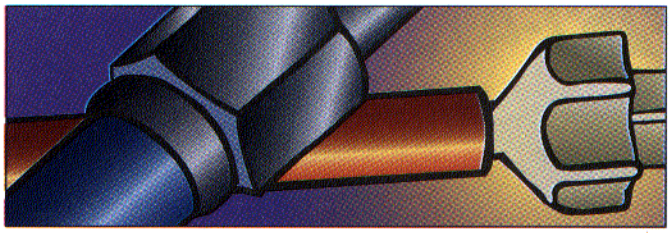
# LC Troubleshooting



## Injection Loop Adsorption

John W. Dolan

What goes into an injector doesn't necessarily come out — at least not in the expected manner.

We often take the performance of our liquid chromatography (LC) systems for granted — until we encounter some problem. We especially rely on the autosampler or manual injector to provide us with the precision we have come to expect in LC methods. Most of us expect to obtain relative standard deviations (RSDs) for replicate injections of 2% or less when injecting 20-µL or larger samples. This month, we'll look at some aspects of sample injectors that can compromise method precision. These problems can occur with both manual injectors and autosamplers, so unless my reference is specific to one type of module, I will refer to injectors generically in this column.

### LOOP-FILLING CHARACTERISTICS

One of the most common problems with injection imprecision is related to the characteristics of loop-based injectors. One valve manufacturer's technical note includes a very good discussion of this problem (1), and Figure 1 illustrates it. In this case, users fitted a 20-µL sample loop to an injector, introduced various volumes of sample into the valve, and observed the detector response (peak height or area). When a 10-µL sample was injected, users saw a 50% response, as expected. Similarly, when 60 µL was forced into the loop, users saw a 100% response because the loop captured 20 µL and the remaining sample was discharged to waste. Theoretically, we'd expect the response curve to be linear to 20 µL and then constant (as shown by the dotted line). The observed behavior, however, shows a region of nonlinearity from approximately 50% of the loop capacity to two- to threefold the loop capacity. This observation means, for example, that injecting 18 µL into a 20-µL loop yields something less than the expected 90% response.

The cause for this nonlinearity can be readily explained by the hydrodynamic behavior of fluids as they pass through tubing. A process called *laminar flow* takes place under conditions in which molecules close to the tubing walls are slowed by frictional forces. The net result is a bullet-shaped profile in which the molecules in the center of the stream travel at roughly twice the velocity of those at the tube walls. This problem is not so great with mobile phase, because large volumes of solvent with a constant composition (or slowly changing composition, as in gradient elution) are involved. For the small volumes used in sample injection, however, laminar flow can play an important role. In the case of the 18-µL injection mentioned above, the faster-moving molecules in the center of the sample stream have left the loop before the loop-filling process is complete, which means that the loop contains less than the expected volume.

Thus, we can see that laminar flow can account for the nonlinearity of loading plots such as the one shown in Figure 1. To avoid the nonlinear portion of the loading curve, we need to inject less than 50% of the loop volume or more than twice the loop volume. From a practical standpoint and for added security, most workers make injections that are at least threefold the loop volume.

Analysts can use clever tricks, such as placing a small bubble at each end of the sample (2), to get around the limitations placed on loop filling by laminar flow. Additionally, the problem of nonlinearity is less of a concern with autosamplers because the syringe mechanisms used to meter samples into the loop are so precise. Although the injected volume may not be exactly what you set, the precision of the mechanism makes the injection volume reproducibility very good.

### ADDITIONAL PROBLEMS

If problems with nonlinear filling of the injection loop were the only things we had to worry about, our jobs wouldn't be so bad. However, several workers reported an additional observation of nonlinearity that is summarized in Figure 2. In this case, a 10-µL loop was fitted to the injection valve and various volumes of sample in aqueous buffer were passed through the loop (3). As can be seen in Figure 2, increasing the flush volume resulted in increased detector response. Although the plot seems to level out as the volume is increased, the signal increased even after flushing with 8 mL of sample — that's 800 loop volumes. The workers identified the problem as sample adsorption on the internal surfaces of the injector, particularly the Vespel rotor seal.

Sample adsorption inside the injector shouldn't come as a great surprise. In fact, it is surprising that we don't encounter more problems with adsorption. After all, the stainless steel of the injection loop and the Vespel of the rotor are just chemical surfaces, and we know that the chromato-

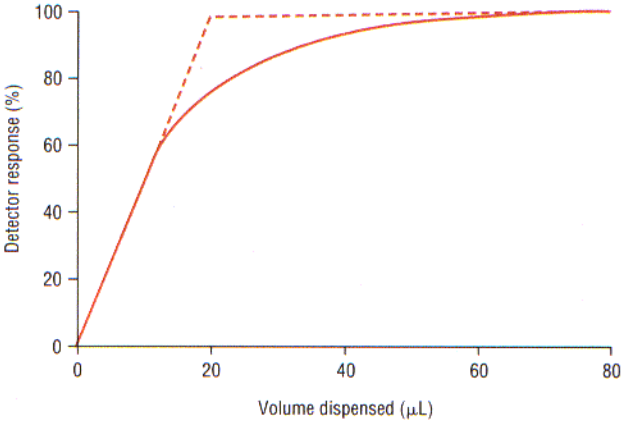



**FIGURE 1:** Plot of detector response vs. amount of sample flushed into a 20-µL sample loop (1). See text for details.

graphic process relies on the differential interaction of sample molecules with such surfaces. We want this interaction to take place in the column, but we assume that the injector is free from those effects.

## INJECTION SOLVENT EFFECTS

The mobile phase affects separations in the column; similarly, we should be able to control injector adsorption by changing the strength of the sample solvent. As shown in Figure 3, increasing the solvent strength reduced the adsorption problem. In this instance, the sample was dissolved in various concentrations of methanol–buffer, 1 mL of buffer was passed through the loop, and then the loop was flushed with 1 mL of buffer. When the loop was switched to the inject position, the mobile phase eluted the sample adsorbed to the interior of the valve. It is clear that increased solvent strength (more methanol) reduced the adsorption problem.

## WHERE IS IT STICKING?

How can we determine where the sample is sticking in the injector? With a cleverly designed series of experiments, Lough and co-workers (3) showed that the primary adsorption site for their sample was the Vespel rotor in the valve, not the stainless steel sample loop. Wiggins and colleagues (4) found that the stainless steel loop was responsible for adsorption, and they successfully eliminated the problem by switching to a PEEK (polyetheretherketone) sample loop.

Is this a universal problem? Various workers have reported differing results (see recommendations for further reading at the end of this column). Some find that all tested samples suffer from adsorptive losses, whereas others report selective adsorption. My guess is that most of us never encounter this problem, so it is likely that different compounds will behave in different ways. These results are what we expect when we realize that this process is chromatographic — not magic. Different compounds will adsorb to differing degrees depending on their chemical nature, the sample solvent, and the chemical nature of the internal surfaces of the injection valve.

## SELECTING THE INJECTION SOLVENT

Chromatographers commonly know that injecting small volumes of sample dissolved in mobile phase yields the best results. When dilute samples need to be concentrated for improved detection, a common practice is to inject large volumes of dilute sample. If the injection solvent is sufficiently dilute when compared to the mobile phase, very large samples (milliliters to liters) can be injected with success. The resulting injection, called *on-column concentration,* is equivalent to a much smaller injection of a more concentrated solution.

The studies summarized in this column suggest that when the on-column concentration technique is used, we should check the method for possible sample adsorption in the injector. If we aren't careful and consistent regarding the volume of sample that passes through the sample loop, adsorption problems may reduce precision.

It is easy to check for adsorptive losses. Just inject a series of samples in which the overfill volume of the loop is changed. If you see an increase of detector response when the overfill is increased, either insufficient sample volumes (as illustrated in Figure 1) or sample adsorption is the problem. The first problem is solved by increasing the degree of overfill. The second problem is addressed by using a fixed overfill volume and by using an injection solvent that is as strong as possible but does not compromise the peak shape.

*First, the more similar the sample solvent is to the mobile phase, the less likely it is that problems will occur. Second, overfilling the sample loop is necessary for the best precision with filled-loop injection, but the operative word is* overfill *not overkill.*

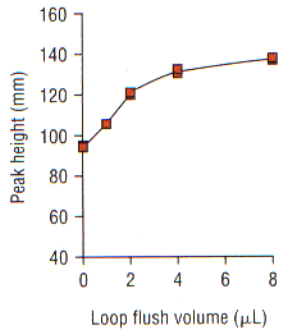



**FIGURE 2:** Plot of peak height vs. the amount of sample flushed through a 10-µL sample loop. The sample was 1 µg/mL indomethacin in 0.02 M phosphate buffer (pH 7). (Reprinted with permission from reference 3.)

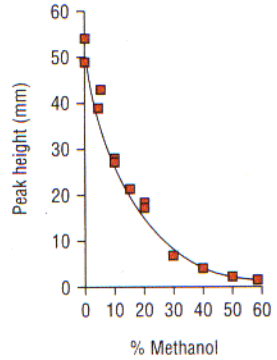



**FIGURE 3:** Plot of peak height vs. injection solvent strength for a 1-mL overfill of a 10-µL sample loop. The loop was flushed with 1 mL of phosphate buffer following filling. The injection solvent was various concentrations of 0.02 M phosphate buffer (pH 7) and methanol, and the sample was the same as in Figure 2. (Reprinted with permission from reference 3.)

## SUMMARY

Unfortunately, because the adsorption problem is very sample-, instrument-, and method-specific, it is difficult to offer specific recommendations for avoiding adsorption problems. However, I can glean several guidelines from the preceding discussion. First, the more similar the sample solvent is to the mobile phase, the less likely it is that problems will occur. Second, overfilling the sample loop is necessary for the best precision with filled-loop injection, but the operative word is *overfill* not *overkill.* Overfilling the loop volume by three- to five-fold is reasonable; 10-fold or greater is unlikely to improve injection precision but instead encourages the appearance of adsorption problems. Finally, because each case is unique, look for injection problems such as those described above. If you suspect injection problems, design a systematic experiment to isolate and identify them and then adjust your method to minimize the adsorption problems.

If you encounter problems that you suspect are related to adsorption of samples in the injector, consult references 1, 3, and 5–8 for further discussion of this problem and its solution.

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*"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Walnut Creek, California, and a member of the Editorial Advisory Board of* LC•GC. *Direct correspondence about this column to "LC Troubleshooting,"* LC•GC, *859 Willamette Street, Eugene, OR 97401, e-mail 76631.2547@compuserve.com.*