

## Instructions for Slurry Packing a uCapillary Column

### Materials needed:

- packing material
- 70% ethanol
- 5% acetic acid
- silica tubing of the desired diameter
- column end fittings, frits, and tubing sleeves
- 1.5ml Eppendorf vials
- small stir bar
- microscope
- light source

### Procedure:

1. Cut a section of silica tubing to approximately 40 cm in length. Inspect ends under a microscope to insure a clean cut, not beveled.
2. To one end attach an end fitting setup with a frit. Flow will exit the column through this end. Connect a small section of tubing to the output of the end fitting. This will allow you to see the liquid flow out the end as you pack the column. The other end of the column will remain without an end fitting until the column is packed.
3. Fill an Eppendorf vial with 70% ethanol almost to the top and cut off the cap with a razor blade. Place the vial into the pressure cell chamber using forceps. Put the top of the pressure cell in place and tighten the four bolts to finger tightness
4. Thread the end of the capillary without a frit into the top of the pressure cell – through the nut, Teflon ferrule and top of the cell. The end of the capillary inside the pressure cell should sit about half way down in the vial of 70% ethanol. This is best achieved by pushing the tubing down until you feel it hit the bottom, and then backing it off a bit. When you have it to the correct height, tighten the nut partially. At this time, fully tighten the four bolts and then go back and tighten the nut on top of the cell tightly with your fingers. Do not use a wrench for this; it will destroy the plastic ferrule. Mark the capillary with a permanent marker so that the position of the capillary inside the cell is easy to set again.
5. Set the two-stage, high pressure regulator to about 1500 psi. You are now ready to wet the column. Slowly turn the pressure on in the cell by turning the valve 180°. You should see liquid coming from the top of the column. Once you see the liquid flowing, turn the valve 180° back to the off position. This will release the pressure from the cell. The column is now wet and ready to be packed.
6. Remove the top from the cell and take out the vial with forceps. Very little solvent should have been used in the wetting process. We will add our packing material to the remaining solvent to make a slurry for packing the column. Using a spatula place

about 50-100ul (dry volume) of packing material in the solvent. Vortex to mix and briefly sonicate (no more than 1 minute). Place a small stir bar in the bottom of the vial and place vial in cell. Turn stir plate below cell on.

**CAUTION: inhalation of derivatized silica can be dangerous to your health**

7. Use the same method as in step 4 to seat the capillary in the cell and tighten the bolts and nut. Using the same method as in step 5, turn the pressure on. Use the light source to observe the beads packing and note the flow of the liquid out the top tube. The packing process may take 20 minutes-1hour, or longer depending on the inner diameter of the tubing being packed.
8. When the packing comes to within 1 cm of the desired length, turn the valve 90°. This stops pressure from the tank but retains pressure built up in the cell. **Very slowly** turn the valve to the off position. This step must be done very gradually to prevent the packing from sliding down the capillary. For best results, turn the valve until you hear a slight hissing from the pressure release and stop. After a few minutes the hissing should stop and you can turn the valve until it hisses again. Repeat this until the valve is all the way off. This step may take 10-15 minutes when done properly.
9. Remove the top from the cell being careful not to disrupt the column. Put a vial with 5% acetic acid in the cell chamber. The acetic acid wash will finish the packing because there remains some unpacked silica in the back of the capillary. It also functions in activating the column. Using the same methods as previously described, seal the pressure cell and turn the valve to the on position. After about 15 minutes, slowly release the pressure as previously described. Cut the open end about 0.5 cm up from the bottom of the packing material and attach the inlet fitting. Cap ends of column.

Things that could go wrong:

**-no flow is coming out the top during packing**

Capillary is probably not far enough into the vial, or is clogged. Release pressure and Check.

**-flow is coming out the end, but column is not packing**

If this happens, it is most likely that the packing material is settling possibly due to the stir bar stopping. Release pressure and vortex slurry again, check stir bar and plate.

**-slurry is sliding down capillary when pressure is released**

Pressure was most likely released from cell too quickly. The integrity of the packing bed is probably compromised if this has happened. Best advised to start again.