PicoClear™ Union Technology Applications for Nano ESI-MS Molecules

Carla J. Marshall-Waggett, Adam W. Perala, Christopher J. Toher, Gary A. Valaskovic
New Objective, Inc., Woburn, MA

Introduction

Optimizing nanobore ESI-MS warrants careful attention to minimizing pre- and post-column volumes. As bores approach 100 µm, insufficient mechanical tolerance of conventional ferrule fittings and adapter sleeves can produce surface defects and particulates from unwanted rotary motion and tightening. Under high operating pressures, microfluidic coupling elements with inert fluoropolymer cores eliminate connector dead volume, maintain axial alignment, and facilitate flush and durable connections between fused-silica tubing.

Methods & Materials

Instrumentation & Components
- 360 µm OD / 75 µm ID fused-silica tubing
- Optical Microscope (Olympus® BH-2)
- PicoClear™ union for 360 µm OD tubing with inert fluoropolymer core (New Objective)
- Harvard Apparatus PHD2000 Infuse/Withdraw Syringe Pump
- 360 µm OD / 75 µm ID Self-Pack IntegraFrit™ column (New Objective)
- Nanobore column with integral frit, 360 µm OD, 75 µm ID, 15 µm tip ID (PicoFrit®, New Objective) with 5 cm and 10 cm beds containing ProteoPep™ II C18 (New Objective)
- Nanobore column, 360 µm OD, 75 µm ID with 5 cm bed containing ProteoPep II C18 (New Objective)
- Nanospray source (Digital PicoView® 150, New Objective)
- High-pressure injection platform (PIP-500, New Objective)

Zero Dead Volume Tubing Connection
- Two identical pieces of 360 µm OD / 75 µm ID fused-silica tubing were joined together via the PicoClear union.
- Optical microscopy was employed to record joint quality.

Inline Particulate Monitoring
- 360 µm OD / 75 µm ID fused-silica tubing with inline silica frit (Self-Pack IntegraFrit, New Objective) connected to a syringe pump
- The fritted end of the IntegraFrit was joined via the PicoClear union to 360 µm OD / 75 µm ID fused-silica tubing
- The PicoClear union was positioned under an optical microscope with the frit-tubing junction in view.
- Particulate collection was monitored by pumping a suspension at 250 L/min through the IntegraFrit column into fused-silica tubing

Bed Augmentation and High-Pressure On-Column Injection
- A 360 µm OD / 75 µm ID / 15 µm tip ID PicoFrit column was cleaved at the 5 cm bed terminus containing ProteoPep II C18.
- The fritted end of a 360 µm OD / 75 µm ID IntegraFrit with 5 cm bed of ProteoPep II was connected to the PicoFrit column via the PicoClear union.
- Chromatographic separation performance of the resulting column combination was assessed via online nanobore ESI-MS.
Results

The PicoClear™ union allowed visual confirmation of flush connectivity between adjoining pieces of fused-silica tubing (Figures 1-3). The visibility of the tubing connection ensures the inexistence of dead volume. Using a Self-Pack IntegraFrit™ connected to fused-silica tubing served as an inline filter and facilitated observation of clog-inducing particulate accumulation (Figure 5).

To confirm dead volume absence, a medium molecule test mixture was first analyzed using a PicoFrit® column containing 10 cm of ProteoPep™ II packing. The same sample was then analyzed on an IntegraFrit column containing 5 cm ProteoPep II packing adjoined via the PicoClear union to a PicoFrit column containing 5 cm of ProteoPep II (Figure 6). Evaluation of retention times and peak responses revealed identical performance for the single and joined columns (Figure 7).

Observations/Conclusions

• The PicoClear™ union creates quality tubing connections with visible absence of dead volume.
• Using a PicoClear union with a Self-Pack IntegraFrit™ and fused-silica tubing facilitates monitoring the collection of inline particulates and enhances troubleshooting capacity.
• Connecting a 5 cm IntegraFrit column to the 5 cm bed terminus of a PicoFrit® column enhances separating capability without compromising chromatographic performance.
• Using the PicoClear union for on-column injections minimizes contamination, consequent column life reduction, and prevents tip damage to the PicoFrit column.