Troubleshooting

Autosamplers, Part I — Design Features

Autosamplers are more than just automated injection valves.

John W. Dolan
LC Troubleshooting Editor

This column is the first of a two-part series about autosamplers used in liquid chromatography (LC) systems. Today, nearly all chromatographers use autosamplers daily. Autosamplers reduce manual labor, increase sample throughput, and improve precision for routine work. This month's "LC Troubleshooting" looks at three common autosampler designs, how they work, and some of their strong and weak points. The next installment of this series will consider common problems encountered with autosampler use.

Injection Valves

Most autosamplers use six-port injection valves as the mechanism to deliver samples to columns. This type of valve comprises a fixed stator and a movable rotor. The stator is connected to the valve body in a manner that allows the attachment of tubing, loops, injection ports, and other peripheral connections. The stator generally is made of a wear-resistant material such as stainless steel or ceramic. The rotor is held closely against the stator and, as the name implies, rotates during operation. The rotor usually is made of a softer material, such as a fluorocarbon polymer, than that used in the stator.

In autosamplers, the rotor is driven by an electric motor, although some older units use compressed air; manual injectors have a lever handle attached to the rotor. The rotor contains small passages that connect the various stator passages as needed. In the drawings used in this discussion, the stator and associated connections are outside the main circle of the valve, whereas the rotor with its U-shaped connecting channels is drawn inside the circle. Figure 1 shows a typical injection valve configuration. Figure 1a shows the valve in the *load* or *fill* position, in which the injection loop is filled with sample. Meanwhile, the pump delivers mobile phase directly to the column and bypasses the loop. Next, the rotor is turned to the *inject* position (Figure 1b), and the connecting passages direct the pump flow through the loop and flush its contents onto the column.

Filled- and Partial-Loop Injection

Conventional six-port valves can deliver sample to a column in the filled-loop or partial-loop mode. In the filled-loop mode, the volume of sample injected is dictated by the volume of the loop. Figure 2a shows this function schematically. For example, a 20-μL loop might be fitted to the valve and then sample would be delivered to the loop until the excess sample exited the waste port. The loop contents (the cross-hatched portion) then are delivered to the column after the rotor is moved to the inject position. Because of the fluid dynamics

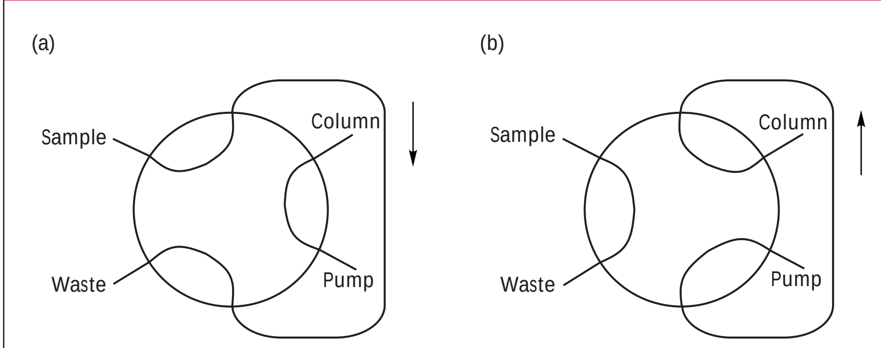

Figure 1: Schematics of a six-port injection valve shown in (a) load and (b) inject positions. Arrows show direction of flow through the loops.

involved, analysts should overfill the loop by at least twofold to ensure uniform loop filling. To change the injection size, users would mount a different volume loop on the injector.

An alternate injection technique is the partial-loop injection. In this technique, illustrated in Figure 2b, a carefully measured volume of sample is delivered to the loop. For example, 20 μL might be placed in a 100-μL loop. Once again, the loop contents are flushed onto the column when the rotor is moved. For the most reproducible injections, it is best to keep the injection volume at less than half the loop volume. This technique is more flexible than the filled-loop injection because it allows chromatographers to inject different volumes without having to install another loop. However, the partial-fill technique requires accurate and precise delivery of sample to the loop for good injection reproducibility.

Note that the loop is backflushed onto the column. That is, the sample is loaded into the loop in one direction and flushed out of the loop in the opposite direction. Backflushing does not affect the results in the filled-loop mode, but it is important for the partial-fill method. Consider the case in which a large loop is fitted to the valve, as might be the case for some autosamplers, for example, a 1-mL loop. If an analyst placed 10 μL of sample in the 1-mL loop and backflushed it onto the column, the sample would be delivered in a discrete plug followed by the remainder of the loop contents (mobile phase), and we would expect good chromatographic performance. With forward flushing, as would be the case if the pump and column connections were reversed in Figure 2b, the 10-μL plug would have to flow through 1 mL of tubing and would be broadened in the process. A broad peak at the point of injection will continue to broaden during the separation, and all the peaks will be wider than expected, especially in isocratic separations. Therefore, it is important that the loop is plumbed to ensure that the loop is backflushed onto the column. This practice generally is no problem unless a user dismantles a valve to service it and reattaches the connections in the wrong positions.

Manual injectors operate as illustrated in Figures 1 and 2, in which the sample is introduced with a syringe, and the valve is rotated by hand (or by pressing an Inject button). Most autosamplers use one of three configurations, in which the sample is pulled or pushed through a fixed loop or a special case in which the injection needle forms part of the loop. Although other designs and hybrids exist, I will discuss only the three basic autosampler variations here.

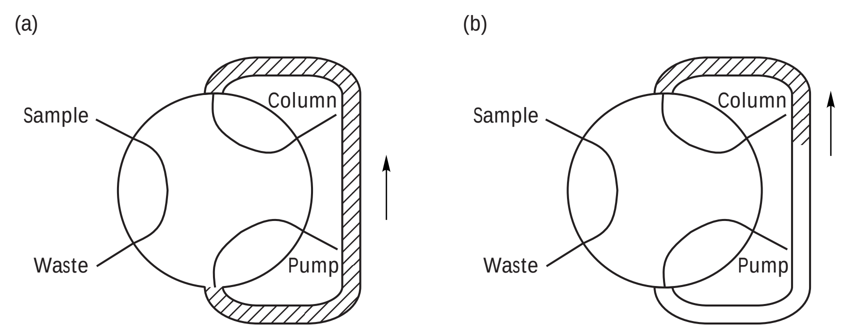

Figure 2: Schematics of (a) filled- and (b) partial-loop autosampler injection modes.

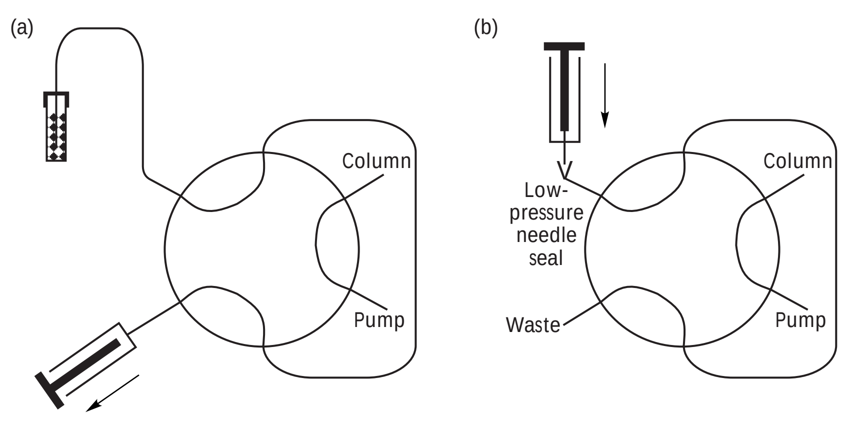

Figure 3: Schematics of (a) pull-to-fill and (b) push-to-fill autosampler designs in the fill mode. Arrows show direction of sample flow during the fill cycle.

Pull-to-Fill Autosamplers

The pull-to-fill autosamplers get their name because the sample is drawn into the sample loop by syringe suction. This type of autosampler was popular in the early days of autosampler development because of its simplicity and reliability, but it has dropped from favor with the advent of the other two designs.

Figure 3a illustrates the pull-to-fill design. In this example, a syringe is attached to the waste port, and a needle with connecting tubing is attached to the sample port. The needle is inserted in a sample vial, and the syringe is drawn back until the loop is filled with sample. The rotation of the valve results in the injection of the loop contents. This autosampler design is quite simple, often with a needle that moves in just one axis (up and down) with a sample tray rotating until the desired vial is below the needle. One popular variation is even simpler — it uses a tight-sealing vial cap that is pressed down into the vial to displace sample into the loop rather than using a syringe at the other end.

If simplicity is the strong point of the pull-to-fill autosampler, sample waste is its weak point. As Figure 3a shows, sample must fill the needle and all the connecting tubing before it reaches the sample loop. This sample, sometimes as much as 10–100 μL, is wasted. This waste may or may not be important, depending on the available volume of sample.

Because of the simple design, most of these autosamplers use a rotating sample tray with a fixed sequence and a predetermined number of injections per vial. The flushing mechanism may require a wash vial between each sample vial or may use a separate wash station.

Push-to-Fill Autosamplers

A more common autosampler design uses the push-to-fill technique. This technique is very similar to manual injection. The syringe moves to the sample vial, pulls up the desired sample volume, moves it to the injection port, and delivers the sample to the loop, as shown in Figure 3b. Typically, users need much less extra sample with this design than for the pull-to-fill design. Syringe filling and dispensing is under the control of a stepping motor, which can provide very precise and accurate sample delivery. As a result, an imprecision of less than 0.5% is common in both filled- and partial-loop modes.

Usually, push-to-fill autosamplers add features to the basic pull-to-fill designs, such as random vial access, more effective flushing, and programmable sample volumes. The tray layout may be round or may use an *x,y,z* axis arm that moves to find the desired vial from a rack of vials or a 96-well plate. The valve can accommodate various loop sizes, although 100–500 μL volumes are the most common. Some older autosamplers used a 5-mL or even a 10-mL sample loop that could not be changed.

Integral-Loop Autosamplers

In recent years, the integral-loop autosampler has become popular. The strong point of this design is that no sample is wasted, and that can be very important for trace analysis when sample volume is limited. The action of the integral-loop autosampler is more complicated, as illustrated in Figure 4. Figure 4a shows the valve in the flush position, in which wash solvent is pulled, pushed, or both through the loop, depending on the system design. Note that the needle is fitted firmly into a high-pressure seal. The needle is moved to the autosampler vial, and sample is pulled into the loop (Figure 4b). Finally, the needle is inserted into the high-pressure seal again, and the rotor is moved to the inject position, as shown in Figure 4c. Because the sample is contained only in the swept portion of the loop, 100% of the sample is injected. The maximum sample volume is dictated by the loop size, which is difficult, if not impossible to change. Typically 100 μL is the maximum injection volume for these autosamplers, whereas the other two designs can incorporate any loop size desired.

One potential weak point of the integral-loop injector is the high-pressure seal. The push-to-fill autosampler design uses a low-pressure seal that generally is trouble-free. Because of problems with the high-pressure seals, some manufacturers incorporate an additional valve instead of a high-pressure seal to perform a similar function, but this change adds cost and complexity. The vial access, injection volume flexibility (to as much as the loop volume), and programmability are equivalent to the push-to-fill designs.

Tradeoffs

Some of the strong and weak points of each autosampler are listed above. Table I compares some aspects of the three autosampler designs.

The pull-to-fill design has the simplest mechanical design, whereas the integral-loop autosampler tends to be the most complex. This feature often translates to the reliability or number of problems that crop up during routine operation.

The pull-to-fill autosamplers may have no independent flushing procedure, so flushing the needle and transfer tubing may require placing a wash vial between each sample. An alternative would be to allow the next sample to provide the flush, but it would require large sample volumes. The other two autosamplers can use a independent wash solvent, and they generally allow multiple flushes between samples.

The mechanical simplicity of the pull-to-fill design often means that the samples are placed in a tray that indexes one position at a time, so the vials must be loaded in the injection order. It may also require that the same number of injections be performed for each vial. The other two designs usually allow random access to vials, a variable number of injections, and even a variable injection volume for each

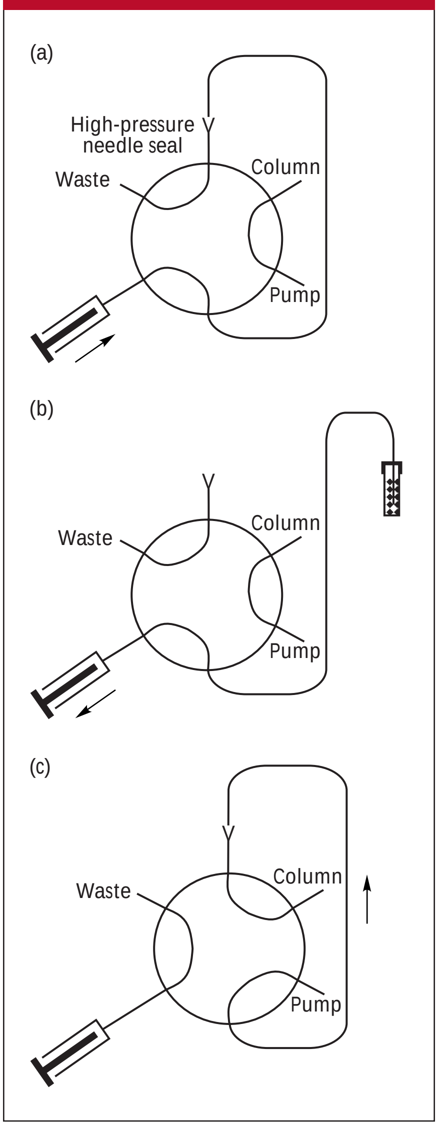

Figure 4: Schematic of an integral-loop autosampler in its (a) needle- and loop-flushing, (b) loop-filling, and (c) injection positions. Arrows show direction of sample or flush solvent flow.

Table I: Comparison of autosampler features*

Features	Pull-to-Fill	Push-to-Fill	Integral Loop
Mechanical simplicity	†	0	–
Ease of flushing	–	+	+
Random access	–	+	+
Conserves sample	–	0	†
Variable injection volume	–	†	+
Needle seal problems	+	0	–
Syringe precision required	+	–	–
Impact on dwell volume	+	0 or –	0

* + is a positive trait, – is a negative trait, 0 is neither a positive nor a negative trait, and † is the autosampler's strongest trait.

vial. Programming is simplest if the vials are injected in order, but the flexibility of random access can be a useful feature, particularly if one or more samples or standards must be injected at intervals during the run sequence.

Because all the sample is contained in the swept needle-loop tubing, the integral-loop autosampler wastes little or no sample. If analysts use microvials or vial inserts, nearly all the sample in the vial can be drawn into the loop and injected. This feature can be attractive in trace analyses in which sample volume is limited. Push-to-fill syringes generally waste little sample, but a small volume of sample usually is left in the syringe and connecting passages and is wasted.

The push-to-fill design generally provides the most flexibility in terms of injection volume. Small volumes of as little as 1–2 μL can be injected if the syringe mechanism is driven precisely. By fitting a large loop on the injector, chromatographers can inject nearly any large volume. The integral-loop design also allows programmed injection volumes as large as the loop volume, but the loop is not changed easily, so the upper end of the injection volume range is more restricted than it is for the push-to-fill models.

The high-pressure needle seal is one of the weak points of the integral-loop autosamplers, and users should take care to ensure that the seal is in good condition. The low-pressure seal of the push-to-fill injector rarely is a problem, and it can be adjusted easily by tightening a fitting in most models. No needle seal is used in the pull-to-fill autosampler.

Both the integral-loop and the push-to-fill models rely on the syringe to determine the injection volume in most cases. Therefore, the syringe control must be precise and accurate for good performance. Fortunately, the mechanical controls in these units are very good, and they should provide an imprecision of less than 0.5% if the autosampler is operating properly.

The dwell volume of an LC system is the volume from the point at which the mobile-phase components are mixed to the head of the column. This volume accounts for an unintentional isocratic hold built into every gradient run. Large dwell volumes can result in methods that are hard to transfer and have longer-than-desired gradient run times. Dwell volume has no practical effect on isocratic separations. Dwell volumes for common LC equipment range from 0.2 mL to 5 mL or more, depending on the equipment design. The dwell volume comprises the solvent mixing chamber and all the connecting tubing between the mixer and the column. For low-pressure mixing systems, the dwell volume includes the pump volume.

A final important portion of the dwell volume is the autosampler loop volume, because the loop in the inject position is part of the volume between the mixer and the column. As long as the loop's volume is no larger than approximately 100 μL, it generally is an unimportant contributor to the dwell volume. However, it is easy to conceive unintentional dwell volume changes when a loop is changed. For example, if the system had a 300-μL dwell volume with a 50-μL loop, then the dwell volume would change to 1250 μL if a 1-mL loop were substituted. This change could affect the appearance of the gradient chromatogram, especially if analytes were eluted within the first few minutes of the run or if the separation used a narrow-bore column. Analysts should consider the effect on the dwell volume and the implications for the separation when they change a loop.

Conclusions

I have described three basic autosampler designs. Some commercial models may use a combination of these designs, so they may not clearly fall into one category or another. Each design has its appropriate uses and, in general, will perform satisfactorily if used within its capabilities. In the second installment of this series, I'll look at some common problems that users may encounter with routine autosampler use.

John W. Dolan
"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Walnut Creek, California, and a member of LCGC*'s editorial advisory board. Direct correspondence about this column to "LC Troubleshooting,"* LCGC*, 859 Willamette Street, Eugene, OR 97401, e-mail John.Dolan@LCResources.com.*

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