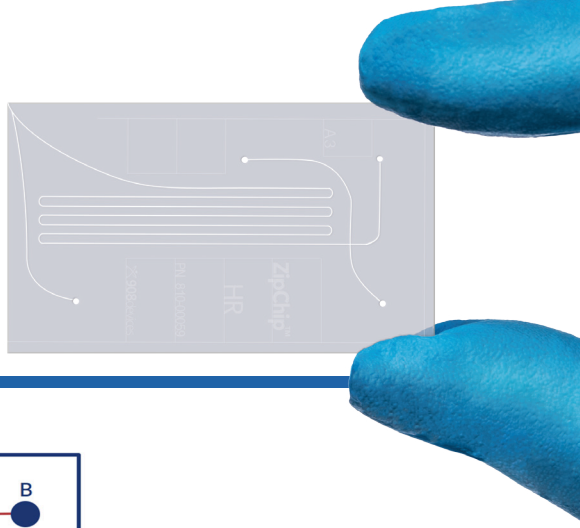
TECH NOTE 1.0

ZipChip: What They Are & How They Work



WHAT THEY ARE:

908 Devices' ZipChip™ is a microfluidic chip that integrates capillary electrophoresis (CE) with electrospray ionization. In 3 minutes or less, biological samples are quickly separated and injected into a Mass Spectrometer (MS) for analysis. ZipChips process a wide range of analytes from small molecules though intact proteins. This includes diverse matrices such as growth media, cell lysates or blood and require only a tiny sample volume for analysis. Separations are extremely efficient, providing sharp well-defined sample peaks with full MS analysis.

Each chip is a single piece of glass, about the same size and shape as a common microscope slide. These chips are fabricated using photolithography and wet chemical etching to produce microfluidic channels in a glass substrate. These channels are then enclosed by fusion bonding of a cover plate over the top of the channels. The cover plate contains small access holes to allow fluidic connection to the microfluidic channels. A schematic diagram of a ZipChip is shown in Figure 1. The design and operation of the chip can be thought of as the seamless integration of 3 major function elements: sample injection, electrophoretic separation, and electrospray ionization (ESI). Sample injections utilize the cross feature shown in the top of Figure 1; the separation occurs in the long, serpentine channel; and ESI occurs at the lower right corner of the device. ZipChip employs free zone CE, where analytes are injected as a small band at the inlet of the separation column; and are then separated by the application of an electric field along the length of the column. For these separations there is no bulk flow of solvent in the separation channel, and no interactions between the analyte molecules and any surfaces. Integration of these components on a single microfluidic chip eliminates junctions, connectors and dead volume between individual functions. These limitations cause increased analysis time and decreased resolution (or band broadening) in other liquid separation approaches. The analytes separate due to differences in electrophoretic mobility, which is simply a function of the charge and size of the analyte.

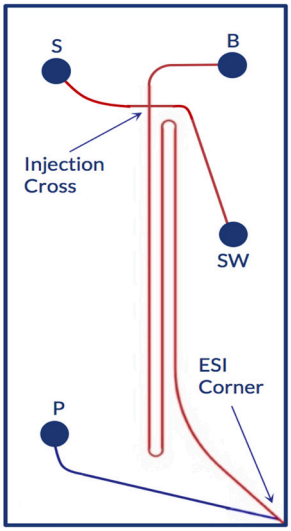


Figure 1. Schematic of a ZipChip HS.

HOW THEY WORK:

Injection: ZipChip uses a novel sample injection method that utilizes pressure-driven flow to precisely deliver aliquots of sample into the separation channel with no injection bias. Figure 2 shows a schematic that describes this injection method. This schematic represents a close up look at the injection cross labeled in Figure 1. The sample to be injected is placed in the sample reservoir labeled "S", in Figure 1. The CE background electrolyte (BGE) is placed in the background reservoir labeled "B". To introduce sample into the separation channel, head pressure is applied to reservoirs S and B, for a period of time called the loading step. The duration of the loading step can be varied (typically from about 3 – 60 seconds) to control the amount of sample loaded and increase LODs. A brief clearing step utilizing the flow of clean BGE from reservoir B, leaves a cleanly defined plug of sample in the separation channel. At this point voltage is applied to perform the electrophoretic separation. In normal operation this precisely controlled sequence is automated within the sample run and is invisible to the operation.

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Separation: Immediately following the sample injection sequence, voltage is applied to reservoirs B and P. These voltages can be controlled to dictate the electrical field strength for the CE separation and the ESI voltage. Typical operation uses a separation field strength of approximately 1,000 V/cm and an ESI voltage of 3 kV. Positively charged analytes migrate in the electric field toward the ESI orifice, while neutral and negatively charged sample matrix components move back out of the separation channel to waste.

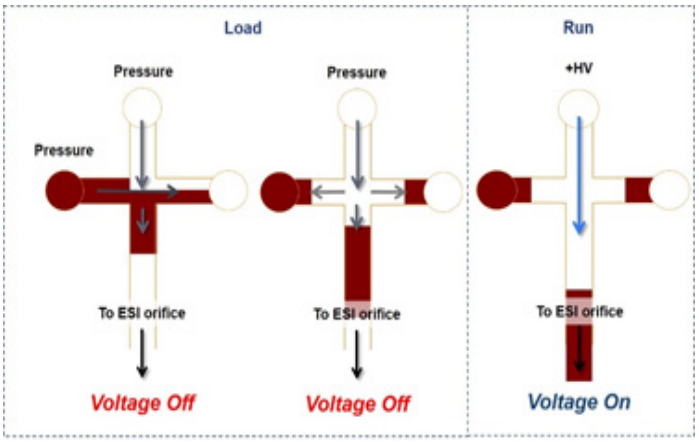


Figure 2. The pressure-driven injection scheme.

The pressure-driven injection method used by ZipChip enables on-chip desalting and on-chip sample stacking – preconcentration of the sample analytes to gain extra sensitivity. This phenomenon is known as transient isotachophoresis (tITP). To implement tITP, we only need the sample to include a component with significantly higher conductivity than the BGE. When biological samples have high salt levels, the salt itself will act as the leading electrolyte – no sample preparation steps are required as desalting happens on-chip. For samples without naturally occurring salt levels, we can add a suitable electrolyte. Ammonium acetate at a concentration of about 100 mM is an ideal choice. tITP works because the electrolyte added to the sample gives the sample a significantly higher conductivity than the BGE. Analyte ions stack up into a narrow, concentrated band at the back edge of the leading electrolyte (e.g. ammonium) as it migrates out of the initial sample zone. When the leading electrolyte fully exits the sample zone, the normal process of free zone CE begins. When biological samples have normal high salt levels, the salt itself will act as the leading electrolyte – no sample preparation steps are required, rather 'de-salting' is happening on chip. From a practical standpoint, we can simply increase the duration of our sample loading step to inject larger bands of sample and generate stronger signals in the MS. Without tITP, this would diminish our separation efficiency, but with tITP we continue to observe very efficient CE peaks with increasing peak concentration. Figure 3 shows an example of this for the analysis of amino acids.

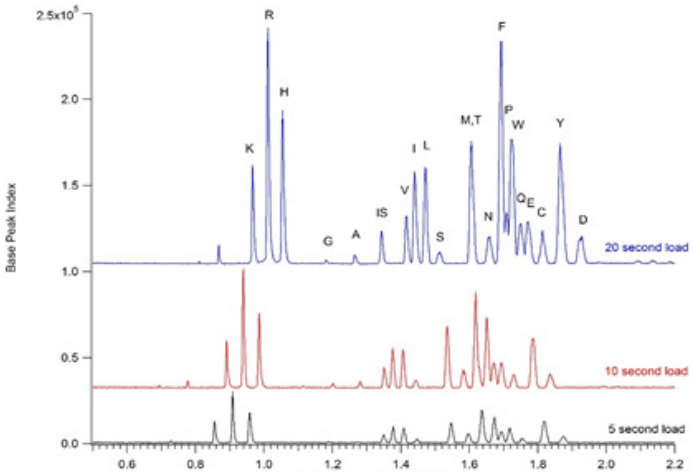


Figure 3. Electropherograms from a ZipChip HS separation of the 20 essential amino acids (5 uM). 100 mM ammonium acetate was added to the sample to enable integrated transient isotacho-phoresis. The BGE was 50% methanol, 2% formic acid (pH 2.2). Increased sample loading times yield more intense peaks with negligible effect on resolution.

ESI: The bulk flow necessary to support ESI is provided by fluid flow from well P (Pump) to the ESI corner. Depending on the application, this flow can be generated by either electoosmotic flow, or by the application of head pressure to well P. ZipChip can be operated to yield stable and sensitivity ESI at flow rates ranging from about 50 to 1000 nL/min, but are typically operated at about 150 nL/min to achieve the best combination of sensitivity and robustness. The ESI emitter of the ZipChip is formed directly on the corner of the microfluidic device. This corner is diced so that the terminal end of the separation channel is centered on this corner. Figure 4 shows an image of a ZipChip electrospraying towards the inlet of a mass spectrometer. At such low flow rates the ESI plume is not visible with normal lighting, so a green diode laser is used for illumination. The opening of the microfluidic channel is more like a wide, shallow slit than a typical cylindrical emitter. The depth of the channel is the critical dimension defining performance that is very

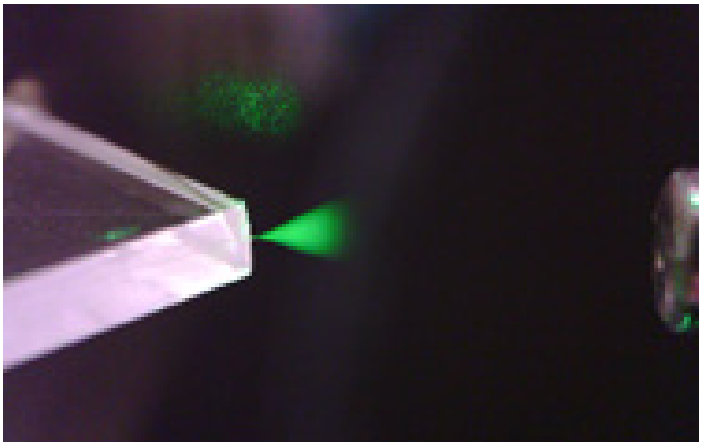
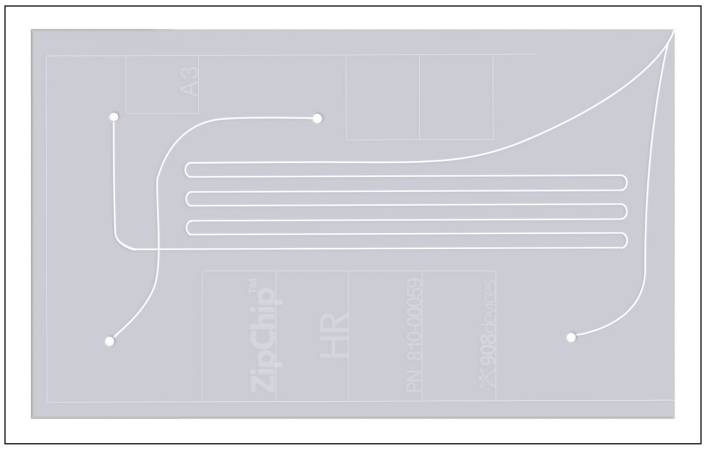


Figure 4. Image taken from the ZipChip optical system showing the ESI plume illuminated with a green laser.



ZipChip provides answers on analytes from small molecules up to intact proteins, antibodies and antibody drug conjugates.

similar to that of a 10um fused silica emitter. However, the ZipChip emitter is far more resistant to clogging than a capillary because the channel is wide and has a cross sectional area equivalent to a 30um i.d. capillary and no tapers.

ZipChip Interface: The interface uses standard mounting to attach to a conventional mass spectrometer and simplifies the analysis of samples with ZipChip. The ZipChip Interface is fully automated for push-button operation. It positions the chip correctly relative to the inlet of the MS, provides for all electrical and fluidic connections, triggers MS data collection, collects waste and even rinses the sample reservoir at the end of a run. Load your sample via pipette or autosampler and Go.

The ZipChip interface mounts easily and quickly to your existing MS instrument.

Zip Chip Benefits

- Fast Analysis – 2-3 minutes typical
- Low Sample Volume – only 10s of nanoliters required
- Analyze a wide Range of Analytes – small molecules and amino acids, peptides, intact proteins, antibodies and antibody drug conjugates (ADCs)
- Analyze a wide Range of Matrices – Cell lysates, growth media, plasma, blood urine, biopharma end-product etc.
- No sample Prep – dilute and shoot for most samples
- On-Chip Desalting – no pre-preparation required for typical biological samples

ZipChip

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