

## High-Pressure, Transparent Microfluidic Coupling Method for Nanobore LC-MS

Jeffrey J. Wynn, Christopher J. Toher, Adam W. Perala, Gary A. Valaskovic

New Objective, Inc., Woburn, MA

### Introduction

Optimizing nanobore ESI-MS necessitates careful attention to minimization of pre- and post-column volumes. As bores go below 100  $\mu\text{m}$ , insufficient mechanical tolerance of conventional ferrule fittings and adapter sleeves can produce surface defects and particulates from induced rotary motion and tightening. Microfluidic coupling elements with inert fluoropolymer cores eliminate connector dead volume, maintain axial alignment, and facilitate flush and durable connections between fused-silica tubing under high operating pressures.

Constructed of optically clear materials, connections can be confirmed by visual inspection while yielding unprecedented analytical efficacy. In addition to creating and verifying a zero-dead-volume connection between two fused-silica tubes of identical inner diameter, clear union performance was examined for monitoring collection of clog-inducing particulates, connecting a nanobore column to a second column with an integrally fritted tip, and extending PicoFrit<sup>®</sup> column life when using high pressure on-column injection methods. A second novel fluoropolymeric-based coupling device combines these advantages with a high-voltage junction contact.

### Methods & Materials

#### *Instrumentation & Components*

- 360  $\mu\text{m}$  OD / 75  $\mu\text{m}$  ID fused-silica tubing (Polymicro Technologies)
- Optical Microscope (Olympus<sup>®</sup> BH-2)
- PicoClear<sup>™</sup> union for 360  $\mu\text{m}$  OD tubing with inert fluoropolymer core (New Objective)
- PicoClear conductive union for 360  $\mu\text{m}$  OD tubing with inert fluoropolymer core and platinum wire for liquid-junction contact (New Objective)
- Harvard Apparatus PHD2000 Infuse / Withdraw Syringe Pump
- 360  $\mu\text{m}$  OD / 75  $\mu\text{m}$  ID Self-Pack IntegraFrit<sup>™</sup> column (New Objective)
- Nanobore column with integral frit, 360  $\mu\text{m}$  OD, 75  $\mu\text{m}$  ID, 15  $\mu\text{m}$  tip ID (PicoFrit<sup>®</sup>, New Objective) with 5 cm and 10 cm beds containing ProteoPep<sup>™</sup> II C18 (New Objective)
- Nanobore column, 360  $\mu\text{m}$  OD, 75  $\mu\text{m}$  ID with 5 cm bed containing ProteoPep II C18
- Nanospray source (Digital PicoView<sup>®</sup> 150, New Objective)
- High-pressure injection platform (PIP-500, New Objective)

#### *Zero Dead Volume Tubing Connection*

- Two identical pieces of 360  $\mu\text{m}$  OD / 75  $\mu\text{m}$  ID fused-silica tubing were joined together via the PicoClear<sup>™</sup> union and PicoClear<sup>™</sup> conductive union.

- Optical microscopy was employed to record joint quality.

#### *Inline Particulate Monitoring*

- 360  $\mu\text{m}$  OD / 75  $\mu\text{m}$  ID fused-silica tubing with inline silica frit (Self-Pack IntegraFrit<sup>™</sup>, New Objective) connected to a syringe pump
- The fritted end of the IntegraFrit was joined via the PicoClear<sup>™</sup> union to 360  $\mu\text{m}$  OD / 75  $\mu\text{m}$  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 nL/min through the IntegraFrit column into fused-silica tubing

#### *Bed Augmentation and High-Pressure On-Column Injection*

- A 360  $\mu\text{m}$  OD / 75  $\mu\text{m}$  ID / 15  $\mu\text{m}$  tip ID PicoFrit<sup>®</sup> column was cleaved at the 5 cm bed terminus containing ProteoPep<sup>™</sup> II C18.
- The fritted end of a 360  $\mu\text{m}$  OD / 75  $\mu\text{m}$  ID IntegraFrit<sup>™</sup> with a 5 cm bed of ProteoPep II C18 was connected to the PicoFrit column via the PicoClear<sup>™</sup> union.
- Chromatographic separation performance of the resulting column combination was assessed via online nanobore ESI-MS.

## Results

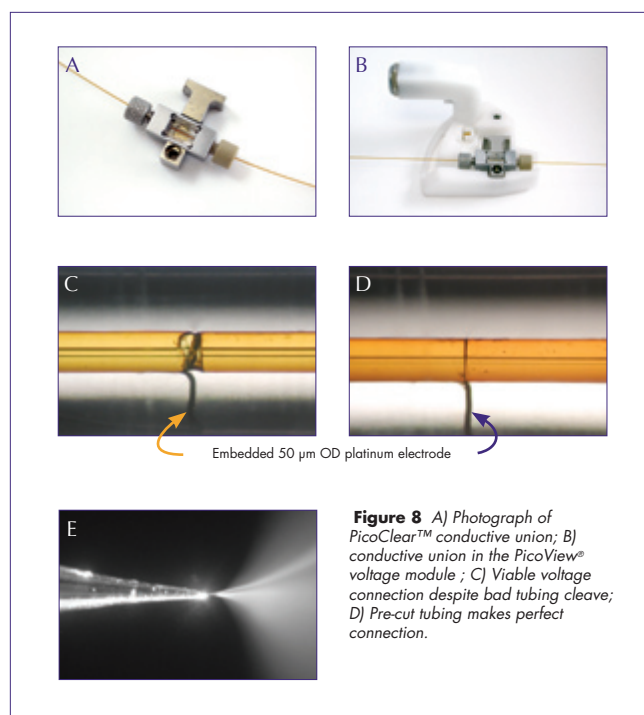
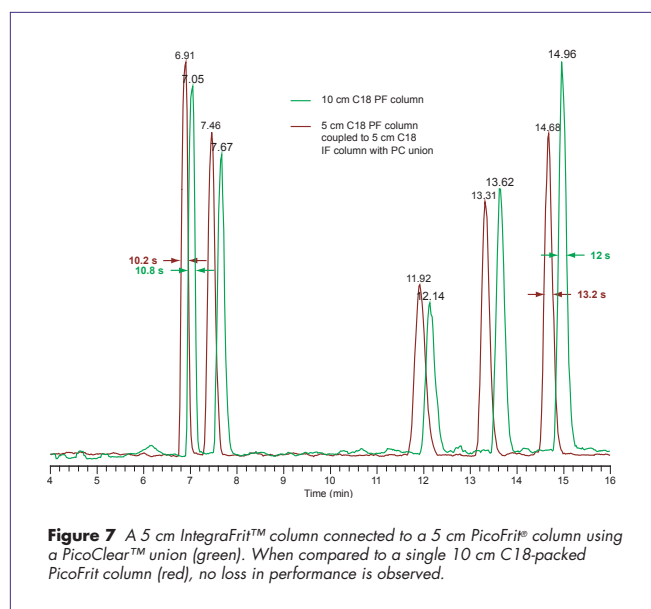
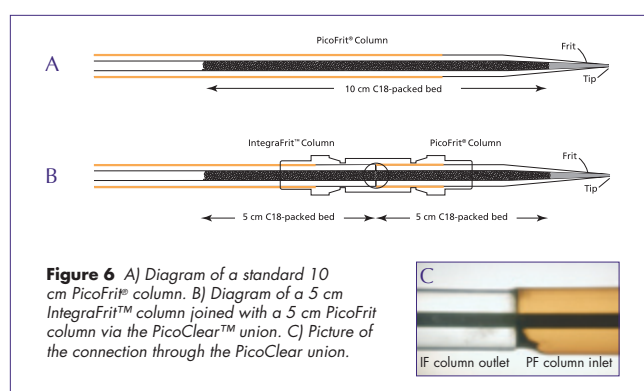
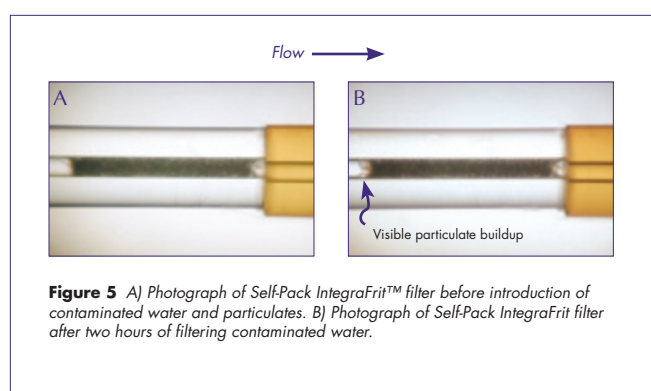
### PicoClear™ Clear Union

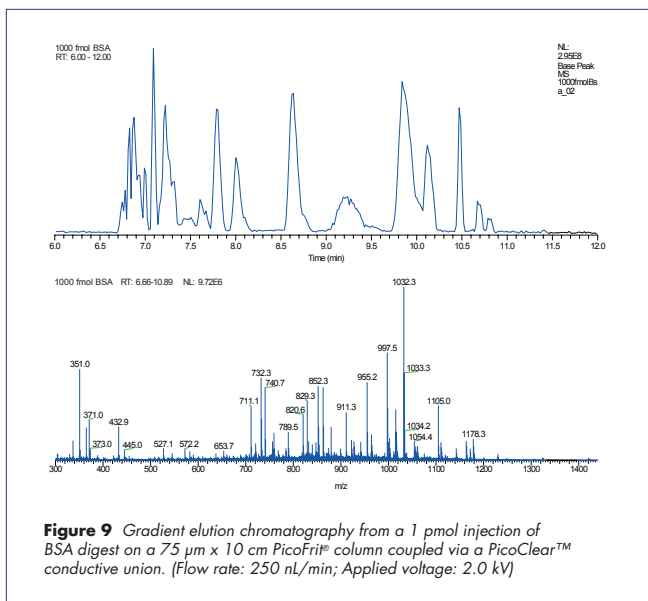
The PicoClear union allowed visual confirmation of flush connectivity between adjoining pieces of fused-silica tubing (Figures 1-3). The visibility of the tubing connection ensures the inexistence of dead volume. A Self-Pack IntegraFrit™ connected to fused-silica tubing served as an inline filter and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

To confirm the absence of dead volume, a medium molecule test mixture was first analyzed using a PicoFrit® column containing 10 cm of ProteoPep™ II C18 packing. The same sample was then analyzed using 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).

### PicoClear™ Conductive Union

A transparent conductive union was connected via fused-silica tubing to a syringe pump producing a 50:50 ACN/water flow at 250 nL/min. When connected to a high-voltage source, leak resistance and electrospray production of the high-voltage junction contact was monitored under a stereomicroscope.





## Observations/Conclusions

- The PicoClear<sup>™</sup> union creates quality tubing connections with visible absence of dead volume.
- Using a PicoClear union with a Self-Pack IntegraFrit<sup>™</sup> 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<sup>®</sup> 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.
- The transparent conductive union displayed superior coupling with no observable dead-volume when two pieces of machine-cut fused silica tubing were joined and inspected under a light microscope.
- In addition to no evidence of leakage, the clear conductive union produced high-quality electrospray with a distinct Taylor cone, jet, and radiant plume.