Using the PATsmart™ ZipChip® Infusion Function

Application Note

Repligen Corp. now owns the life sciences PAT product portfolio of 908 Devices Inc. Please contact Repligen for further inquiries.

Overview

The v1.3.0 release of the PATsmart™ ZipChip® software gives users the ability to use their existing ZipChip system as a static nanospray source to infuse sample for extended ESI-MS. This function can be particularly useful for long-duration mass spectrometry experiments, such as top down MS of proteins, or for tuning or optimizing your MS settings or methods.

How ZipChip Infusions Work

The new infusion function pushes sample into the microfluidic separation channel at the same time that the electric field is applied. So rather than forming a discreet band of sample which separates as it migrates down the channel, infusions generate a constant stream of sample for as long as the infusion is running. The infusion function can be run with any of the existing types of ZipChip chips, but the process will be faster on High Speed (HS) ZipChip chips. A screenshot of the infusion page of ZipChip application is shown in Figure 1.

ZipChip infusions push all components of the sample matrix into the separation channel; and the infusion process does not separate analytes from salts or surfactants the way that ZipChip separations do. Therefore, it is very important that you follow the Infusion Rules posted on the infusion page of the ZipChip app (Figure 1).

Figure 1. Screen shot of the infusion page of the ZipChip application, version 1.3.0. The image is from a ZipChip System with an autosampler. Note that the *Rinse Sample Well* button is not available for manual units.

The sample needs to be diluted into the same background electrolyte that was used to prime the chip, otherwise mismatches in electrical conductivity and pH could damage the chip. High concentrations of proteins can also lead to obstruction of the ESI orifice. We therefore recommend that limiting the concentration of proteins to 0.1 mg/mL or less, and promptly clearing the separation channel at the end of the infusion.

Example ZipChip Infusion: Native NIST mAb on an HRN Chip

The infusion process is particularly useful for optimizing mass spec settings for native protein analysis. Our application scientists run this specific infusion process whenever they attempt native antibody work on a new mass spec for the first time. Because it uses the same BGE and chip type as our ZipChip native antibody separation methods, users can switch from infusion to separation without having to re-prime a chip.

Preparing the chip and the sample

Begin by priming an HRN chip with Charge Variant Analysis BGE*. Dilute 10 µL of the NIST Monoclonal Antibody Reference Material with 90 µL of LC-MS grade water. This creates a 1 mg/mL sample which can later be used for ZipChip separations. **Do not attempt to use this sample for infusions**. It is too concentrated, and its sample matrix does not match the BGE in the chip. Dilute 10 µL of the 1 mg/mL sample with 90 µL of Charge Variant Analysis BGE.

**The Charge Variant Analysis BGE has replaced the Native Antibody BGE since publication.*

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This creates a 0.1 mg/mL sample which is appropriate for ZipChip infusion. The sample must be manually loaded into the chip. Slide the chip out of the chip manifold and manually remove any liquid that is present in the sample well (see Figure 2) of the chip. Pipet 20 to 40 µL of the 0.1 mg/mL sample into the sample well, then lock the chip back into the chip manifold.

Acquiring data during the infusion process

To demonstrate all of the features of the ZipChip infusion, this example includes acquisition of MS data for the entire process. The Acquisition settings shown in Figure 3 were used. Note that a method file was not used so that the settings could be adjusted in real time. Also note that the acquisition time was set to *continuously*, and *On start* was set to *don't wait*. The ZipChip infusion process does not trigger the mass spec to start data collection the way that a separation method does, so do not set the MS to wait for a contact closure signal. The continuous acquisition means that the data acquisition must be manually stopped when finished.

Running the Infusion

On the infusion page of the ZipChip app (Figure 1), set the *Infusion Field Strength* to 500 V/cm, then press the *Start Infusion* button. The electrospray process begins immediately. For this example, the *Start* button on the MS Tune Acquisition

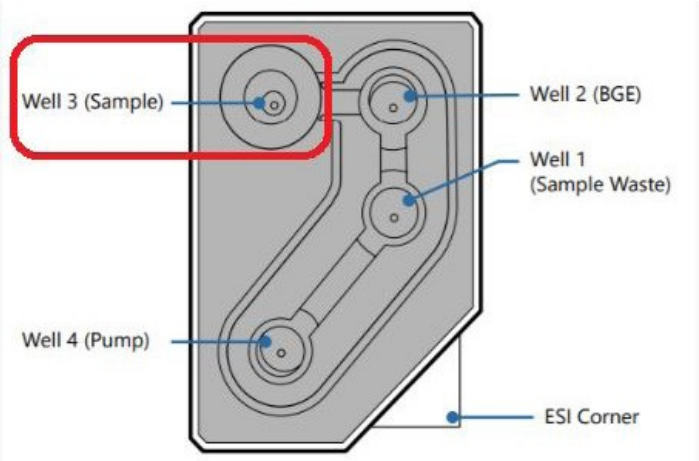

Figure 2. ZipChip schematic showing location of the sample well.

was pressed immediately after starting the infusion. Figure 4 shows the base peak ion signal recorded during the infusion process. Note that it takes about five minutes before the antibody signal is detected by the mass spectrometer. This is the time it takes for the antibody molecules to migrate down the separation channel. Once the antibody reaches the ESI orifice, a constant mass spec signal is detected for as long as the infusion process is run. Figure 5 shows a mass spectrum from this example infusion of the NIST mAb. For this example, the *Stop* button on the infusion page was pressed 15 minutes after the infusion was started and about 10 minutes after the antibody was first detected. If necessary, the sample can be infused for longer times, but it is recommended to refresh the BGE and replace the sample every hour. As seen in Figure 4, the MS signal immediately dropped to zero when the *Stop* button was pressed, since the electrospray process stopped. At that point the entire separation channel was still full of sample, so the *Clear Channel* button was immediately pressed to begin emptying the channel. It took an additional 6–7 minutes for the residual antibody to fully clear out of the separation channel. At this point, the microfluidic channels of the chip are clean. To proceed with ZipChip separations or

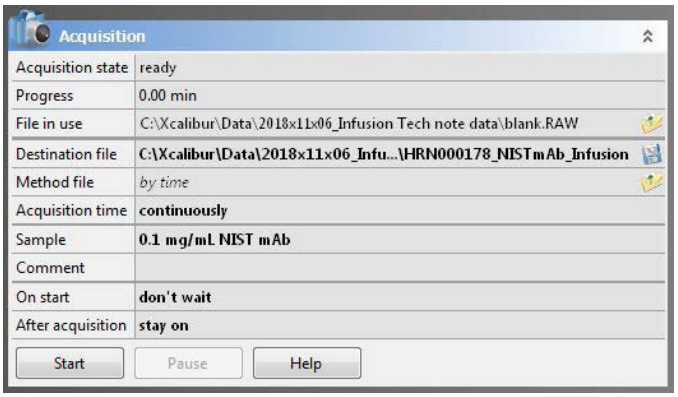

Figure 3. MS Tune Acquisition settings.

another infusion experiment, simply rinse the sample well and refresh the BGE.

Summary

The ZipChip system can now be used as a static nanospray source for infusing samples. The new infusion page in the ZipChip software provides a quick and easy way to infuse samples with the same hardware and consumables used for ZipChip separations. ZipChip infusions can be used for a wide variety of applications such as topdown MS/MS fragmentation, ultra-high-resolution MS characterization, or optimization of MS settings for new assays. This feature, combined with ZipChip separations, makes the system a powerful and versatile tool well-suited for in-depth characterization of complex samples.

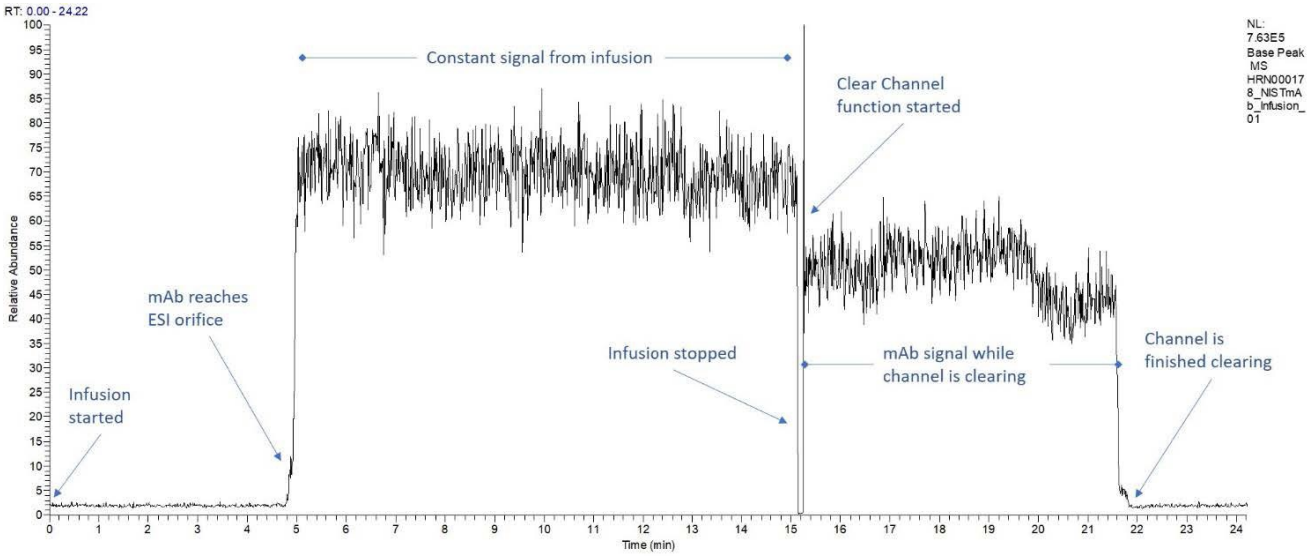

Figure 4. Mass spec base peak intensity observed during the example infusion of the NIST mAb on an HRN chip.

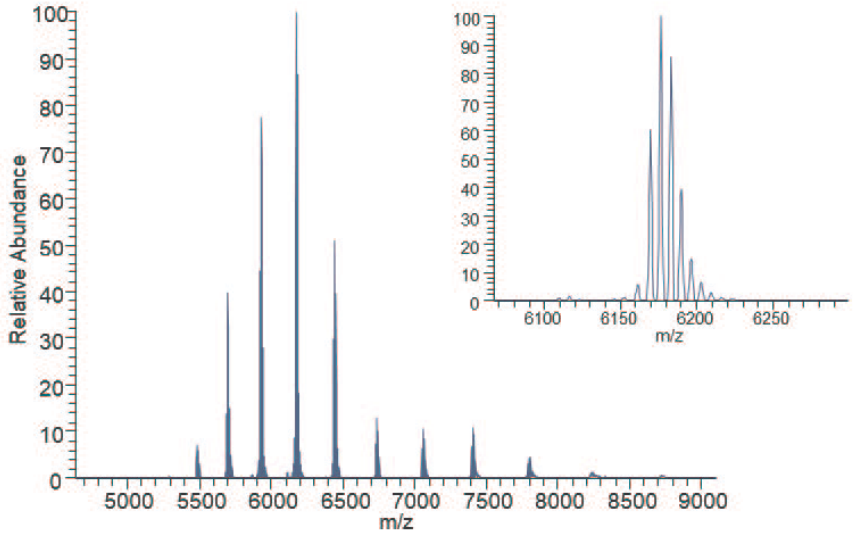

Figure 5. Mass Spectrum from NIST mAb infusion. The inset is a zoomed view of the most intense charge state.

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