Handling IntegraFrit™ Columns

There are unique considerations in the handling and use of IntegraFrit™ columns. IntegraFrit columns are fabricated from 360 µm OD, polyimide-coated, fused-silica tubing as shown in Figure 1. The frit end of the column has an integral high-porosity frit. The edge of the fused-silica tubing at the frit end has been polished flat. Behind the frit is the packed chromatography bed. There is no frit at the distal, or back, end of the bed, only unpacked fused-silica tubing.

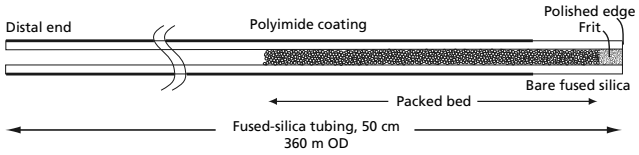

FIGURE 1 IntegraFrit™ Column

Packaging

The IntegraFrit™ column is packaged as a loop, with the frit and distal ends connected and protected by a sleeve of snug-fitting FEP (Teflon®) tubing as shown in Figure 2. The clear FEP sleeve keeps the column hydrated with methanol during shipping and storage.

Guidelines for Handling IntegraFrit™ Columns

- Never bend the clear FEP sleeve. Bending the sleeve will result in column failure.
- The column loop should not be opened until the column is being prepared for installation and conditioning.
- Mobile phase flow must always be directed towards the frit. Reversing the flow may result in partial or complete unpacking of the chromatography bed.
- For long term storage, remove the column from the box and hang the loop vertically with the FEP sleeve nearest to the floor.

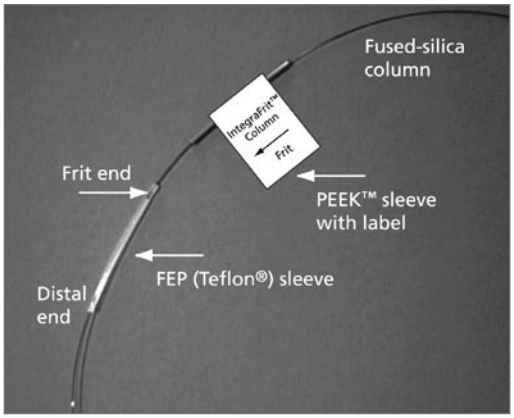

FIGURE 2 Packaging of an IntegraFrit™ Column

Connecting to the IntegraFrit™ Column Outlet

You have two choices for connecting the outlet of the IntegraFrit™ column to your detector (UV, MS etc.):

- Remove the IntegraFrit column from the FEP sleeve and use a MicroTight® or similar union designed to connect two pieces of capillary tubing. To follow this option use the directions below, “Removing the FEP Sleeve from the IntegraFrit Column”.
- Leave the FEP sleeve in place on the column outlet and modify it for use as a direct-connect, zero dead volume (ZDV) union. To follow this option use the directions on the facing page, “Converting the FEP Sleeve to a ZDV Union”.

Sample Trap Injection for Improved MS Spectra & Hardware Longevity

Optimizing sensitivity, spectral quality, and hardware longevity for nanobore ESI-MS applications depends largely on sample composition and purity. Where many factors require consideration in obtaining a desirable spray (see Technical Note PF-4, "Spray Optimization"), signal-suppressing salt removal and pre-injection analyte concentration can further enhance spectroscopic data collection and even extend the service time of exisiting hardware.

Advantages of sample trap injections

New Objective provides 2.5 cm sample trap columns available in 75, 100, and 150 µm inner diameters (IDs). Columns are packed with New Objective's premier chemistries: ProteoPep II™, ProteoPep™, BioBasic®, AQUASIL, or strong cation exchange (SCX). With the exception of AQUASIL, all sorbents have an average particle size of 5 µm and a 300Å pore size. AQUASIL has a pore size of 100Å. Custom column packing is available by special order. The column is inserted into the UpChurch® Scientific Trap Cartridge prior to its installation in an online configuration. See Technical Note IF-2 on using the cartridge with the sample trap.

Because nanobore ESI-MS applications measure analytes at subfemtomole levels, obtaining requisite sample concentration for analyte detection is of paramount concern. Sample injection onto a sample trap promotes analyte adsorption onto the reverse-phase sorbent, thereby concentrating it within the trap. In addition, ionic species which can bind to the analyte and suppress its detection are washed away from the adsorbed sample and jettisoned via the waste outlet. While optimal wash times vary between samples, a 3–5 minute minimum is common practice. Further, the sample trap acts as an additional particulate filter immediately prior to column introduction, significantly reducing clog formation and extending column lifetime.

Achieving maximum product performance and longevity

Concentrating and desalting the analyte by sample trap injection provide noticeable differences in chromtographic peak shape and resolution. Figure 1A displays the chromatogram of a bovine serum albumen (BSA) sample injected via a sample loop without a trap; Figure 1B displays the same BSA sample injected onto a sample loop with a sample trap. Methanol was employed as an organic modifier in both experiments[1].

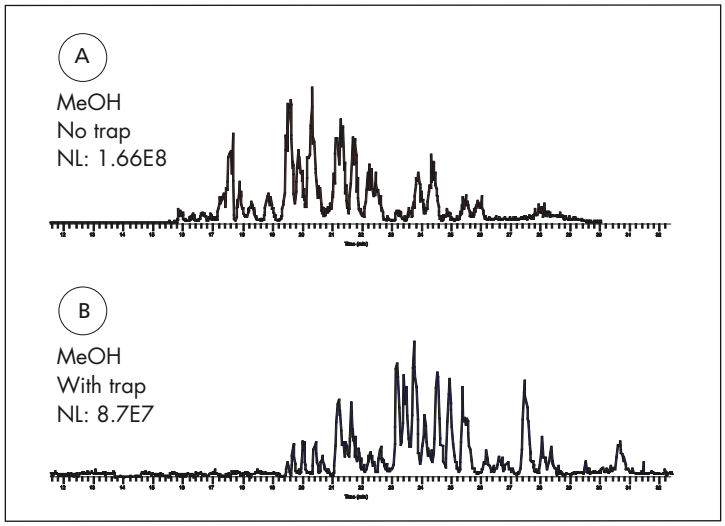

FIGURE 1

When coupled with the inline microfilter and nanofilter recommended in Tech Note PF-4 “Spray Optimization”, sample trap injections can prevent the formation of clogs and dramatically extend the service time of the nanobore LC column. Figure 2 displays nanobore LC-MS data collected after two days (Figure 2A - Injection #2) and after months of continuous operation (Figure 2B - Injection #1630).

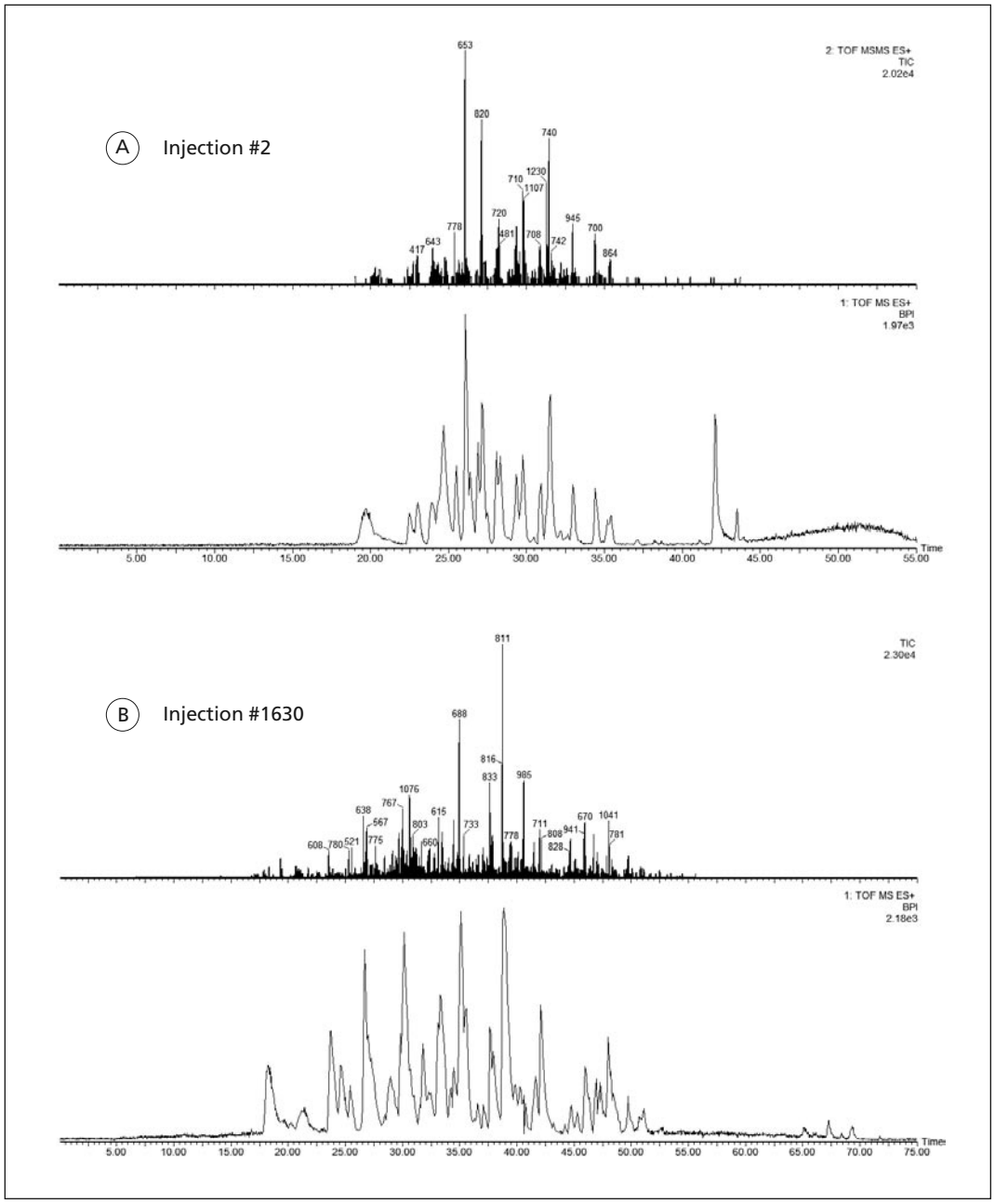

FIGURE 2 *LC-MS data collected over a period of several months with a single PicoFrit® column*

The data were collected with a 75 µm x 4 µm x 10 cm C18 PicoFrit® column with an inline nanofilter, microfilter, and 1 mm C18 trap cartridge on a Micromass® Q-Tof™ mass spectrometer. Figure 3 displays the complete configuration used in data acquisition.

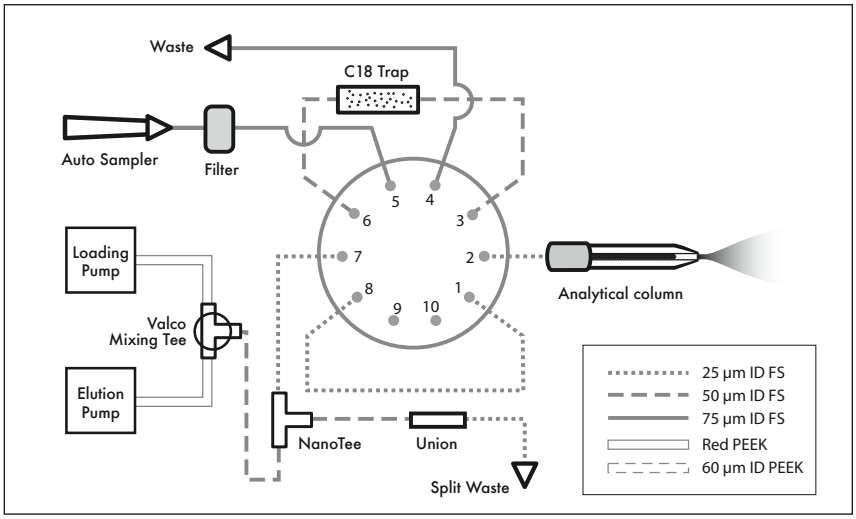

FIGURE 3 *Nanobore LC-MS configuration for data collected in Figure 2*

Procedure for sample trap injection

The following steps illustrate the loading, washing, and injection procedure using a sample trap. For this task, a 10-port valve is assumed; Positions 1 and 2 are the *load* and *inject* settings, respectively.

1) With the valve configured in Position 1, load the sample into the sample loop (Figure 4).
2) Switch the valve to Position 2. High flow rate carries the sample from the sample loop to the sample trap for washing, desalting, and analyte concentration (Figure 5).
3) After running the the mobile phase through the sample trap for several minutes, restore the valve to Position 1 (Figure 6).
4) Activate the LC gradient to introduce the analyte onto the column from the sample trap. The electrospray emitter introduces the sample into the MS inlet for analysis.

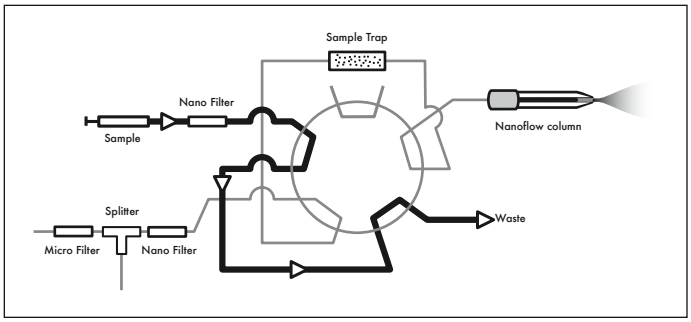

FIGURE 4 *Loading the sample loop (valve in Position 1)*

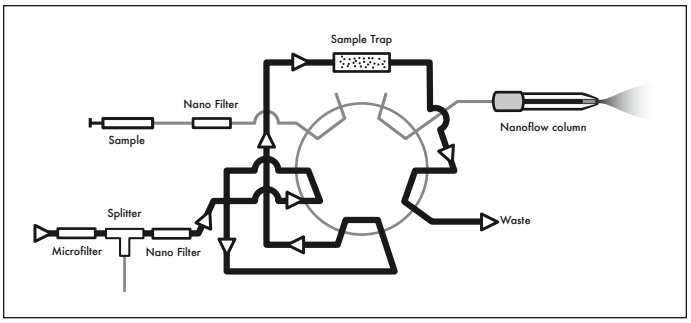

FIGURE 5 *Loading sample onto trap for concentration and desalting (valve in Position 2)*

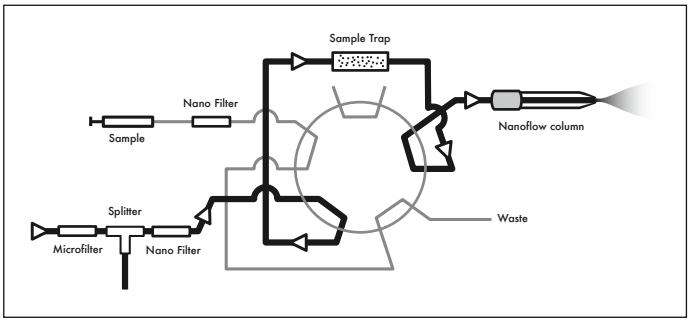

FIGURE 6 *Loading sample onto column for analysis (valve in Position 1)*

New Objective graciously acknowledges Dr. Daniel Boismenu of McGill University for LC-MS data and system configuration details.

1. Perala, A.W.; Toher, C.J.; Valaskovic, G.A. "Enhanced Nanobore LC-MS Using Methanol Gradient Elution With Peptide Mixture." Poster presented at The American Society for Mass Spectrometry, San Antonio, Texas, 2005

Handling Self-Pack IntegraFrit™ Columns

Self-Pack IntegraFrit™ Columns are designed for customers custom-packing their own nanobore liquid chromatography columns. Self-Pack IntegraFrit Columns are 50cm-long 360 µm outer diameter (OD) fused-silica tubes with a porous, sintered glass frit at one end. The edge of the fused-silica tubing at the frit has been polished flat; the distal or back end of the fused-silica capillary is open and contains no frit (Figure 1). Depending on desired flow rate, Self-Pack IntegraFritTM Columns are available in 50 µm, 75 µm, 100 µm, and 150 µm inner diameters (IDs).

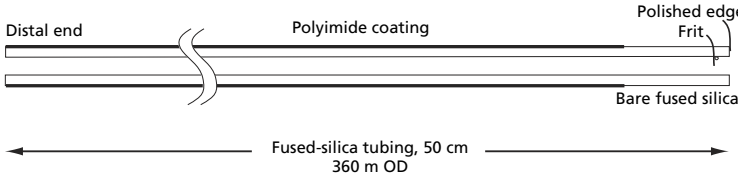

FIGURE 1 IntegraFrit™ Self-Pack Column prior to packing

Once packed, the chromatography bed extends from immediately behind the frit and terminates at a bed length of the user's designation (Figure 2).

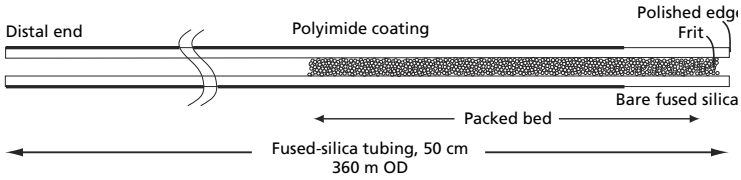

FIGURE 2 IntegraFrit™ Self-Pack Column packed

Packaging of Self-Pack IntegraFrit™ Columns

Self-Pack IntegraFrit™ Columns are packaged in a plastic box (Figure 3) with the delicate fritted ends threaded through an elevated plastic mounting block to avoid damage (Figure 4). An FEP sleeve surrounds the tubing emerging from the back of the mounting block and extends 25 cm over the length of the tubing; remaining fused-silica tubing is curled into a loop near the distal end of the IntegraFrit.

FIGURE 3

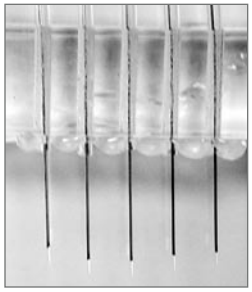

FIGURE 4

Guidelines for Handling Self-Pack IntegraFrit™ Columns

1). Never bend the clear FEP sleeve

2). Once packed with stationary phase by the user, mobile phase flow must always be directed towards the frit. Reversing the flow may result in partial or complete unpacking of the bed.

CAUTION: Handling fused-silica tubing can result in serious personal injury, including eye and skin injury. Use safety goggles meeting ANSI Z87.1-1989 requirements, or the equivalent. Puncture and chemical-resistant gloves should also be worn at all times.

Removing Self-Pack IntegraFrit™ Columns from Packaging

1). Apply a pair of forceps to a location about 2cm away from the fritted end.

2). Pull the column forward until the distal end slides out of the FEP tubing and out of the block (Figure 5).

WARNING: Do not touch the fritted end of the IntegraFrit column to any surface. It is extremely delicate and breaks easily.

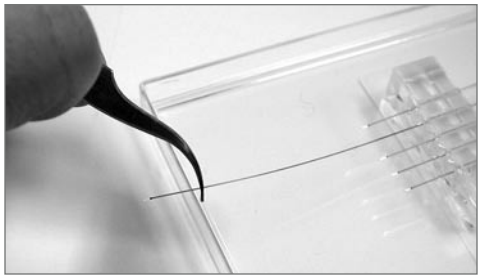

FIGURE 5 Removing Self-Pack IntegraFrit™ Columns from packaging

Cleaving Fused Silica

Proper cleaving of fused-silica tubing is a critical but often overlooked operation in the preparation of emitters and columns prior to use. A flat, smooth cleave is essential for maintaining low dead volume connections with other sections of fused-silica tubing. It is also critical that cleaving does not generate flow-stopping particulate matter. Cleaving is best accomplished with a high-quality diamond chip or sapphire cleaving tool. New Objective's 1 mm wide diamond-blade cleaving tool, shown in Figure 6, has been selected to provide a consistent, flat cleave with a minimum of particulate generation. Inexpensive carbide scribing tools are not recommended, since they generally result in poor-quality (i.e., ragged) cleaved end faces that generate many fine particles.

CAUTION: Handling of fused-silica tubing and emitters can result in serious personal injury, including skin and eye injury. Use safety glasses or goggles meeting ANSI Z87.1-1989 requirements or the equivalent. Puncture- and chemical-resistant gloves should be worn at all times.

Procedure

1. Place the tubing to be cut on a flat, clean surface and position the cleaving tool perpendicular to the tubing surface, as shown in Figures 7 & 8B. The long axis of the blade should be perpendicular to the tubing bore.
2. Press down gently (Figure 8B); DO NOT use a sawing motion when pressing the blade. You only need to nick the surface of the polyimide coating (Figure 8C). Be careful not to force the blade through the tubing, which will generate a ragged end and many particles (Figure 8D).
3. Pull gently on the tubing along its axis; it should easily separate at the point of contact. If it does not, repeat the procedure with a little more force. A typical cleave of 360 µm OD, 75 µm ID fused-silica tubing is shown in Figure 12. Residual surface irregularity is on average less than or equal to 10 µm.

Inspection of the distal end of the tip for particle contamination using a light microscope with transmitted light at 100x magnification is highly recommended. New Objective sells an accessory kit that contains all the high-quality tools (cleaver, special forceps, ruler, etc.) you will need to properly handle fused-silica emitters, columns, and tubing. Please see our catalog or Web site for a full description of our Micro Tool Kit (stock number TIP-KIT).

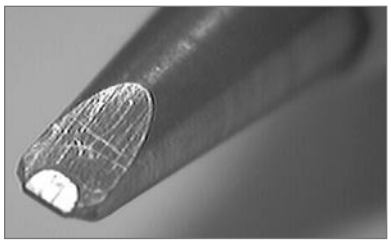

FIGURE 6 Close-up view of diamond-blade cleaving tool

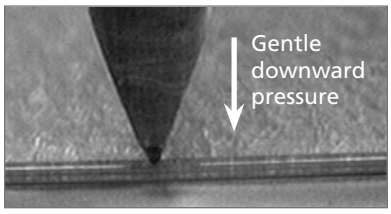

FIGURE 7 Cleaving tool in proper position

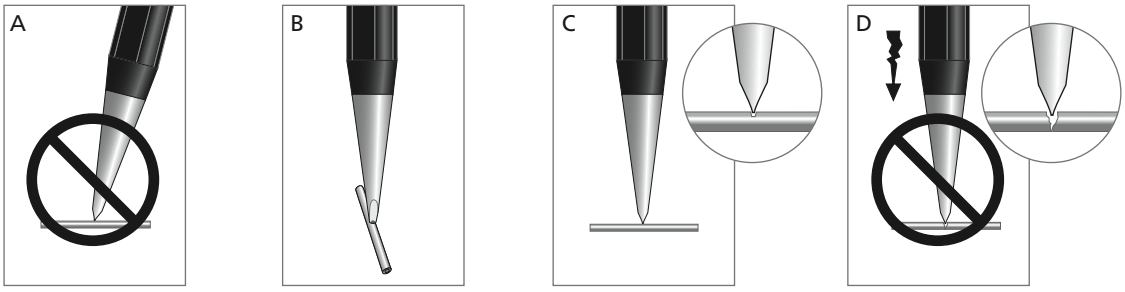

FIGURE 8 (A) Improper cutting angle (B) Align cleaving tool perpendicular to tubing (C) Press down gently, scoring tubing (D) Too much downward pressure will crush tubing, producing particles that can cause tubing to clog

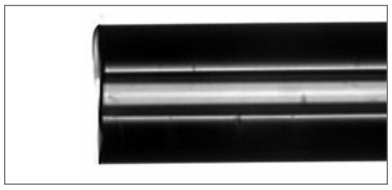

FIGURE 9 Typical cleave. Polyimide coating was removed after cleaving for clarity of image.

Using IntegraFrit™ Sample Traps with the Upchurch™ Sample Trap Column Assembly

IntegraFrit™ sample traps work with the UpChurch Scientific® Sample Trap Column Assembly to concentrate and purify LC-MS samples. **Proper assembly technique is critical to achieving a good seal and avoiding leakage.**

IMPORTANT: These instructions have been revised to accommodate recent modifications to the Upchurch Sample Trap Assembly hardware. Please read all instructions carefully before using the sample trap assembly to avoid damage to both columns and the assembly.

WARNING: Handling of fused-silica tubing and emitters can result in serious personal injury, including skin and eye injury. Use safety glasses or goggles meeting ANSI Z87.1-1989 requirements or the equivalent. Powder-free, puncture- and chemical-resistant gloves should be worn at all times.

Preparing the IntegraFrit Column for Insertion

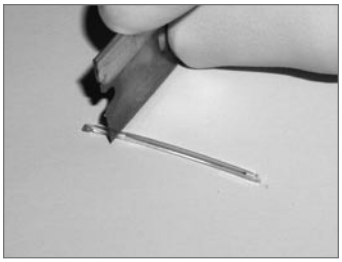

FIGURE 1

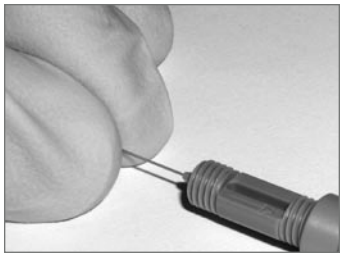

FIGURE 2

1) Using a razor blade or scalpel, carefully cut both ends of the protective sleeve around the IntegraFrit column without cutting into the fused silica column (Figure 1)
2) Using fused silica tubing, push the column out of the sleeve (Figure 2)

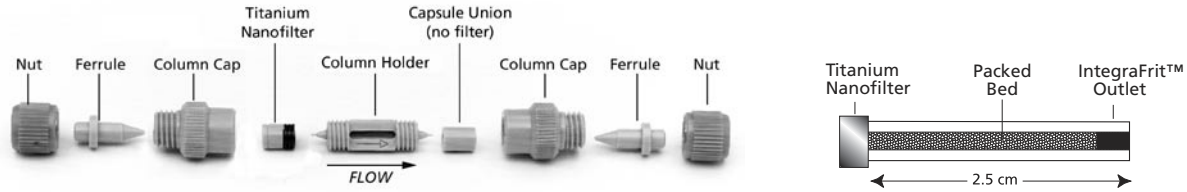

FIGURE 3

Product Description

The IntegraFrit™ Sample Trap contains multiple parts and is assembled using the component sequence illustrated in Figure 3.

Located in the center, the column holder contains an interior channel through which the IntegraFrit column is inserted; the arrow next to the channel indicates flow direction. A titanium nanofilter is located prior to the column holder (inlet), and a capsule union is inserted on the outlet side of the column holder inside the opposite column cap. Both sides of the sample trap contain a ferrule followed by a nut.

Initializing New Nanofilter Capsules for Use

Nanofilter capsules must be pre-swaged prior to use with the sample trap assembly in order to provide a proper seal and without damaging the sample trap column. Failure to do so may result in leaking, crushed fused-silica and contamination of the nanofilter.

You will want to have an old, or unusable column holder on hand as a swaging tool.

1) Without inserting any tubing, place a new, unused nanofilter into one of the assembly end caps. Insert an EMPTY column holder into the end cap and tighten the fitting until very snug. You may notice a slight audible cracking sound.
2) Loosen and remove the column holder. The nanofilter is ready to be used.

NOTE: The column holder used to perform the swage is now unusable, as compression of the ferruled-end has effectively sealed the through-path. Retain this column holder for future nanofilter swaging.

Inserting the IntegraFrit™ Column

The IntegraFrit™ column, complete with its packed bed and fritted outlet, is specifically designed for the sample trap assembly. It arrives in a ready-to-use form with no need for cutting or adjustment.

1) With the flow arrow pointing to the right, remove both column caps with the ferrules, and nuts still attached
2) Insert the clear (fritted) end of the IntegraFrit column into the column holder so it enters in a direction parallel to the arrow (Figure 4)

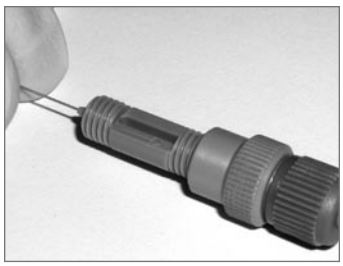

FIGURE 4

NOTE: The fritted end contains no polyimide coating and is fragile.

3) Inspect the outlet end of the column holder so the frit end of the column slightly protrudes (Figure 5)

FIGURE 5

Sealing the Column in the Column Holder

1) With the IntegraFrit™ column in place inside the column holder, insert the capsule union onto the outlet end of the column cap as shown in Figure 6. Place an end cap over the capsule union, and screw onto the column holder only until minimum resistance is felt. (The sample trap column inside the column holder must be able to rotate within the column holder and end cap.)
2) Insert the nanofilter onto the inlet end of the column cap as shown in Figure 7. Place the second end cap over the nanofilter and tighten securely onto the column holder. This end should be fully tightened (Figure 8).
3) Proceed to tighten the end cap on the outlet end of the assembly until fingertight (Figure 9).

CAUTION: Prematurely tightening the outlet end of the trap results in poor sealing and leakage and can cause damage to the column.

4) Inspect the channel for a slight bend in the IntegraFrit column. This indicates a successful watertight seal (Figure 10).

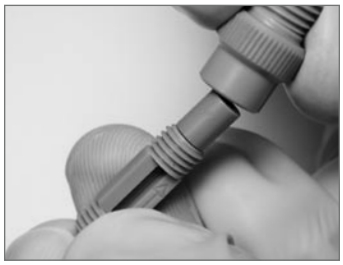

FIGURE 6 Set the capsule union on the inlet end of the column holder. Cover with an end cap and very loosely tighten.

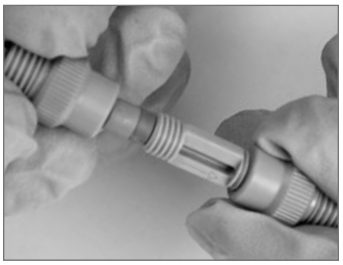

FIGURE 7 Set the nanofilter on the outlet end of the column holder assembly. Cover with the second end cap.

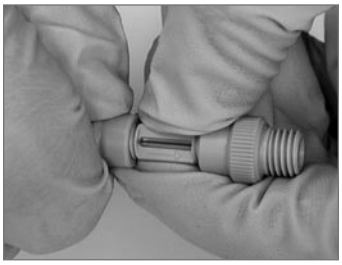

FIGURE 8 Gripping the column holder, securely tighten the end cap containing the nanofilter (inlet end of the assembly)

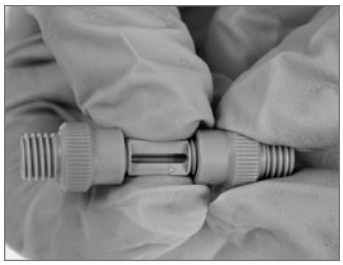

FIGURE 9 Gripping the column holder, securely tighten the end cap on the outlet end of the assembly

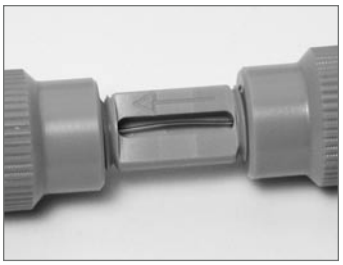

FIGURE 10 A slight bend in the installed column indicates a successful seal

Installing the Sample Trap in Your System

1) Thread a segment of fused silica tubing through the nut and ferrule until the end of the tubing protrudes from the pointed end of the ferrule
2) Insert Ferrule into the end cap
3) Press lightly against the fused silica so it butts against the inside of the end cap
4) Tighten the nut around the fused-silica tubing while holding the end cap so the cap does not move (Figure 12)
5) Repeat Steps (1)-(4) for the other side of the sample trap assembly

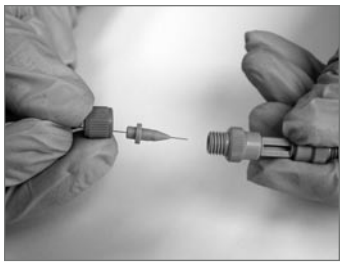

FIGURE 11

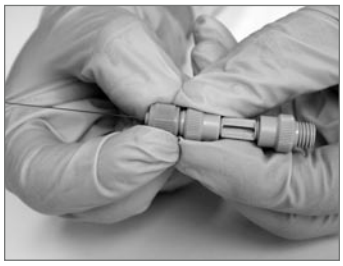

FIGURE 12

WARNING: Handling of fused-silica tubing can result in serious personal injury, including eye and skin injury. Use safety glasses or goggles meeting ANSI Z87.1-1989 requirements, or the equivalent. Puncture and chemical resistant gloves should also be worn at all times.

Removing the FEP Sleeve from the IntegraFrit™ Column

Care must be taken when removing the FEP sleeve to prevent damaging the column. Bending the FEP sleeve can fracture the frit and render the column useless. Do not attempt to remove the sleeve by pulling it off the end of the column. Use the free-sliding PEEK™ sleeve to push the FEP sleeve off the fused-silica tubing in the following manner:

- Remove the distal end of the column from the FEP sleeve by either pulling it free of the sleeve, or (preferably) cleaving the fused-silica tubing near the terminus.
- Slide the PEEK sleeve towards the frit end until it butts up against the FEP sleeve as shown in Figure 3.
- In one hand, firmly grasp the fused-silica column. It is helpful to wrap some of the fused-silica tubing around the palm of your hand for a firm grip. The polyimide coating is quite durable and will withstand considerable handling.
- Orient the FEP sleeve vertically, frit facing down towards the floor. This way, when the FEP sleeve slides free, it will fall towards the ground without damaging the column.
- Using your other hand, push the PEEK sleeve against the FEP sleeve until the FEP sleeve falls off. This may take a LOT of force. Do not be afraid to push hard. You will have to move the PEEK sleeve about 3 to 5 mm. Figure 4 shows the column after the FEP sleeve has been pushed off.
- Remove PEEK sleeve by sliding it over the back end of the column.

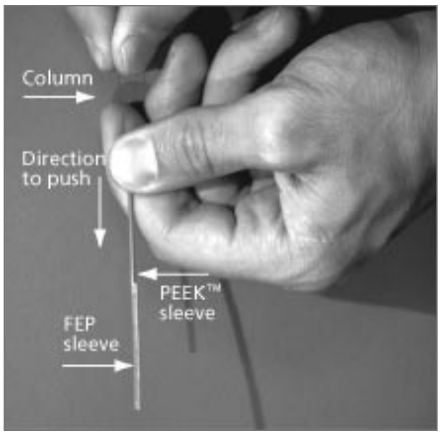

FIGURE 3 Sliding the PEEK™ sleeve

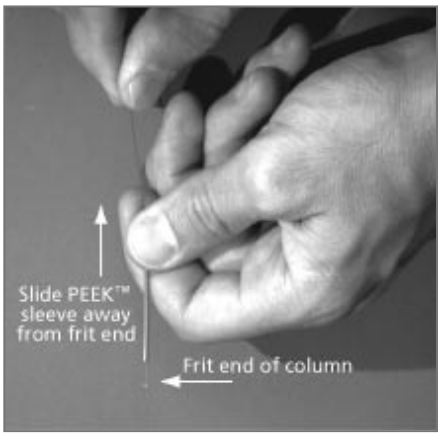

FIGURE 4 IntegraFrit™ column after the FEP sleeve has been removed

Converting the FEP Sleeve to a ZDV Union

- Locate the fritted end of the column inside the FEP sleeve.
- Within the FEP sleeve there is a gap between the frit end and the distal end of the column. Measure 5 mm into this gap, towards the distal end of the column, from the end of the frit. Cut the FEP sleeve using a single edge razor blade or similar sharp cutting tool as shown in Figure 5A. Use caution because bending the FEP sleeve can fracture the frit and render the column useless. A properly cut FEP sleeve is shown in Figure 5B.
- Using a dissecting needle, such as that found in New Objective's Micro Tool Kit (order no. TIP-KIT), swage the inside of the cut FEP sleeve as in Figure 5C. The needle should come no closer than 2 mm to the frit. Do not contact the frit with the sharp end of the needle. Remove the needle from the sleeve.

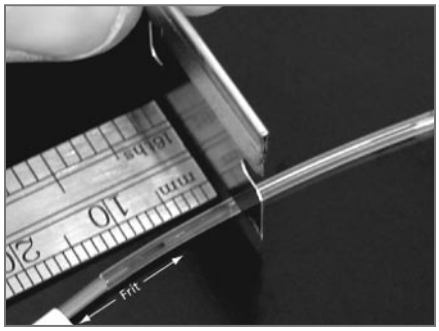

FIGURE 5A Cutting the FEP sleeve

- The sleeve is now ready for the insertion of a square cut length of fused-silica tubing or, for example, the distal end of a New Objective PicoTip®. Push the fused-silica tubing into the sleeve until the tubing contacts the end of the frit as in Figure 6.
- Inspection of a good connection in a FEP sleeve, now a union, under a light microscope is shown in Figure 7. This union is a true zero dead volume connection and will offer good chromatographic performance. A properly prepared FEP union will typically hold pressure to 200 psi or more.

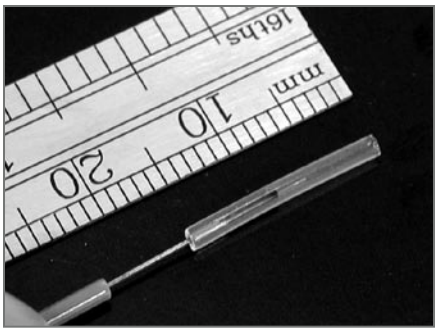

FIGURE 5B Properly cut sleeve

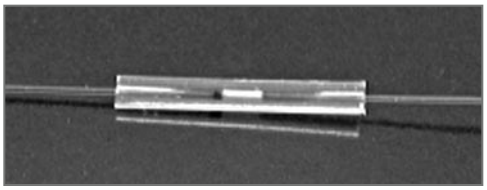

FIGURE 6 Properly loaded FEP union

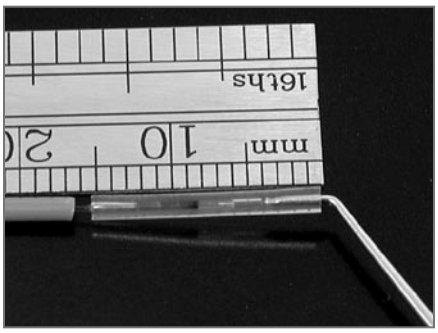

FIGURE 5C Swaging the sleeve

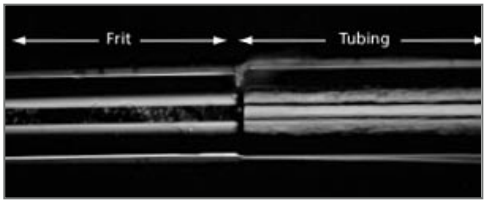

FIGURE 7 Close-up of a good connection

Cleaving Fused Silica

Proper cleaving of fused-silica tubing is a critical but often overlooked operation in the preparation of emitters and columns prior to use. A flat, smooth cleave is essential for maintaining low dead volume connections with other sections of fused-silica tubing. It is also critical that cleaving does not generate flow-stopping particulate matter. Cleaving is best accomplished with a high-quality diamond chip or sapphire cleaving tool. New Objective's 1 mm wide diamond-blade cleaving tool, shown in Figure 8, has been selected to provide a consistent, flat cleave with a minimum of particulate generation. Inexpensive carbide scribing tools are not recommended, since they generally result in poor-quality (i.e., ragged) cleaved end faces that generate many fine particles.

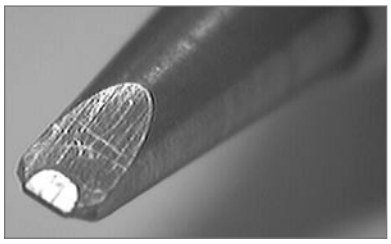

FIGURE 8 Close-up view of diamond-blade cleaving tool

WARNING: Handling of fused-silica tubing and emitters can result in serious personal injury, including skin and eye injury. Use safety glasses or goggles meeting ANSI Z87.1-1989 requirements or the equivalent. Puncture- and chemical-resistant gloves should be worn at all times.

Procedure

1. Place the tubing to be cut on a flat, clean surface and position the cleaving tool perpendicular to the tubing surface, as shown in Figures 9 & 10B. The long axis of the blade should be perpendicular to the tubing bore.
2. Press down gently (Figure 10B); DO NOT use a sawing motion when pressing the blade. You only need to nick the surface of the polyimide coating (Figure 10C). Be careful not to force the blade through the tubing, which will generate a ragged end and many particles (Figure 10D).
3. Pull gently on the tubing along its axis; it should easily separate at the point of contact. If it does not, repeat the procedure with a little more force. A typical cleave of 360 µm OD, 75 µm ID fused-silica tubing is shown in Figure 11. Residual surface irregularity is on average less than or equal to 10 µm.

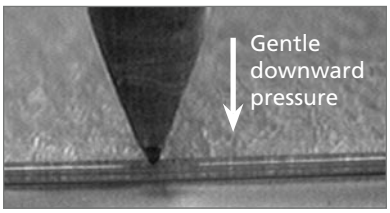

FIGURE 9 Cleaving tool in proper position

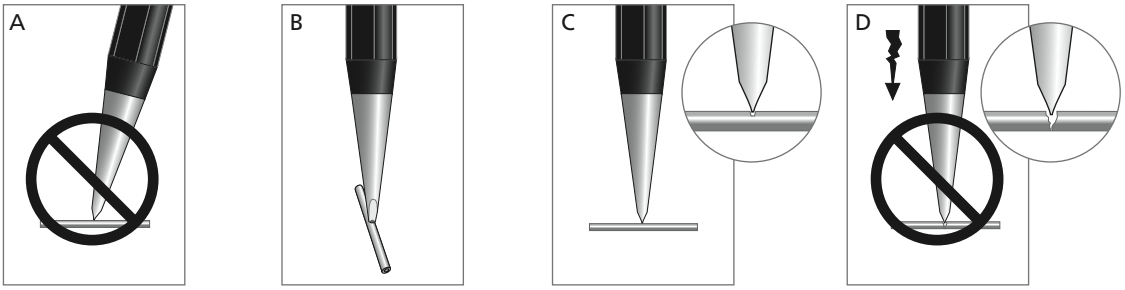

FIGURE 10 (A) Improper cutting angle (B) Align cleaving tool perpendicular to tubing (C) Press down gently, scoring tubing (D) Too much downward pressure will crush tubing, producing particles that can cause tubing to clog

Inspection of the distal end of the tip for particle contamination using a light microscope with transmitted light at 100x magnification is highly recommended. New Objective sells an accessory kit that contains all the high-quality tools (cleaver, special forceps, ruler, etc.) you will need to properly handle fused-silica emitters, columns and tubing. Please see our catalog or Web site for a full description of our accessory kit (stock number TIP-KIT).

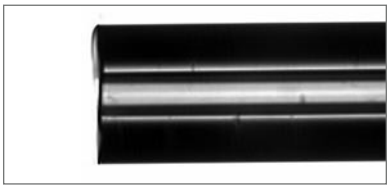

FIGURE 11 Typical cleave. Polyimide coating was removed after cleaving for clarity of image.