subunits are separated in ~2.5 minutes.

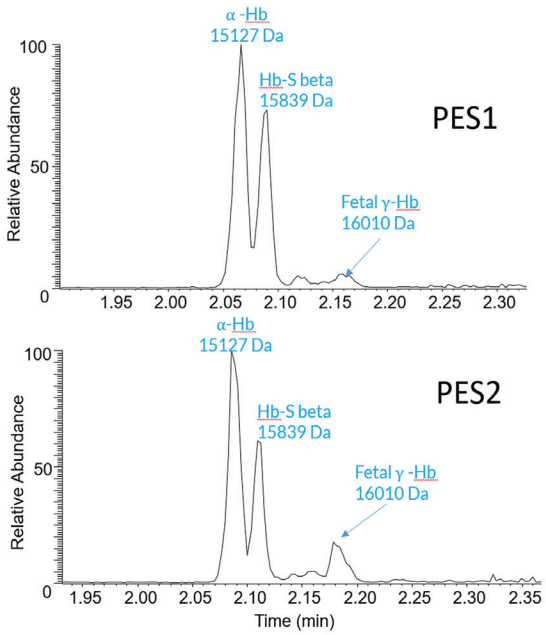

Figure 5. Microfluidic CE-MS analysis of erythrocyte lysates. In these samples Hb-S and γ-Hb were separated and detected.

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To assess the utility of microfluidic CE-MS for characterizing other hemoglobin variants, four sets of purified erythroid cells from patients suffering from Sickle Cell disease were prepared and analyzed. As shown in Figure 4, a similar pattern of subunit variants was observed, but in these samples the mass of the second peak agreed with the Sickle Cell variant of β-Hb, Hb-S. A peak for the fetal variant, γ-Hb was also observed. The levels of γ-Hb and Hb-S were found to vary between samples and the % γ-Hb was calculated for each according to the equation below. Higher levels of γ-Hb have been shown to correlate with milder symptoms in adults with Sickle Cell disease.

$$\%gamma\ Hb = \frac{Deconvoluted\ Intensity\ \gamma Hb}{\sum Deconvoluted\ Intensity\ \beta Hb\ Species}$$

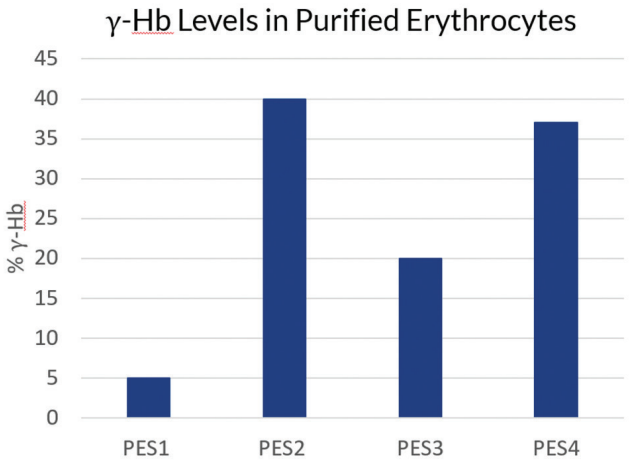

Figure 6. Calculated levels of γ -Hb with respect to the total amount of β -Hb in the samples. The amount varied from ~5% γ -Hb to ~40% γ -Hb

CONCLUSIONS

In a single microfluidic CE-MS run, multiple small molecules can be monitored to assess or identify biomarkers of disease states. In this work DBS extracts were analyzed to demonstrate applicability to a variety of real world samples. Additionally, the same system can be used to monitor protein biomarkers. To demonstrate this, hemoglobin variants from erythrocyte samples were analyzed. Alpha, beta, and gamma subunits were separated and identified. In samples from patients with Sickle Cell disease, Hb-S and γ-Hb were detected; and the %γ-Hb was calculated in each. Overall, the simple sample prep and fast run times demonstrate that microfluidic CE-MS can be an effective tool for performing assays related to small and large molecule biomarker monitoring or identification.

References:
1. Banta-Wright, S.A. et al., *Perinat Neonat Nurs*Vol. 18, No. 1, 41–58 2. Charrow, J. et al., *Genetics in Medicine*, 2000, Vol. 2 No. 4, 267-269
3. E.A. Redman et al., *Anal.Chem.*2016, 88, 5324−5330

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