Tips on Tips - Offline PicoTips®

Thank you for ordering from New Objective's line of PicoTip® emitters for offline nanospray. Consisting of GlassTips™, EconoTips™, and QuartzTips™, they represent the most advanced precision emitters available for nanospray.

Given the wide variety of electrospray ionization (ESI) sources produced by different manufacturers, the exact implementation of the PicoTip emitters on your system may affect utility and performance. This "tip sheet" gives a few pointers on the successful use of PicoTips. Please observe all manufacturer safety recommendations and read the safety statement at the end of this document.

Unpacking and handling your PicoTips®

NOTE: Please wear ANSI-approved safety glasses when handling PicoTip® emitters.

Coated PicoTips® have a special enhanced conductive multilayer coating (U.S. Patent 5,788,166) that provides for excellent electrochemical stability and durability against ESI solvent exposure and arcing. Coated tips should be handled with care, as mechanical abrasion can remove the coatings. No attempt should ever be made to handle the tips with bare hands. The highest quality fine, non-serrated forceps are recommended. New Objective sells an accessory kit containing all the high-quality tools (cleaver, special forceps, ruler, etc.) you will need to properly handle PicoTips. Please see our catalog or Web site for a full description of our accessory kit (stock number TIP-KIT).

Inside the box, the PicoTips are held down by adhesion. When ready to use, pull the PicoTip off with a pair of fine forceps, taking care not to touch the tip or scrape off the conductive coating, as the coating can be ruined by improper or rough handling. Lift from the tip end of the emitter, keeping the tip away from the base of the packaging. The emitter is fairly durable, but the end of the tip must not make physical contact with any surface.

Sample loading and coupling

Given the wide variety of applications, there is no one best method for the loading of samples into a PicoTip® emitter. Since PicoTips have open tips, filling with a tabletop centrifuge is not recommended. Unless rotor rpm is kept very low, your sample will be ejected from the tip as the rotor spins up. If you must utilize this procedure, test your filling technique with a blank solution. Offline PicoTips are fabricated from tubing with a special cross-sectional shape that greatly enhances filling by capillary action. This shape assists in the filling of the tapered region of the tip, preventing "vapor lock" from occurring by allowing the liquid to flow around any air bubbles. Fused-silica needles and gel-loading tips are two of the most common devices used for sample introduction and will be detailed in this Technical Note.

Carbon, TiO_2, and ZrO_2 Wall-Coated Trap'nTips™ for Online & Offline Phosphopeptide Analysis

The design of carbon, TiO_2, and ZrO_2 wall-coated pipette tips facilitates phosphopeptide enrichment and manual aspiration through the pipette tip followed by expulsion of purified solution for immediate nanobore column injection or direct loading into an offline nanospray emitter. As previously demonstrated, these substrate-specific resins effectively concentrate, desalt, and enhance the MS signal of phosphopeptides from trypic digests[1]. The novel design of the Trap'nTip™ eliminates geometric constraints of sample preparation, pipette tip-coupling, and pressurized back-loading associated with conventional practice (See Technical Note PT-6).

FIGURE 1 Carbon-, TiO_2-, and ZrO_2-coated Trap'nTips™

Product Description

The Trap'nTip is comprised of a standard gel-loader pipette tip containing one of three phosphopeptide-specific sorbents adsorbed to the inner wall. New Objective offers Trap'nTips for phosphopeptide analysis containing titania (TiO_2), zirconia (ZrO_2), and carbon sorbents.

Trap'nTip™ Conditioning and Sample Loading

Prior to the application, Trap'nTips coated with phosphopeptide-specific sorbents require a conditioning step. The complete preparation and sample-loading procedure is delineated below:

1. Conduct 5 aspriation/expulsion cycles of HPLC-grade water with a 0.5-10 µL Eppendorf® Single-Channel Research Pipette.
2. Load phosphopeptide analyte onto the Trap'nTip using 10 aspiration/expulsion cycles of 10 µL each.
3. Wash loaded samples via ten 10 µL aspiration/expulsion cycles of HPLC-grade water.
4. Aspirate 2 µL aqueous solution containing 50 mM NH_4HCO_3 and 50 mM triethylamine (TEA) to elute analyte from the Trap'nTip.
5. Expel eluent from the Trap'nTip into a clean vial.
6. Add 2 µL 50 mM TEA in CH_3OH to vial containing eluted sample.
7. Mix eluent with CH_3OH by centrifugation

Loading an Offline GlassTip™ Using the Trap'nTip™

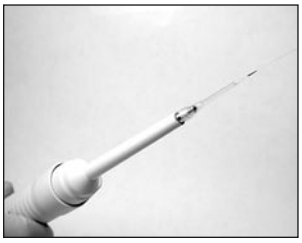


Figure 2 Trap'nTip™ fitted onto 10 µL pipette

Figure 3 Sample loaded into the Trap'nTip

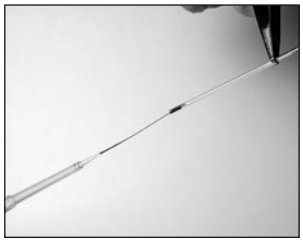


Figure 4 Insert the Trap'nTip into the distal end of the PicoTip®

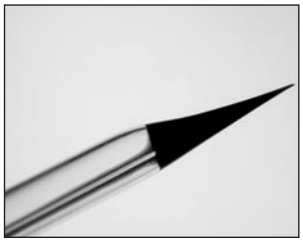


Figure 5 Capillary action draws the sample into the tip-end of the PicoTip emitter

Online Chromatography of Phosphopeptides Using the Trap'nTip™

The following procedure successfully separates phosphopeptide analytes for online analysis.

1. Conduct 5 aspriation/expulsion cycles of HPLC-grade water using a 0.5-10 µL Eppendorf® Single-Channel Research Pipette.
2. Load phosphopeptide analyte onto the Trap'nTip using 10 aspiration/expulsion cycles of 10 µL each.
3. Wash loaded samples through ten 10 µL aspiration/expulsion cycles of HPLC-grade water.
4. Aspirate 10 µL aqueous solution containing 250 mM NH_4HCO_3 (pH 9) to elute analyte from the Trap'nTip.
5. Expel eluent from Trap'nTip into a vial.
6. Conduct 9-10 additional eluent aspiration/expulsion cycles through the Trap'nTip
7. Deliver sample to injection port using conventional method (10 µL syringe, autosampler, etc.)

MassPREP™ standards were obtained from Waters. The first standard was a combined enolase digest/phosphopeptide mixture, and the second standard was a phosphopeptide standard. Both solutions contained phosphopeptides listed in Table 1.

Peptide	Sequence	$[M+H]^+$	$[M+2H]^{2+}$
T18 1P	NCPLpY K	813.3912	407.1995
T19 1P	HLADL pSK	863.4028	432.2053
T43 1P	VNQIG pTLSES IK	1368.6776	684.8428
T43 2P	VNQIG TLpSEpS IK	1448.6439	724.8259

TABLE 1

Figure 6 displays scans of the enolase digest (Figure 6A) and phosphopeptide standard (Figure 6B) prior to Trap'nTip purification. Figures 6C and 6D display the enolase digest after treatment with the TiO_2 and ZrO_2 Trap'nTips. Due to trace-level presence, the T43 2P phosphopeptide is not visible in the latter two figures.

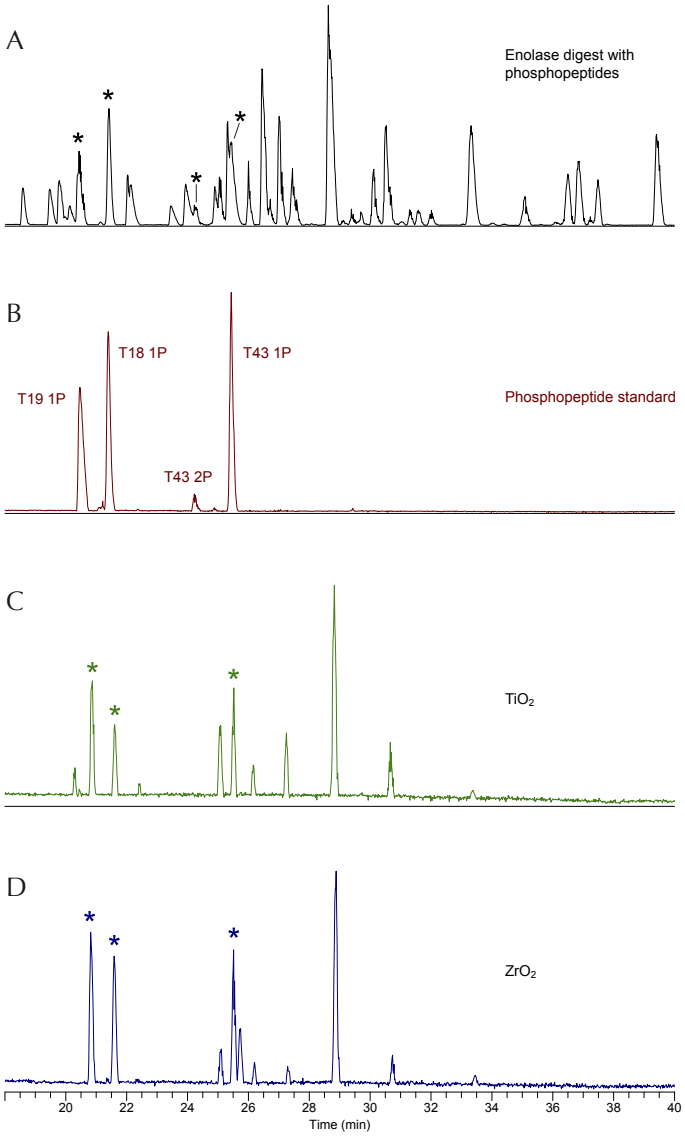


FIGURE 6

References

1. Toher, C.J.; Perala, A.W.; Shukla, A.K.; Valaskovic, G.A.; Oetting, A.A.; Shukla, M.M. "Offline Nano-ESI Phosphopeptide Analysis with Carbon, TiO_2, and ZrO_2 Wall-Coated Trap'nTips". Poster presented at Association of Biomolecular Research Facilities Conference, Long Beach, CA, 2006.
2. Toher, C.J.; Perala, A.W.; Shukla, A.K.; Valaskovic, G.A.; Oetting, A.A.; Shukla, M.M., Marshall-Waggett, C.J. "Online and Offline Nanoelectrospray Analysis of Phosphopeptides Purified by TiO_2, ZrO_2, and Carbon Wall-Coated Pipette Tips". Poster presented at American Society for Mass Spectrometry, Seattle, WA, 2006.

Setup and Measurement of Flow Rate for Online Nanobore LC-MS

Eluent flow rate provides crucial implications for configuring your nanobore ESI application. The flow rate dictates recommended tubing and tip inner diameters (IDs) for standard LC columns, PicoTip® online emitters, and PicoFrit® columns. Figure 1 displays recommended tip and column dimensions for given flow rates.

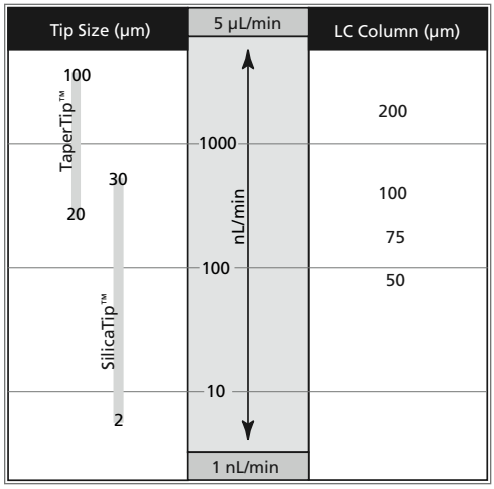


FIGURE 1

While many LC pumps accommodate flow in the µL/min range, few are specifically designed for the nL/min level. Employing a flow-splitter (Figure 2) between the LC pump and column helps to significantly reduce flow rate to accommodate nanobore LC. A flow-splitter is comprised of a "T" junction where the fused silica LC line from the pump is plumbed immediately opposite the LC line leading to the column. A third fused-silica line (waste tubing) is plumbed orthogonally to the flow path, providing a second mobile phase outlet (Figure 2 - Outlet 2).

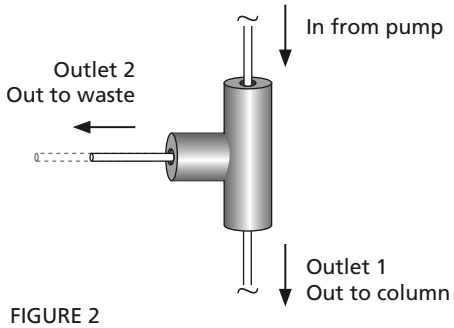


FIGURE 2

In general, the shorter the piece of fused silica for Outlet 2, above, the greater the flow rate reduction at the tip. Best practice involves starting with a long piece of fused silica for Outlet 2, measuring the flow rate at the column tip, and systematically reducing outlet 2 length with an appropriate fused-silica cutter (refer to Technical Note FS-1) to attain the desired flow rate. Installation of an inline microfilter immediately before the flow-splitter and an inline nanofilter between the flow-splitter and column helps minimize clogs for this configuration. Loading a sample onto the column by sample trap injection further purifies the sample and prevents the entry of particulates into the system. See Technical Note IF-3 for information of effective sample trap use.

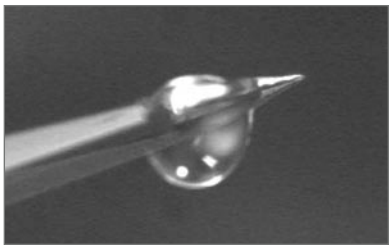


FIGURE 3

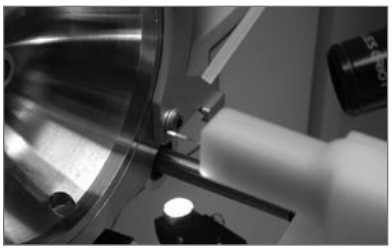


FIGURE 4

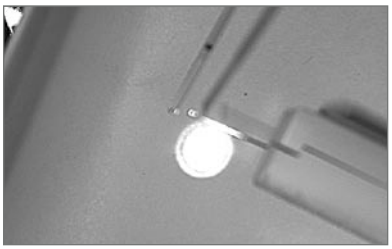


FIGURE 5

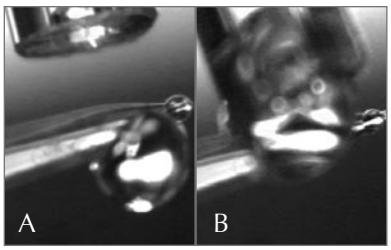


FIGURE 6

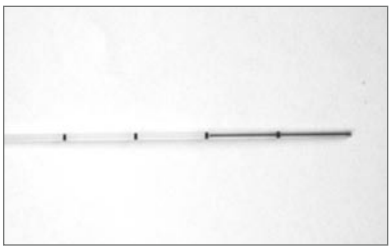


FIGURE 7

Flow-Rate Measurement

New Objective provides Calibrated Micropipettes (Order Number NO2-000-001) designed to measure flow rate at the tip of columns or online emitters. This technical note describes how to use these micropipettes to measure flow rate while minimizing tip damage.

WARNING: Electrospray ionization involves the use of potentially lethal high-voltage electrical current. Observe all manufacturers' safety recommendations in the use of such equipment. No equipment modifications should be made except by trained personnel using methods approved by the manufacturer in accordance with all safety requirements. Installation of equipment should be performed by qualified personnel in accordance with all applicable electrical codes.

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.

1. Following recommended procedures from your mass spectrometer manufacturer, turn off the voltage supply to your nanospray source
2. Activate your pump to begin eluent flow
3. Allow a droplet to form at the emitter tip (Figure 3)
4. From a distance of approximately 2 cm, aim the nozzle of canned air spray at the emitter tip (Figure 4)
5. With a stop watch ready, spray the canned air directly on the tip to remove the initial droplet
6. Immediately upon droplet removal, start the stopwatch and allow 4-5 minutes to elapse as a new droplet forms.
7. As the end of the 4-5 minute measurement interval approaches, hold the calibrated micropipette so the end points toward the growing droplet (Figure 5)
8. Immediately upon concluding the 4-5 minute measurement interval, lightly touch the tip of the micropipette to the droplet and collect fluid (Figures 6A - 6B)
9. Remove the micropipette from the tip and measure the fluid volume in the pipette
10. Use the formula below to calculate flow your rate:

$$\text{Eluent flow rate (µL/min)} = \frac{\text{Fluid volume in micropipette (µL)}}{\text{Total time of fluid collection (min)}}$$

11. Flow rate can be adjusted by increasing/decreasing the diameter/length of the waste tubing at the flow splitter. Depending on mobile phase composition and the inner diameters of both the tubing and emitter tip, typical nanospray flow rates range between 100 - 500 nL/min.

Using Trap'nTips™ for Offline Sample Purification

Trap'nTips™ provide the combined advantage of aspirating liquid samples directly into a pipette tip followed by immediate sample desalting and concentration onto an inner coating of reverse-phase sorbent. The Trap'nTip's novel capacity for offline LC-MS facilitates manual aspiration-expulsion cycles with increasing organic modifier concentrations and subsequent MS analysis by static nanospray.

Product Description

The Trap'nTip is comprised of a standard gel-loader pipette tip containing one of three reverse-phase sorbents adsorbed to the inner wall; New Objective offers Trap'nTips containing C18, carbon, and a C18/carbon moiety. Figure I illustrates components of the Trap'nTip.

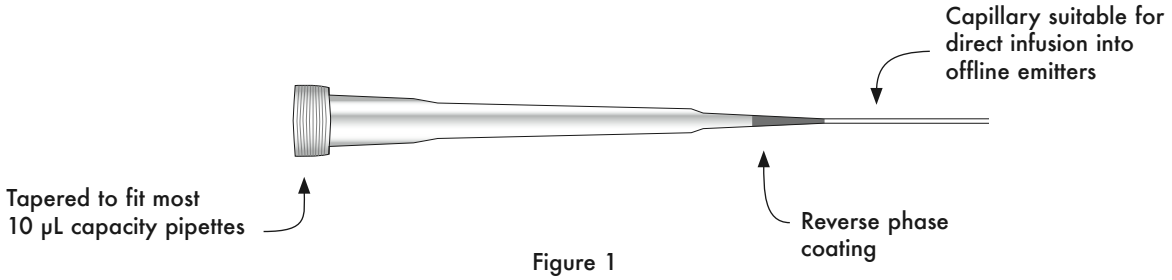


Figure 1

Tip Conditioning

Trap'nTips™ require a brief conditioning step before sample loading. Trap'nTip conditioning requires both binding solution (~2% organic modifier) and an eluent (10-60% organic modifier). New Objective recommends the Trap'nTip conditioning procedure below.

1.) Aspirate and expel five consecutive 10 µL aliquots of eluent into the Trap'nTip.
2.) Follow eluent flush with five consecutive aspiration/expulsion cycles of 10 µL binding solution (2% organic modifier).

Sample Loading

Trap'nTips™ are ready for sample loading immediately after conditioning. The following steps illustrate the sample-loading procedure.

1.) Aspirate 10 µL sample onto the Trap'nTip coating and expel into a waste receptacle. Repeat ten times.
2.) With analyte bound to the tip, desalt by aspirating 10 µL binding solution and expelling back into the sample vial. Repeat ten or more times.

Loading a PicoTip® Using the Trap'nTip™

Trap'nTips™ can be used to load sample into any New Objective Offline PicoTip®. The following steps describe filling a PicoTip with the Trap'nTip.

1.) Aspirate 2 µL eluent into the Trap'nTip™ containing loaded sample
2.) Insert the end of the Trap'nTip into the distal end of the PicoTip
3.) Exert slow pressure on pipette plunger to empty sample into the PicoTip
4.) Invert loaded offline emitter and allow the self-filling capillary action of the PicoTip® to guide the liquid sample to the tip
5.) Mount the offline emitter onto the nanospray source with voltage supply
6.) Proceed with static nanospray analysis

Figure 2 Trap'nTip™ fitted onto 10 µL pipette

Offline Chromatography with the Trap'nTip™

The presence of reverse-phase sorbent permits the use of Trap'nTips™ in manual chromatographic separations. For analytes loaded onto the Trap'nTip, aspirating and expelling eluents of different organic modifier concentrations through Trap'nTips produced excellent peptide separation ability[1].

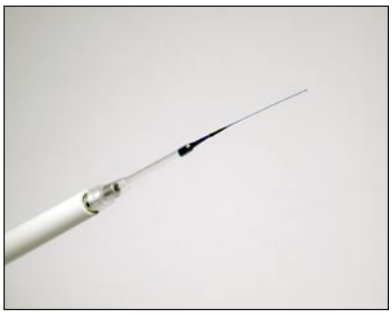


Figure 3 Sample loaded into the Trap'nTip

The procedure below illustrates how to employ the Trap'nTip in the offline chromatographic separation of a peptide. This manual gradient begins with an eluent of low organic modifier concentration (i.e. 10% ACN) and concludes with an eluent of higher organic modifier concentration (i.e. 60% ACN).

1.) Repeat the conditioning and sample-loading procedures previously outlined
2.) Repeat steps 1-6 under *Loading a PicoTip® Using the Trap'nTip* with an eluent containing 10% organic
3.) For the same Trap'nTip, repeat steps 1-2 above using eluents of successively higher organic concentrations (i.e. 20%, 40%, 60%)

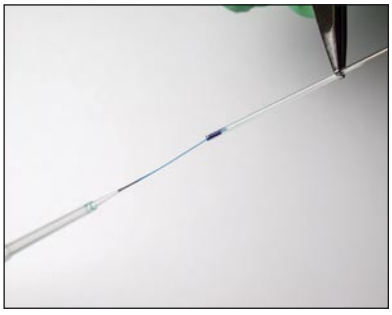


Figure 4 Insert the Trap'nTip into the distal end of the PicoTip®

For more information on filling New Objective offline nanospray emitters, please refer to Tech Note PT-1 "Tips on Tips: Using Offline PicoTips®."

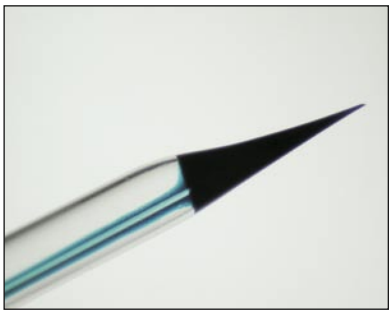


Figure 5 Capillary action draws the sample into the tip-end of the PicoTip emitter

Reference
1. Toher, C.J.; Perala, A.W.; Shukla, A.K.; Valaskovic, G.A. "Sample Purification for Static Nanospray MS Using Wall-Coated Pipette Trap'nTips," Poster presented at American Society for Mass Spectrometry, San Antonio, TX, **2005**.

Higher Flow Rate Operation Using TaperTips™

Introduction

Traditionally, commercial electrospray ionization (ESI) mass spectrometers utilize flow rates from tens of microliters per minute (10 µL/min) to milliliters per minute (1 mL/min). Because of the relatively large volume of liquid exiting the emitter, aerosol formation must be assisted by pneumatic nebulization and/or by thermal heating in an effort to obtain
a stable spray. The efficiency of ionization, however, improves as the flow rate is lowered and a lower volume of mobile phase passes through the emitter, producing smaller aerosol droplets. Working at the lower flow rates of nanoliters per minute (below 500 nL/min) is commonly referred to as "nanospray" and has become a popular method employed in protein analysis. The lower flow rates in nanospray also allow for longer analysis time, providing opportunity to
perform novel mass spectrometer scan functions and obtain structural information of an analyte.

New Objective manufactures PicoTip® emitters to operate with flow rates from 20 nL/min to 3µL/min. While flow rates from 10 to 500 nL/min are best served by SilicaTip™ emitters, TaperTip™ emitters have been developed to bridge the gap between traditional electrospray and nanospray techniques. TaperTips give exceptional performance from 200 nL/min to 3 µL/min, making them ideal for researchers who want to make the transition into nanospray or for experienced nanospray researchers looking for the flexibility of operating at higher flow rates.

TaperTips are designed with an external taper similar to SilicaTips, but without an accompanying internal taper (Figure 1). This unique design eliminates restrictions to flow, making the emitter extremely robust and less prone to particle clogging while providing the peak performance associated with SilicaTips.

TaperTips can be implemented with any mounting hardware designed to handle fused-silica ESI components. Users of the PicoView® series or adapter models ADPT-LTQ or ADPT-PRO can take full advantage of operation at these higher flow rates with the proper choice of TaperTip.

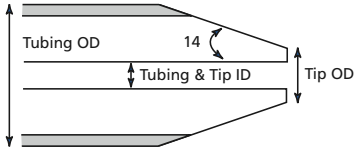


FIGURE 1 TaperTip™ emitter

Coating options

TaperTips™ are available either uncoated or coated. While many users obtain suitable performance with our standard coating (-CE- in the stock number), some find that the emitter performance can be compromised by excessive arcing during tuning due to an overvoltage condition. Although great improvements have been made with emitter coatings, constant arcing may still damage a coated tip, reducing or preventing stable operation. A good solution for those experiencing tuning problems is to use emitters with a distal coating (-D- in the stock number). The high-voltage contact is made through a junction-style contact inside the PEEK™ union. Since the distal coating is only applied to the non-tip end of the emitter, it is immune to arcing.

Mounting

Distal-coated TaperTips™ are mounted identically to distal-coated SilicaTips™. Follow the mounting instructions for junction contact provided in the manual supplied with your mounting system hardware.

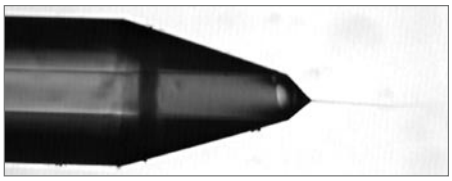
Tuning

TaperTips™ will typically employ a higher applied voltage to maintain electrospray ionization compared to other fused-silica PicoTips™. For example, a 50 µm ID TaperTip running at 1 µL/min may require an applied voltage in excess of 3.5 kV. Figure 2 provides an example of Taylor cone performance under different tuning parameters.

Troubleshooting

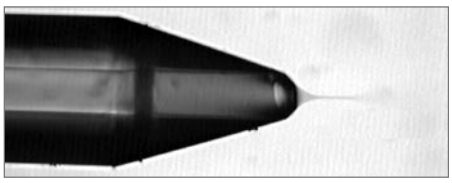
A repeating dropout of ion current
TaperTips™ operate in an intermediate flow range, which may require some special attention to find an optimal electrospray potential. A less-than-optimal applied voltage and/or flow rate may lead to a dropout in ion current and may require some fine tuning. Start by adjusting the voltage up or down in 100-volt increments. If instability persists, set the voltage at a "moderate" level where a signal (albeit unstable) is obtained, then turn off the pumping system. If good stability is observed as the pressure bleeds off, it is a good indication that the flow rate is too high. Reduce the system flow rate 25–50% and repeat the tuning procedure. If turning the pump off does not produce a stable signal, increase the system flow rate 25–50%. Figure 2 shows some of the possible modes of ESI emission. Optimal signal is usually obtained with a stable Taylor cone, which also produces smaller droplets with the highest charge-to-mass ratio for efficient desolvation.

An erratic drop in ion intensity caused by gas bubbles in the system
Gas bubbles can wreak havoc with spray stability, as pictured in Figure 3. Small bubbles can originate from trapped air pockets within a coupling union, electrolysis at the high-voltage contact, or dissolved gasses in the solvent. Bubbles can be minimized by making certain all fittings are sufficiently gas- and liquid-tight. Allow time for any residual gas to bleed out of the system. If air bubbles persist,
try using a TaperTip™ with a smaller inner diameter than that of the transfer line. This can create sufficient back-pressure and reduce or eliminate outgassing from solvents and electrolysis.



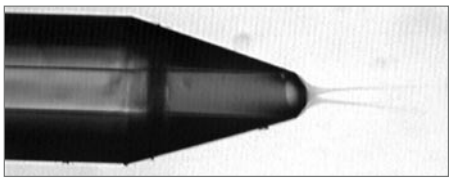
1 µl/min @ 2800 V
Excellent stability, optimal signal; stable Taylor cone

Decrease voltage



1 µl/min @ 2500 V
Reasonable stability, decreased signal; jet plus droplets

Increase flow rate



3 µl/min @ 2800 V
Diminished stability; multiple jets plus droplets

FIGURE 2 Taylor cone performance of a 50 µm distal-coated TaperTip™ (TT360-50-5-D), pumped with 75% MeOH, 2% HOAc, having a counter-electrode distance of about 6.5 mm

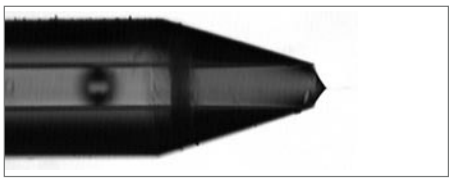
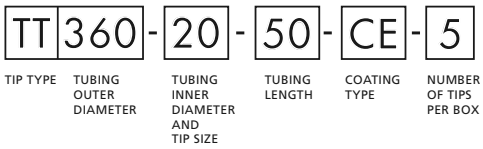


FIGURE 3 A stability-reducing gas bubble flowing toward the end of a TaperTip™

Product Specifications



See our catalog or Web site for available sizes and coating options for TaperTip™ emitters.

If you ordered the TaperKit-360, you have been supplied with five tips:

Order Number	Tubing OD	Tip ID	Quantity
TT360-20	360 µm	20 µm	2
TT360-50	360 µm	50 µm	1
TT360-75	360 µm	75 µm	1
TT360-100	360 µm	100 µm	1

Using Standard-Coated PicoTips® with the Thermo Finnigan Dynamic Flow Nanospray Ionization Source

New Objective has introduced the ADPT-TNS Probe Modification Kit to enable the use of industry standard 5 cm coated PicoTips® emitters with your existing Thermo Finnigan LCQ™-series nanospray ionization source. Use of both standard- and distal-coated SilicaTips™ or TaperTips™ are supported by a MicroTight® union that fits easily into the source's probe assembly. This slight modification allows emitters of various lengths to be used in the source. The MicroTight union virtually eliminates dead volume disturbances to maintain chromatographic integrity by circumventing band broadening.

FIGURE 1 MicroTight® union holding a standard-length PicoTip® emitter

Contents of ADPT-TNS

The Probe Modification Kit contains:

- MicroTight® union, fittings, and sleeves
- New Objective's diamond cleaving tool
- Fused-silica tubing (50 µm ID, 2 m long)
- Conductive elastomer tubing

Coated PicoTips® are primarily used for continuous infusion at nanospray flow rates, for nanoscale flow injection, or for connection to conventional capillary LC columns at microspray flow rates. Figure 2 details the length specifications for the two types of coatings. Emitters with a coating on the "tip end," or standard coating (-CE- in the stock number), may be subject to arcing at higher applied voltages. Arcing can be difficult to avoid, so the use of distal-coated tips (-D- in the stock number) is generally recommended. The distal coating provides a junction-style contact and is immune to arcing.

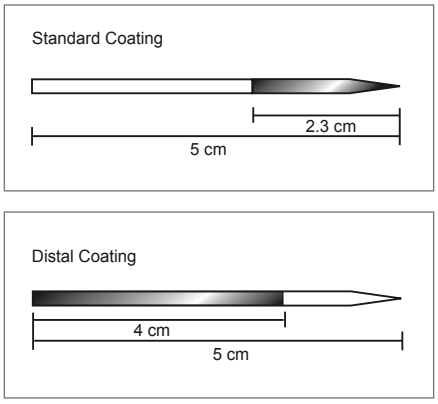


FIGURE 2 Standard PicoTip® specifications

The 20 µm ID distal-coated TaperTips™ are ideal for 100–200 µm ID capillary LC columns or for continuous infusion at microspray flow rates. TaperTips feature a clog-free design for extra ruggedness and serve as the recommended starting point. The featured 10 µm ID distal-coated SilicaTips™ are ideal for connection to 75 µm nanobore LC columns or for low flow rate continuous infusion.

Table 1 provides recommended flow rates for different standard tip IDs. Note that actual performance also depends on a variety of other experimental parameters, including applied voltage, mobile phase composition, and source design. Distal-coated SilicaTips (10 µm ID) and TaperTips (20 µm ID) have been selected for inclusion in this kit.

The adapter kit includes a zero dead volume union. Making proper connections within this union is essential to maintaining the best sensitivity.

Table 1

PicoTip® Style	Tubing Size (OD/ID)	Tip Size (µm)	Flow Rate* (nL/min)
TaperTip™	360/20	20	200–500
TaperTip™	360/75	75	300–2000
TaperTip™	360/100	100	400–3000
SilicaTip™	360/20	10	100–400
SilicaTip™	360/75	15	200–500
SilicaTip™	360/75	30	300–1000

*Typical range of ESI cone stability. Actual performance may vary.

Making a zero dead-volume connection

Remove the fittings from both ends of the MicroTight® union. Insert the white gauge plug into one side of the union and tighten until finger-tight, as shown in Figure 3A.

Thread the fused-silica transfer line tubing through a green PEEK™ MicroTight tubing sleeve.

Insert the sleeved tubing completely through one of the MicroTight fittings and carefully cleave the end. See Technical Note FS-1, available on our Web site, for instructions on cleaving fused silica.

Slide the assembled sleeved tubing and fitting into the open end of the union, as shown in Figure 3B. Press the tubing and sleeve firmly against the gauge plug to ensure they are both properly seated. Finger-tighten the fitting.

Remove the white gauge plug.

CAUTION: Coated tips should be handled with care, as mechanical abrasion can deteriorate the coatings. Wear ANSI-approved safety glasses for protection and use non-powder gloves and a pair of fine tweezers when handling the emitters and transfer lines.

Select another PEEK tubing sleeve and cut it to 15 mm. Remove a PicoTips® from its box and slide it through the cut sleeve, with the distal, non-tip end going first. Remove 15 mm from the distal end of the tip with the diamond cleaving tool (see Technical Note FS-1 for cleaving instructions) and repeat steps for connecting the emitter to the other side of the union. Figures 3C and 3D show the PicoTip before and after cleaving. Ensure that both tubing ends are firmly "butted" together within the union and that the fittings are sufficiently tight by gently pulling on the tubing. Check for leaks by running solvent through the tubing at the anticipated operating pressure. Leaks will be apparent if solvent collects at the exposed ends of the MicroTight sleeves. Figure 3E shows a fully assembled MicroTight union ready for installation.

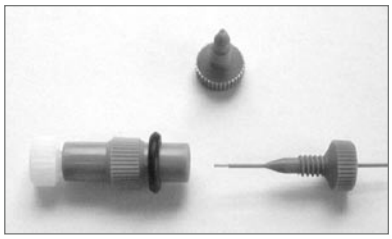


FIGURE 3A MicroTight® union with gauge plug in left side and assembled sleeved tubing with fitting on right

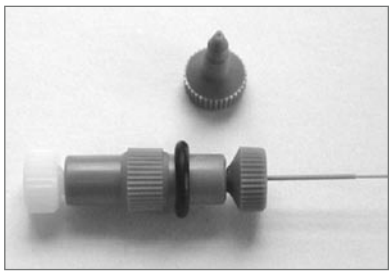


FIGURE 3B Union with assembled sleeve, tubing, and fitting

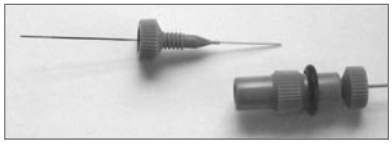


FIGURE 3C PicoTip® assembled with sleeve and fitting before cleaving...

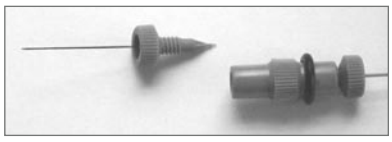


FIGURE 3D ...and after cleaving the distal end of the emitter

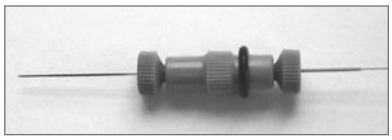


FIGURE 3E Fully assembled MicroTight® union, shown with the PicoTip® on the left

Installing the assembled union

To avoid electrical shock, put the LCQ™ in Standby mode in the Tune Plus window and remove the high-voltage leads from the nanospray (NSI) source. Loosen the two retaining knobs on the spray shield and pull the NSI source slide adapter back along the rails. Remove any existing emitter from the NSI probe assembly.

Use a 5/64 Allen key to loosen the 2-56 socket screw holding the emitter clip in place. Remove the emitter clip (see Figure 4A) and gently slide a piece of the conductive elastomer tubing 3–4 mm over the end of the clip where the screw hole is located (sliding the tubing over the bent end is more difficult and is not recommended). Trim the excess tubing at the end of the clip and slide the tubing over the clip until it covers the clip bend, as shown in Figure 4B.

Being sure not to touch the tip to any surface, carefully grasp the assembled union with one hand, and with the other feed the transfer line through the opening in the API spray shield, from the instrument side, out through the notch on the left side of the probe cover (or over the top of the probe cover). When the bulk of the transfer line is through the spray shield, position the union in the large groove in the NSI body assembly (as shown in Figure 5A), angling the tip end up slightly. Slide the union toward the instrument until it is pressed against the forward edge of the groove; then, gently align the PicoTips® in the tip groove, as shown in Figure 5B. If it is necessary to adjust the position of the PicoTip, use fine forceps so as not to scratch the coating. The PicoTip should extend approximately 5 mm beyond the end of the probe assembly when properly positioned.

Install the emitter clip with the elastomer tubing, as shown in Figure 6, and tighten the screw holding the clip in place. Secure the union in place with a small piece of tape.

Attach the free end of the transfer line to the ZDV ground union and subsequently to your pumping element, as described in the Thermo Finnigan manual.

Inspect the end of the probe to make certain that the MicroTight® sleeve is not preventing electrical contact between the conductive coating and probe. Reattach the high-voltage leads.

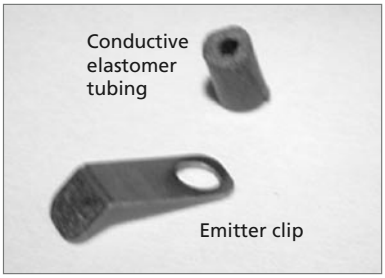


FIGURE 4A Conductive elastomer tubing and emitter clip

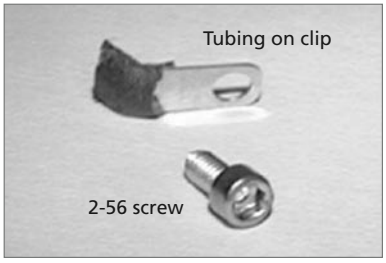


FIGURE 4B Conductive elastomer tubing on the emitter clip and the socket screw

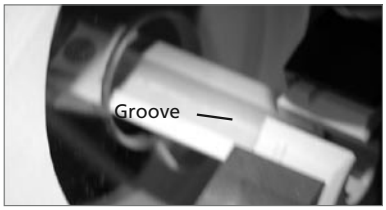


FIGURE 5A Front view of probe assembly groove

FIGURE 5B Top view of assembled union in position

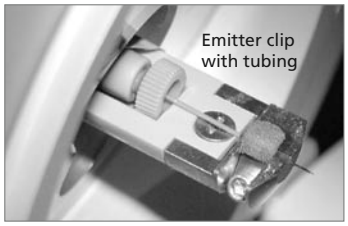


FIGURE 6 Emitter clip and tubing shown with assembled union

Making Connections

This note provides instructions on connecting standard-length 5 cm distal or standard coated PicoTips® to a transfer line or capillary column. "PicoTip" refers to any of New Objective's high-quality tips for electrospray ionization, such as SilicaTips™, PicoFrits®, and TaperTips™.

NOTE: Users must take care when tightening the MicroTight® fittings, making sure to only tighten enough to prevent leaks from occurring. Due to the delicate nature of some fused-silica tubing, it is possible to damage the tubing if the fittings are overtightened.

Loading the MicroTight® Union

1) Remove the MicroTight® Union from the PicoView® components box. Unscrew and remove the compression fittings from both ends of the union.
2) Screw the white gauge plug finger-tight on to one end of the union. Thread the fused-silica transfer line through a green MicroTight sleeve. The appropriate sleeve size is 0.002-0.003 inches greater than the OD of the capillary tubing. Use the green MicroTight sleeves with 360 um OD tubing. After the transfer line passes through a green sleeve, thread it through one of the compression fittings. Figure 2 shows the union loaded with the white gauge plug on the right and fused-silica tubing threaded through a tubing sleeve and a compression fitting. Cleave the end of the tubing and slip it into the union until both the tubing and the sleeve ends seat against the gauge plug inside the union. Screw the compression fitting finger-tight into the union, as shown in Figure 3.
3) Remove the gauge plug and return it to the PicoView components box.
4) Carefully trim a new green MicroTight sleeve to a length of approximately 14 mm, as shown in Figure 4. The shorter sleeve will allow the coating on the PicoTip to contact the conductive elastomer inside the CTM.
5) Choose a PicoTip from the assortment sent with PicoView. Although either coating style, the standard coating (-CE-) or the distal coating (-D-), will work, if flow rates permit, the distal coating is recommended due to its immunity to arcing.
6) Insert the back, or distal, end of the PicoTip through the trimmed sleeve and through the other compression fitting. When properly installed, the tip end should extend 15-20 mm past the end of the fitting when it is tight. This will afford optimal positioning of the PicoTip within the adjustment range of the stage plate. Using a ruler, measure and note the distance the tip extends from the fitting. Remove the fitting/sleeve/PicoTip assembly and carefully trim the back end of the PicoTip so the extension of the tip beyond the fitting is 15-20 mm. Cleave the remaining portion from the back end of the PicoTip. See Figures 5A and B.

Figure 1 MicroTight® union, gauge plug, and compression fittings

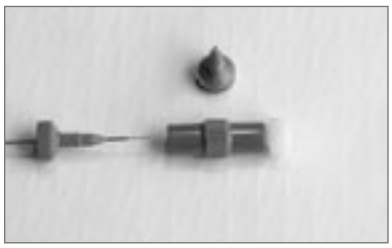


Figure 2 MicroTight® union with gauge plug and compression assembly ready to load

Figure 3 MicroTight® union with gauge plug and compression assembly loaded

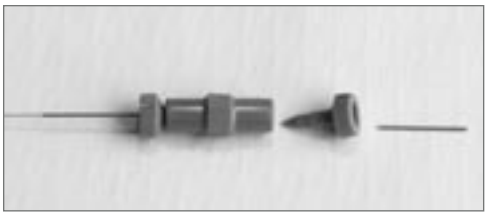


Figure 4 Union, compression fitting, and MicroTight® sleeve cut to 14 mm

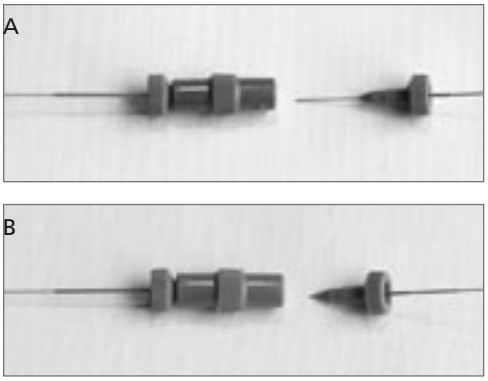


Figure 5 PicoTip™ in fitting, before (A) and after (B) trimming to length

Figure 6 A fully assembled MicroTight® union

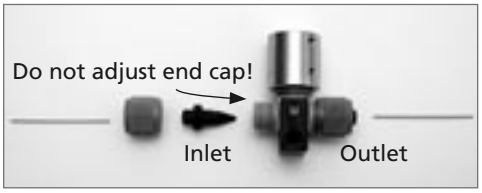


Figure 7 MicroTee with sleeves

Figure 8 Fitting nut, MicroFerrule, and sleeve threaded by PicoTip

7) After trimming, reinsert the assembly into the union, seat the PicoTip and sleeve against the transfer line or column tubing, and tighten the compression fitting finger-tight. Pull gently on the tubing to ensure the connection is tight. Figure 6 depicts the fully assembled union.

CAUTION: Always use chemical- and puncture-resistant gloves and ASNI-approved safety glasses when handling fused-silica tubing.

Supplying High Voltage

Plumbing the MicroTee

1) The MicroTee joins the transfer line to the PicoTip® and supplies the high voltage. Orient the MicroTee as shown in Figure 7 so that the platinum electrode is facing away from the user and the setscrews are visible. Unscrew the nuts and remove the black MicroFerrules from the posts of the MicroTee.

WARNING: Do not loosen the setscrews or remove the electrode cap, as this may damage the electrode. The solvent will not become charged and an electrospray will not form.

2) Thread the end of the PicoTip tubing through a green MicroTight sleeve, which is used for assembly with 360 um OD tubing. Make sure the PicoTip does not extend past the tubing sleeve end that will be inserted into the MicroTee. Thread the sleeved PicoTip through the fitting nut and a black MicroFerrule, as shown in Figure 8.

3) Cleave the end of the PicoTip after the tubing is threaded through the sleeve, nut, and ferrule. (Refer to Tech Note FS-1 for cleaving technique.) Slip the end of the tubing through the right post of the MicroTee, as viewed in Figure 9A, until the tubing and sleeve seat against the bottom ledge inside the post, as shown in Figure 9B. Screw the nut finger-tight onto the MicroTee.

4) Insert the distal end of the fused-silica transfer line through a green MicroTight sleeve, then through the nut and the black MicroFerrule, as shown in Figure 10A. Carefully trim the end of the transfer line. After trimming, insert the assembly back into the MicroTee, seat the transfer line, ferrule, and sleeve against the PicoTip, and finger-tighten the nut, as shown in Figure 10B. Gently pull on the tubing ends to ensure the connection is tight. Check for leaks by running solvent through the tubing at the expected operating pressure. Leaks will be apparent if solvent collects at the exposed ends of the sleeves.

Plumbing the ZDV Union

NOTE: This option is not recommended with PicoFrit® columns.

1) Remove the ZDV union from the PicoView® components box. Orient the union as shown in Figure 11 so that the "T" bracket is facing away from the user. Unscrew the nuts and remove the ferrules from the union.

2) Insert a green SealTight™ sleeve through the ferrule and nut. Thread a PicoTip through the sleeve/ferrule/nut assembly, as shown in Figure 12A. Cleave the end of the PicoTip after the tubing is threaded through the sleeve, ferrule, and nut.

3) Slip the end of the tubing into the right side of the union until the ferrule and sleeve seat against the bottom ledge inside the union (Figure 12B). Screw the nut finger-tight into the union. Do not tighten enough to compress the sleeve.

NOTE: Be careful not to touch the tip to any surface.

4) Trim 5 mm from the end of a green SealTight™ sleeve and insert the sleeve through the ferrule and nut. Thread the back end of the fused-silica transfer line through the sleeve/ferrule/nut assembly. Cleave the back end of the transfer line and slip it into the left side of the union until the transfer line/ferrule/sleeve assembly seats against the PicoTip. Tighten the nuts on both ends of the union. Pull gently on the tubing to ensure the connections are tight. Figure 13 depicts a fully assembled ZDV union.

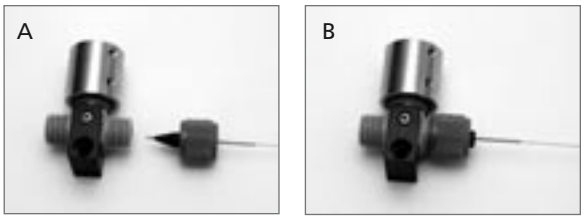


Figure 9 Assembling the nut, ferrule, sleeve, and PicoTip® (A) and securing finger-tight into the MicroTee (B)

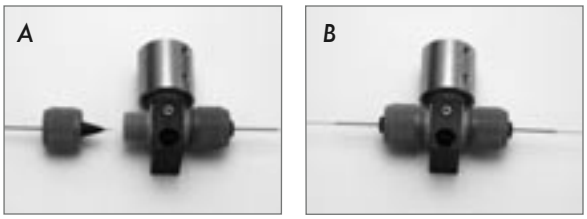


Figure 10 (A) Assembling the nut, ferrule, sleeve, and transfer line and (B) securing into the MicroTee

Figure 11 ZDV union assembly

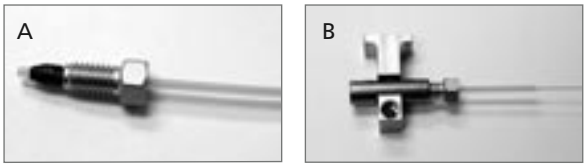


Figure 12 Assemble the nut, ferrule, sleeve, and PicoTip (A) and secure finger-tight into union (B)

Figure 13 ZDV union assembled

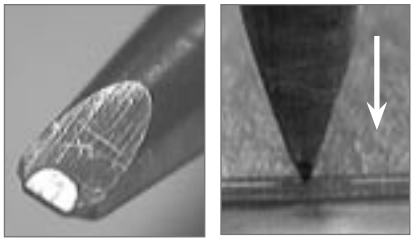


FIGURE 14 (A) Close-up view of diamond-blade cleaving tool, and (B) Cleaving tool in proper position

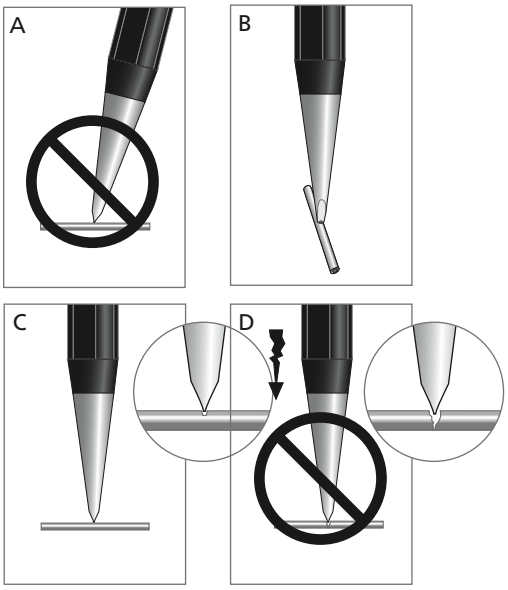


FIGURE 15 (A) Improper cutting angle (B) Align cleaving tool perpendicular to tubing (C) Press down gently, scoring tubing (D) Too much downward pressure will crush tubing, producing particles that can cause tubing to clog

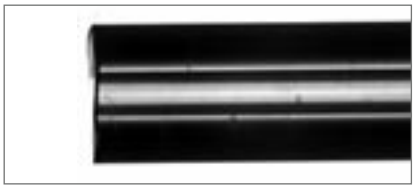


FIGURE 16 Typical cleave. (Polyimide coating was removed after cleaving for clarity of image.)

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 14A, 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.

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 15B and 15C. The long axis of the blade should be perpendicular to the tubing bore (Figure 15B).
2. Gently press straight down; DO NOT use a sawing motion when pressing the blade. You only need to nick the surface of the polyimide coating (Figure 15C). Be careful not to force the blade through the tubing, which will generate a ragged end and many particles (Figure 15D).
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 16. 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 (order number TIP-KIT). A more precise rotary cutting tool is also available from New Objective (order number FSC-001). This tool utilizes a diamond blade with a thumb wheel mechanism to properly score the fused-silica tubing.

Tips on Tips - Online PicoTips®

Thank you for ordering from New Objective's line of PicoTip® emitters for online nanospray. Consisting of SilicaTips™, TaperTips™, and PicoFrits™, they represent the most advanced precision emitters available for nanospray.

Given the wide variety of electrospray ionization (ESI) sources produced by different manufacturers, the exact implementation of PicoTip emitters on your system may affect utility and performance. This "tip sheet" gives a few pointers on the successful use of PicoTips. Please observe all manufacturer safety recommendations and read the safety statement at the end of this document.

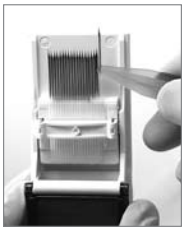
Unpacking and handling your PicoTips®

NOTE: Please wear ANSI-approved safety glasses when handling PicoTip® emitters.

Coated PicoTips® have a special enhanced conductive multilayer coating (U.S. Patent 5,788,166) that provides for excellent electrochemical stability and durability against ESI solvent exposure and arcing. Coated tips should be handled with care, as mechanical abrasion can remove the coatings. No attempt should ever be made to handle emitters with bare hands. Non-serrated forceps are recommended for handling all varieties of emitters. A rubber-tipped disposable pair is included with each package of PicoTips. New Objective carries an accessory kit containing a complete assortment of high-quality tools (cleaver, special forceps, ruler, etc.) needed to properly handle PicoTips. Please see our web site for a full description of our accessory kit (stock number TIP-KIT).

Open package by grasping lid sides

Inside the box, the PicoTips are held in place by a padded pressure bar. When ready to use, pull the PicoTip out from behind the holding bar with a pair of forceps, taking care not to touch the tip or scrape off the conductive coating, as the coating can be ruined by improper or rough handling. Grasp the shaft towards the tip end of the emitter and pull the emitter directly through the holding bar being particularly careful not to bend the emitter. Bending may cause damage to the emitter. The end of the tip must not make physical contact with any surface.



Pull emitter directly up to remove

Cleaving fused silica

Proper cleaving of fused-silica tubing is a critical but often overlooked operation in the preparation of PicoTip® emitters for use. A flat, smooth cleave is critical 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 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.

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 Figure 1. The long axis of the blade should be perpendicular to the tubing bore.
2) Press down gently; DO NOT use a sawing motion when pressing the blade. You only need to nick the surface of the polyimide coating. Be careful not to force the blade through the tubing, which will generate a ragged end and many particles.

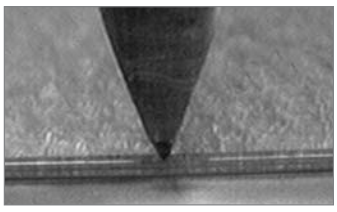


FIGURE 1 Cleaving tool in proper position

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.

Inspection of the distal end of the tip for particle contamination with a light microscope at 100x magnification is highly recommended.

Mounting PicoTips®

Fused-silica PicoTips® can be easily coupled to the "outside world" using any variety of zero or low dead volume unions available from a number of manufacturers. We recommend the use of Upchurch components (MicroTight® series) specifically designed for connecting different diameters of fused-silica tubing. Standard 1/16" HPLC hardware can be used for connecting fused-silica tubing by using 1/16" outer diameter PEEK™ sleeves with an inner diameter appropriate for the silica tubing outer diameter. Avoid the use of graphite ferrules, which can generate particulate matter when handled. New Objective offers a fittings kit (stock number FSFK-1) of assorted Upchurch components. Please see our catalog or Web site for a full description.

Coating style

While many users obtain suitable performance with our standard coated PicoTips® (-CE- in the stock number), some find that emitter performance can be compromised by excessive arcing during tuning due to an overvoltage condition. Although great improvements have been made with emitter coatings, constant arcing may still damage a coated tip, reducing or preventing stable operation. A good solution for those experiencing tuning problems is to use emitters with a distal coating (-D- in the stock number). The high-voltage contact is made through a junction-style contact inside the PEEK™ union. Since the distal coating is only applied to the non-tip end of the emitter, it is immune to arcing.

Spraying

CAUTION: Make certain that all electrical voltages are at ground potential before attempting to insert or remove a PicoTip® on your inlet system.

Before use, emitters should be properly and safely mounted on your ESI emitter mounting system. Make sure there is robust electrical contact between the conductive coating on the coated PicoTip® and your applied voltage "contact point." The final position of the tip should be 1-5 mm from the mass spectrometer inlet.

Applying high voltage

Starting from zero (ground) potential, slowly increase the voltage of the ESI system while monitoring ion or spray current, if your system provides a monitoring point. Although it varies greatly depending upon the exact geometry of your ESI system, spray should initiate at a potential difference between 1000 and 1500 volts. To optimize the applied voltage, monitor ion current while increasing the ESI potential(s). With most systems, a plateau in current is obtainable. The optimal set point is generally found at a voltage just before the onset of the plateau. Occasionally, and especially when spraying solutions that carry no organic solvent, the voltage required to initiate ESI current is quite high (greater than 2.0 kV); such a high voltage generally wastes sample. The voltage can usually be lowered after initiation of stable spray with no expense in ion current and a concurrent reduction in sample flow rate.

In general, the maximum voltage the tips can handle before a stable corona occurs is 3.0-3.5 kV. The fine wall structure of the tip cannot withstand prolonged arcing between the tip and inlet. Potentials that cause arcing should be avoiding when using PicoTips®. Excessive potentials result in higher required flow rates with little gain in total ion current.

Flow rates

Performance varies greatly from instrument to instrument and is highly dependent upon solution characteristics. The most significant influences on flow rate performance are solvent composition, electric field strength, and backing pressure. For operation at lower flow rates, choose smaller diameter PicoTips®. Consult our product literature or Web site for a listing of tip sizes. PicoTips can generally support stable ESI over a range of flow rates. For example, a 5 um tip can operate at rates from less than 25 nL/min to nearly 100 nL/min.

Approximate flow characteristics of the most common sizes of SilicaTips™ are:

Stock Number	Flow Range (nL/min)
FS360-75-30	300-1000
FS360-75-15	200-500
FS360-50-8	50-300
FS360-20-5	20-100

See our website (www.newobjective.com) for more information on flow ranges.

Troubleshooting

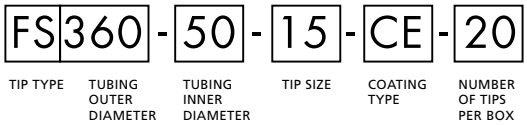
An erratic drop in ion intensity caused by gas bubbles in the system
Gas bubbles can wreak havoc with spray stability. Small bubbles can originate from trapped air pockets within a coupling union, electrolysis at a high-voltage contact, or dissolved gasses in the solvent. Bubbles can be minimized by making certain all fittings are sufficiently gas- and liquid-tight. Allow time for any residual gas to bleed out of the system. If air bubbles persist, try using a PicoTip® with a smaller inner diameter than that of the transfer line. This can create sufficient back-pressure and reduce or eliminate outgassing from solvents and electrolysis.

A droplet forms on the emitter tip
Droplets will form on the tip of an emitter when the applied voltage is not sufficient to maintain a stable spray. Droplets commonly form during the aqueous portion of an LC gradient, as the optimal potential is highest under aqueous conditions. Because most analytes do not elute under highly aqueous conditions, this should not degrade the performance of your system. If increasing the voltage does not help, it may mean that the flow rate is too high. Many customers report fewer problems with droplet formation when using smaller PicoTips®.

Ion signal is diminished
Ion signal can also be diminished with the deterioration of the conductive coating. As a rule of thumb, we suggest you change your tip when this occurs. If using a distal-coated PicoTip®, electrical contact can be reestablished by cleaving a small piece of fused silica from the back end of the tip. The cleaving removes the segment of tubing with an uneven coating and exposes a fresh piece of coating within the union to establish a spray.

Unable to see any flow through the emitter
The primary cause of tip failure is clogging due to particulates. Particles can be seen using a light microscope at 100x magnification. Inline filtration can effectively reduce clogging and extend emitter lifetime. We have obtained best results using HPLC-grade bottled water that has been distilled in glass.

Product Specifications



If you ordered the SilicaKit-360, you have been supplied with nine tips:

Order Number	Tubing OD	Tubing ID	Nominal Tip ID	Quantity
FS360-75-15	360 um	75 um	15 +/-1.5 um	3
FS360-50-8	360 um	50 um	8 +/-1 um	3
FS360-20-5	360 um	20 um	5 +/-1 um	3

Safety Precautions

CAUTION: Electrospray ionization involves the use of potentially lethal high-voltage electrical current. Observe all manufacturers' safety recommendations in the use of such equipment. No equipment modifications should be made except as authorized by the manufacturer in accordance with all safety requirements. Never use this product in defective, damaged, or faulty equipment. Serious personal injury or death could result.

Installation of such equipment should be performed by a qualified contractor in accordance with all applicable electrical codes. This product should be used only by experienced personnel.

Provide a safe workplace and all necessary safety equipment. Follow all safety recommendations of the equipment manufacturer(s). Inspect all equipment and ionization emitters carefully prior to use. Any damaged, chipped, or cracked emitters should not be used. 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.

Fused-silica needles

PicoTips® can be conveniently filled with conventional syringes that substitute a fused-silica needle for a stainless-steel needle. These syringes are available from Hamilton and other suppliers. (Order needle style “RNFS” from Hamilton -- for example, Hamilton syringe model 1701-RNFS, part number 87404, which has a 10 cm long, 170 um OD flexible fused-silica needle.) Fused-silica syringe needles can reach within 0.2 mm of the tip. The OD of the filling needle must be less than the ID of the PicoTip glass tubing.

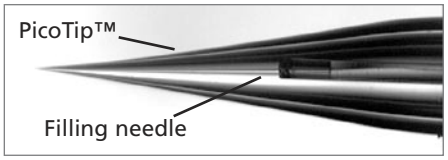
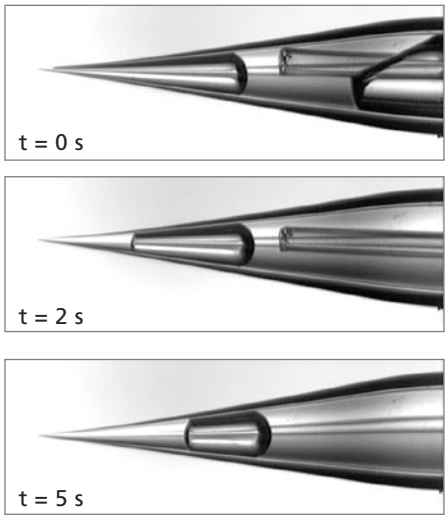


FIGURE 1 Loading an offline PicoTip™ with a fused-silica needle

Insert the filling needle into the distal end of the PicoTip, as seen in Figure 1. Push the needle as far into the PicoTip as possible without damaging either the filling needle or the PicoTip. (It is not critical to reach far into the tapered region, since capillary action will fill the tip. The closer the initial loading of sample is to the taper region, however, the faster the filling action.)

Slowly inject the liquid into the PicoTip. Careless, rapid injection can lead to an excessive number of air bubbles, or “foaming” of the sample. This foaming is especially problematic with concentrated protein and peptide samples. (To prevent this, 1-5 uL of sample should be injected over approximately 5 seconds.) Most syringes are limited to a maximum volume of 10 uL.

Slowly withdraw the filling needle from the PicoTip. The tip will fill by capillary action. Do not be alarmed if you see air bubbles along the shank of the tip. As the sample sprays from the tip, capillary action will provide a continuous feed and eliminate air bubbles in the taper region, as shown in Figure 2.



Gel-loader tips

Another device convenient for tip filling is a gel-loader type disposable pipette tip. Choose the smallest OD gel-loader tip available for your pipetter. Gel-loader tips with an OD of less than or equal to 0.35 mm are particularly good for filling offline PicoTips®. Micro-gel loader tips designed for loading samples onto thin gels are available from Eppendorf(R) (pipette tip number 2235-165-6) and other suppliers.

Insert the pipette as far as possible into the distal end of the PicoTip and deliver 1-5 uL of the liquid slowly into the emitter while removing the pipette tip. A typical gel-loader tip is not long enough to extend all the way to the tip of the emitter. The sample will fill only the back end of the PicoTip, but capillary action will bring the sample into the tip. It is a good practice to wait a few minutes for this filling action to take place. Inspection for proper filling with a transmitted light (rather than reflected light) microscope at 50-100x is recommended.

Spraying

CAUTION: Make certain that all electrical voltages are at ground potential before attempting to insert or remove a PicoTip® on your inlet system.

Before use, PicoTips® should be properly and safely mounted on your emitter mounting system. Make sure there is robust electrical contact between the conductive coating on the coated PicoTip and your applied voltage "contact point." The final position of the tip should be 1-5 mm from the mass spectrometer inlet.

Since PicoTips (U.S. Patent 5,788,166) are fabricated with a precision geometry specifically tailored for low-flow ESI, there is no need to break the tip end into the inlet prior to use. Such actions result in uncontrolled tip diameter, wall thickness, and tip shape. We do not recommend or endorse this practice with PicoTips.

Applying high voltage

Starting from zero (ground) potential, slowly increase the voltage of the ESI system while monitoring ion or spray current, if your system provides a monitoring point. Although it varies greatly depending upon the exact geometry of your ESI system, spray should initiate at a potential difference between 600 and 1000 volts. To optimize the applied voltage, monitor ion current while increasing the ESI potential(s). With most systems, a plateau in current is obtainable. The optimal set-point is generally found at a voltage just before the onset of the plateau.Occasionally, and especially when spraying solutions that carry no organic solvent, the voltage required to initiate ESI current is quite high (greater than 1.5 kV); such a high voltage generally wastes sample. The voltage can usually be lowered after initiation of stable spray with no expense in ion current and a concurrent reduction in sample flow rate.

In general, the maximum voltage the tips can handle before a stable corona occurs is 1.6-2.5 kV. The fine wall structure of the glass tip and conductive coating generally cannot withstand prolonged arcing between the tip and inlet. Potentials that cause arcing should be avoided when using PicoTips®. Excessive potentials will only result in a rapid consumption of sample with little gain in total ion current.

Solvents

The proportion of organic solvent can be reduced to 0-30% from "conventional" levels of 70-80%. A lower proportion of organic cosolvent generally results in a slower rate of residue buildup at the tip and hence extended tip lifetime.

With pure, particle-free water (2% acetic acid, no protein or other analyte), tip lifetime is generally more than 50 hours of continuous spray-time. HPLC-grade or better solvents are recommended for optimal performance.

Flow rates

Performance varies greatly from instrument to instrument and is highly dependent upon solution characteristics. The approximate range of optimal flow rates are:

Stock Number	Optimal Flow (nL/min)
Econo10	20-80
Econo12	20-80
BG##-##-2-CE	20-80
BG##-##-4-CE	40-100
QT##-##-2-CE	20-80

The most significant influences on flow rate performance are solvent composition, electric field strength, and backing pressure. For operation at lower flow rates, choose smaller diameter PicoTips®. Consult our product literature or Web site for a listing of tip sizes.

PicoTips can generally support stable ESI over a range of flow rates. For example, a 4 um tip can operate at rates from less than 25 nL/min to nearly 100 nL/min.

Product specifications

Safety precautions

CAUTION: Electrospray ionization involves the use of potentially lethal high-voltage electrical current. Observe all manufacturers' safety recommendations in the use of such equipment. No equipment modifications should be made except as authorized by the manufacturer in accordance with all safety requirements. Never use this product in defective, damaged, or faulty equipment. Serious personal injury or death could result.

Installation of such equipment should be performed by a qualified contractor in accordance with all applicable electrical codes. This product should be used only by experienced personnel.

Provide a safe workplace and all necessary safety equipment. Follow all safety recommendations of the equipment manufacturer(s). Inspect all equipment and ionization emitters carefully prior to use. Any damaged, chipped, or cracked emitters should not be used. Handling of glass 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.