

PicoClear™ Union Technology Applications for Nano ESI-MS Molecules

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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.



FIGURE 1 A PicoClear™ Union

FIGURE 2 The PicoClear™ union provides visual confirmation of tubing connection

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® column life when using high pressure on-column injection methods.

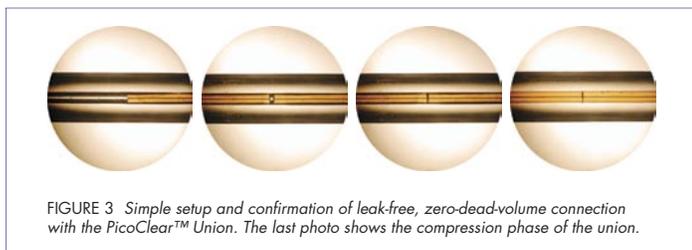


FIGURE 3 Simple setup and confirmation of leak-free, zero-dead-volume connection with the PicoClear™ Union. The last photo shows the compression phase of the union.

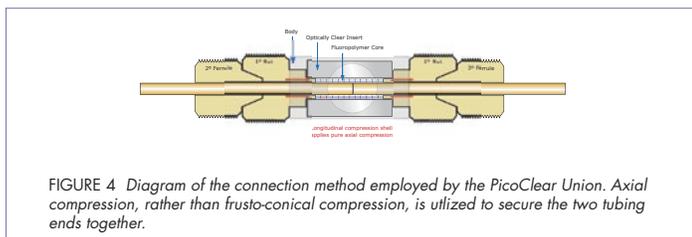


FIGURE 4 Diagram of the connection method employed by the PicoClear Union. Axial compression, rather than frusto-conical compression, is utilized to secure the two tubing ends together.

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 5cm 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).

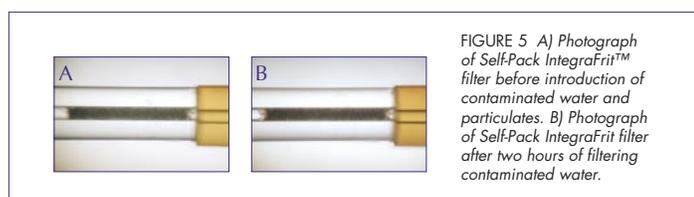


FIGURE 5 A) Photograph of Self-Pack IntegraFrit™ filter before introduction of contaminated water and particulates. B) Photograph of Self-Pack IntegraFrit filter after two hours of filtering contaminated water.

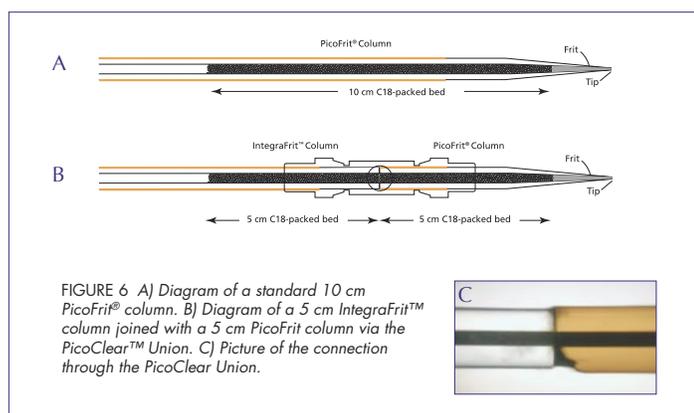


FIGURE 6 A) Diagram of a standard 10 cm PicoFrit® column. B) Diagram of a 5 cm IntegraFrit™ column joined with a 5 cm PicoFrit column via the PicoClear™ Union. C) Picture of the connection through the PicoClear Union.

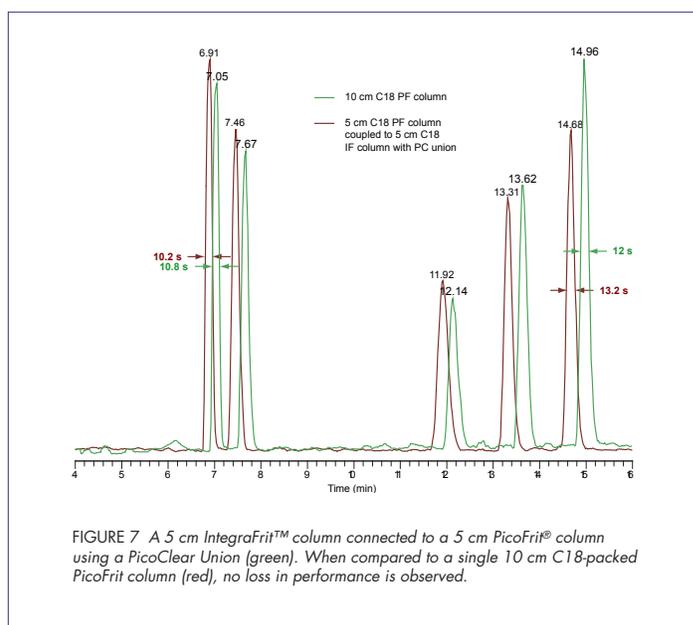


FIGURE 7 A 5 cm IntegraFrit™ column connected to a 5 cm PicoFrit® column using a PicoClear Union (green). When compared to a single 10 cm C18-packed PicoFrit column (red), no loss in performance is observed.

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.