Rheodyne Tech Tip 7:
Sample Loops Loading

Q: "Why can I load only up to half of the volume of the loop in partial-filling method?"

A: Sample occupies 2 µL of loop for every 1 µL loaded from the syringe. For example, 10 µL of sample spreads out over the entire length of a 20-µL loop. Any more sample loaded will overflow the end of the loop and exit out to waste. Reproducibility is poor because the volume of sample in the loop is different from the known volume originally loaded by your syringe.

Fluid spreads in a parabolic shape through a tube instead of moving in one plug because the velocity is different at the center of the tube than at the walls. The velocity at the center of the tube is twice the average velocity, and near the wall the velocity is almost zero, creating a parabolic shape. This fluidic movement is called laminar flow. See Figure 1.

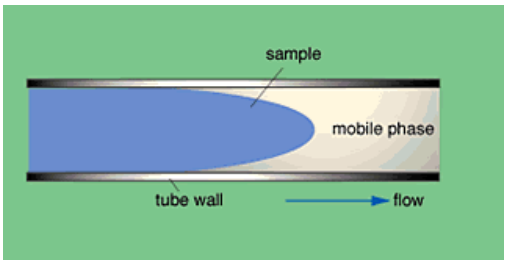

Figure 1. Schematic of sample flow through mobile phase between tubing walls.

In dual-mode injectors (see Tech Tip 6) the sample from the syringe needle loads directly into the sample loop. The sample volume is known since there is no sample waste. The laminar flow phenomenon accounts for the shape of the plot as shown in Figure 2. Note that the plot has three regions:

a) Partial-Filling Region
When the volume dispensed is less than half the loop volume, the curve is linear. Sample has not reached the end of the loop. Within this region, performance depends on the syringe and operator.

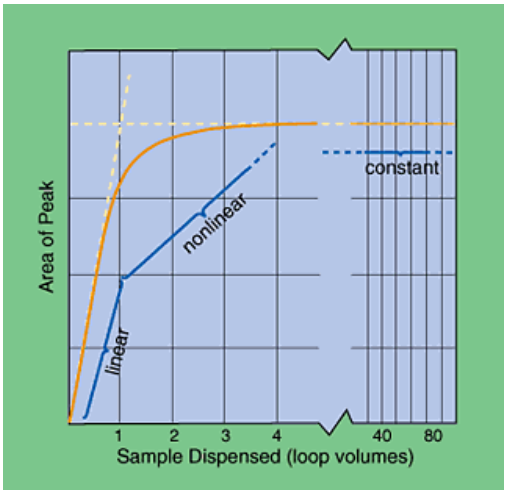

Figure 2. Sample mass (observed peak area) vs. volume of sample dispensed from the syringe, in units of loop volumes, injected onto the column from a Rheodyne® dual mode injector such as Model 7725.

b) Nonlinear Region
When the volume dispensed is between half the loop volume and about two loop volumes, the curve is nonlinear. Sample is lost from the loop, so reproducibility is poor. If you dispense a volume equal to the loop size, you are in this region of poor performance.

c) Complete-Filling Region
When the volume of sample dispensed is several loop volumes, the loop contains only pure sample undiluted by residual mobile phase. Within this region, reproducibility is highest.

In the single mode injectors, the sample must pass through a connecting passage before it reaches the sample loop. Since some of the sample dispensed from the syringe remains in the connecting passageway, an unknown amount enters the sample loop. Therefore, single mode injectors achieve high reproducibility only by using the complete-filling method. See Tech Tip 6.