Production of packed nanospray emitters with integrated emitter-end frits for liquid-chromatography mass spectrometry

Leo Wang (lewang@coh.org)
Wang Lab, City of Hope
Nathan Hendricks
Wang Lab, City of Hope

Method Article

Keywords: mass spectrometry, proteomics, nanospray,

Posted Date: January 2nd, 2018

DOI: https://doi.org/10.1038/protex.2017.154

Abstract

This protocol describes how to frit the emitter-end of a pulled silica capillary and subsequently pack it to produce a robust integrated column. Integrating liquid chromatography separations directly into the emitter of a nanospray source for mass spectrometry offers improved sensitivity and resolution. This is achieved by reducing post-separation dead volume. The method described here provides an optimized setup to maximize sensitivity and separation.

Introduction

The sensitivity of mass spectrometry-based proteomics is enhanced by limiting the diameter of the electrospray emitter to a few micrometers. Additionally, capillary liquid chromatography with small internal diameters \(50 µm or less) enhances the detection of low-level species in complex samples. To maximize the benefit of this style of column, dead volume after the separation of compounds should be minimized. Current protocols for custom-packed fritted columns[1] and pulled silica emitters with distal-end frits[2] can be joined by a union to offer a setup appropriate for nanospray ionization mass spectrometry; however, this introduces dead volume post-separation. Ideally, the column should extend to the tip of the emitter. Emitters packed without a frit may be unsuitable for smaller particle size resins which can escape the opening or be prone to clogging. Consequently, we have developed a protocol to put a frit in the emitter-end of a pulled silica nanospray emitter.

Reagents

Emitter fritting: Polyimide-coated fused silica capillary with dimensions: 360 µm outer diameter \(O.D.) / 50 µm internal diameter \(I.D.) Kimwipes KASIL 1624 potassium silicate solution Formamide Hydrofluoric acid \(HF) Emitter packing: Chromatography resin Methanol

Equipment

P2000 laser puller Ceramic scribe for fused silica Lighter Diamond cutter for fused silica Vortex mixer Microcentrifuge Microscope Oven Capillary packing pressure bomb with stir plate and appropriate gas hookup Stir bar \(3mm) Erlenmeyer flask Nanoflow capable HPLC

Procedure

Fused silica emitter production This section of the procedure is modified from Cifani, et al \[2]. 1. Cut a portion of polyimide-coated fused silica capillary that is twice the length of your desired final column using the ceramic scribe. 2. Burn away approximately 1-2 cm of the polyimide coating at the middle of the silica piece with a lighter. 3. Gently remove the charred residue of the coating using methanol and a Kimwipe. 4. Mount the piece of fused silica on the laser puller; carefully feed the middle section where the silica is exposed into the shielded mirror target of the puller. 5. Close the cover of the puller. **TIP:**

Longer lengths of silica may extend past the cover, so take care not to crush them when closing it. You should be able to bend the silica into the cover so that it sits completely enclosed by the shield. 6. Enter the following program, then press “pull”: HEAT=250, FIL= , VEL=25, DEL=180, PULL=25 HEAT=250, FIL= , VEL=25, DEL=180, PULL=25 HEAT=250, FIL= , VEL=25, DEL=180, PULL=25 HEAT=250, FIL= , VEL=25, DEL=180, PULL=25 7. Inspect the emitter under a microscope. The final emitter should appear similar in geometry to figure 1a. **TIP:** Verify that the emitter tip is open by submerging the tip in water for a second. An open tip should have a visible solvent front in the internal capillary \(see figure 1b). Allow the water to evaporate before proceeding with the next step. **Fritting the emitter** 1. Add 75 µL of KASIL 1624 to a 1.5 mL polystyrene tube. 2. Add 25 µL of formamide. 3. Vortex for 10 seconds. 4. Spin down in a centrifuge for 10 seconds. 5. Take the pulled silica emitter and dip the pointed end into the frit solution for ~5 seconds, keeping the capillary vertical. **TIP:** The emitter should barely touch the fluid’s surface and not be submerged in the solution any more than necessary. 6. Verify that the capillary has soaked up a small plug of frit solution under a microscope \(figure 2a). 7. Put the emitter in an Erlenmeyer flask with the tip pointed upwards. Heat the flask at 100°C overnight. 8. Remove the emitter from the oven and let it cool before proceeding. 9. Check the emitter under a microscope to verify that a solid frit has formed in the tip of the emitter \(figure 2b) **NOTE:** A thin film of frit solution on the exterior of the emitter may have formed to cause opacity. This will be removed in the following steps. _Caution: the next step utilizes HF. Take all necessary safety precautions and perform the next steps in a fume hood._ **Emitter packing** 1. Suspend the chromatography material of your choice in methanol in a 1.5 mL polystyrene tube. 2. Place a 3 mm magnetic stir bar into the vial. 3. Place the vial into the capillary packing pressure bomb and secure the lid. 4. Turn on stirring. 5. In a fume hood, insert the fritted emitter into a capillary packing pressure bomb. Tighten the nut to secure it in place. 6. Gradually pressurize the bomb to 500 psi. 7. Bend the emitter to dip the tip into a small vial of HF. Hold it in place for 10 seconds. **NOTE:** Buildup of the solid frit will likely have obstructed the small opening of the emitter. The emitter should have a small but visible solvent drop that forms at the tip after HF etching. Flow will also be indicated by slow movement of the packing material towards the tip. 8. If no flow is apparent, repeat step 7. 9. Increase pressure to 1500 psi. 10. Allow the column to pack. **NOTE:** Small particle sizes with small I.D. columns may take several hours. 11. Once fully packed. Close the valve from the gas canister and allow the column to depressurize slowly. 12. Remove the column from the packing bomb. 13. Connect the emitter to an HPLC and run the column at 80% ACN with an appropriate flow rate for the I.D. and packing material for 1 hour, or until the pressure stabilizes.

Timing

Emitter production: 30 minutes Emitter fritting: 8.5 hours Emitter packing: variable

Anticipated Results

The procedure results in an emitter with an integrated frit that is packed with chromatography material.

References

1. Dhabaria, A., Cifani, P. & Kentsis, A. Fabrication of Capillary Columns with Integrated Frits for Mass Spectrometry. Protoc. Exch. \(2015). doi:10.1038/protex.2015.049 2. Cifani, P., Dhabaria, A. & Kentsis, A. Fabrication of Nanoelectrospray Emitters for LC-MS : Protocol Exchange. Protoc. Exch. \(2015). doi:10.1038/protex.2015.053

Acknowledgements

Thanks to the City of Hope Mass Spectrometry and Proteomics Core Facility for use of their equipment and instruments.

Figures

Figure 1

Pulled silica emitter appearance. Representative laser-pulled emitter (a) and the appearance of a water test demonstrating the tip is open (b). 10x magnification.

Figure 2

Fritting the emitter Visible confirmation frit solution has entered the tip (a) and the appearance of a solidified frit post-baking (b). 10x magnification.