Technical Bulletin A0210: Manual Calibration of Solvent Push Volume for Flow Injection NMR

Objective

This document describes how to determine the most appropriate manual push volume, V_{push}, to maximize S/N for a given sample injection volume, V_{inj}. The following procedure is best performed with a model compound before the analyte of interest is employed. A reasonable sample injection volume is 3-5 µL, with 3 µL preferred. The resultant V_{push} is dependent on the sample injection volume used, so for careful work, it is necessary to perform the following procedure for each V_{inj} you choose to use.

Parts and Apparatus

- 250-µL syringe
- 5-mL syringe
- 10-µL syringe (needle dead volume is 0.9 µL)
- 25-µL syringe (needle dead volume is 0.9 µL)
- Injection port connected to probe:

Manual Injection with MIM or HPMI	Manual Inject
- Upchurch F-287 black nut and ferrule	- Upchurch P-779-01 union
- F-247 sleeve, green; for FEP tubing	- F-331N nut and ferrule
- F-242 sleeve, green; for fused silica capillary	- F-242 (for capillary) and F-247 (for needle) sleeves

For a summary on how to assemble the fittings, see the following tech bulletin at www.microNMR.com:
For FEP tubing, see Tech Bulletin G0113 - Fused Silica Capillary Fluidic Connections
For Fused Silica Capillary, see Tech Bulletin G0310 - FEP Tubing Fluidic Tutorial

Procedures

Starting with a Solvent-Filled Probe (Recommended for $V_{inj} \leq 5$ µL)

- Rinse the probe with solvent alone (250-µL syringe and syringe vise; no port); leave solvent in probe.

- Rinse the 25-µL syringe with solvent.
- Connect syringe to injection port, and inject 25 µL of solvent into probe. Leave syringe in port.
- Rinse another 25-µL syringe with solvent, and empty the syringe barrel leaving its needle filled with solvent.
- Draw sample (model compound) up into the second 25-µL syringe; be sure bubbles are eliminated.
- Inject sample into probe (slowly push plunger all the way down to zero).
- Acquire a spectrum, preferably a 1-scan spectrum. This spectrum should be blank, or indicate very little sample.

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- Remove the sample 25-μL syringe and replace it with the first 25-μL syringe, filled with solvent to the 20 μL mark.
- Measure the amount of tubing/capillary from the base of the probe to your injection port. At 4.5 uL/meter for 75 um tubing/capillary, calculate how much volume there is between your injection port at the base of the probe. This volume (volume=X) will ensure you are before the maximum by only pushing the sample to the base of the probe.
- Slowly inject volume X; leave syringe in port.
- Acquire under the same conditions and measure S/N as before. Measure S/N of a distinct spectral feature under well-relaxed conditions.

- Take the 25-μL syringe out and refill with solvent.
- Draw the same exact volume of sample (model compound) up into the second 25-μL syringe; be sure bubbles are eliminated.
- Mount 25-μL syringe in port by placing the fitting onto the syringe before placing the syringe tip into the injection port and tightening the fitting.
- Inject sample into probe (slowly push plunger all the way down to zero).
- Remove the sample 25-μL syringe and replace it with the solvent 25-μL syringe being careful to place the fitting onto the syringe before placing the syringe tip into the injection port to prevent any air bubbles.
- Slowly inject volume (X + 1uL); leave syringe in port. This additional 1 uL will position the sample 1 uL (4.5 meters) closer to the flowcell.
- Acquire under the same conditions and measure S/N as before.
- Repeat these steps (wash out probe with solvent, inject sample, inject previous push volume of solvent plus incrementally adding an microliter to the push volume until the S/N value goes through a distinct maximum.

- Repeat this entire exercise using 0.5-μL increments around the S/N maximum to more carefully determine the V_{push} that yields the largest S/N.

Use the V_{push} determined above to push the sample volume of interest into the flow cell to yield the maximum S/N.

Starting with an Empty Probe (Recommended for V_{inj} > 5 μL)

- Rinse the probe with solvent alone (250-μL syringe and syringe vise; no port).
- Blow all the solvent from the probe with air (5-mL syringe and syringe clamp; no port).

- Rinse 25-μL syringe with solvent, and empty the syringe barrel leaving its needle filled with solvent.
- Draw sample up into the 25-μL syringe; be sure bubbles are eliminated.
- Attach syringe to injection port and inject sample into probe (push plunger all the way down to zero).
- Acquire a spectrum, preferably a 1-scan spectrum. This spectrum be blank, or indicate very little sample. If the fluid has not slightly overflowed the flow cell, the condition may be unshimmable. If so, proceed to the next step.

- Measure the amount of tubing/capillary from the base of the probe to your injection port. At 4.5 uL/meter for 75 um tubing/capillary, calculate how much volume there is between your injection port at the base of the probe. This volume (volume=X) will ensure you are before the maximum by only pushing the sample to the base of the probe.
- Slowly inject volume X; leave syringe in port.
- Acquire under the same conditions and measure S/N as before. Measure S/N of a distinct spectral feature under well-relaxed conditions.

- Using the solvent syringe, rinse the probe entirely with solvent 25-50 uL.
- Blow all the solvent from the probe with air (5-mL syringe and syringe clamp; no port).

- Draw sample up into the 25-μL syringe; be sure bubbles are eliminated.
- Attach syringe to injection port and inject sample into probe (push plunger all the way down to zero).
- Slowly inject volume (X + 1uL); leave syringe in port. This additional 1 uL will position the sample 1 uL (4.5 meters) closer to the flowcell.
- Repeat these steps (rinse, inject sample, inject push volume plus additional microliter) until the S/N value goes through a distinct maximum.

- Repeat this entire exercise using 0.5-μL increments around the S/N maximum to more carefully determine the V_{push} that yields the largest S/N.

- Use the V_{push} determined above to push the sample volume of interest into the flow cell to yield the maximum S/N.

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Sample Data

See the attached data set as an example of how data may appear.