T R O U B L E S H O O T I N G

Sample-Injection Valves

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Sample-injection valves introduce measured amounts of sample onto LC columns. By injecting directly into the high-pressure mobile phase stream, injection valves eliminate the need to stop mobile phase flow during injection (as with on-column syringe injection). Injectors can be operated manually or automatically, or can be designed as an integral part of an autosampler. A properly operating valve is critical to the quality of the chromatographic data because it provides precise and accurate sample injections. Symptoms of problems associated with the injection valve include broadened peaks, poor precision, leaks, and increases in system pressure.

VALVE DESIGN AND OPERATION

The six-port valve in Figure 1 illustrates the basic design of all sample-injection valves. The valve consists of a rotating **seal** (or **rotor**) and a fixed **body.** An **injection port** facilitates filling of the **sample loop** from a syringe, and the **waste port** vents the loop and allows excess sample to be discarded. The pump and column are connected to the two remaining ports. Injection valves are made of stainless steel (generally type 316 or Nitronic 60) and use seals made of fluorocarbon or a fluorocarbon–polyimide blend, such as Vespel. If the sample loop is mounted on the outside of the valve body as shown in Figure 1, it is called an **external loop** valve. The loops on **internal loop** valves are mounted inside the valve body or in the rotor itself. Generally, internal loops are used for injection of volumes of <5 μL.

To fill the loop with sample, move the rotor to the *load* position (Figure 1a) so that the loop is connected to the injection and waste ports; flow of mobile phase from the pump goes directly to the column. Insert a syringe into the injection port and fill the loop with sample (see below for discussion of proper injection techniques). Next, turn the rotor to the *inject* position (Figure 1b); the contents of the loop are flushed onto the column with mobile phase from the pump. The valve can now be returned to the load position in preparation for the next sample injection.

Please note: In the October "Troubleshooting" column (Vol. 3, No. 10, pp. 868–874), I mentioned that the Kontron 420 pump was designed for flushing behind the pump seal. I have since learned that the IBM 9560, the Tracor 951 and 955, and the LKB 2150 pumps also provide this feature.

In the position intermediate between *load* and *inject,* the rotor seal blocks flow to the column. This blockage causes a pressure pulse at the head of the column because the mobile phase flow drops to zero. Flow/pressure pulses are undesirable, so the valve should be turned as rapidly as possible. Some injectors (for example, the Waters U6K, Waters Chromatography Division, Milford, Massachusetts) are designed with a bypass that shunts the flow around the valve in the intermediate position in order to avoid a switching pulse.

Injection techniques: Typically, the size of the sample loop dictates the injection volume, although sample volumes less than the loop volume can be injected. With the **filled loop** injection technique, the loop is entirely filled with sample. For maximum precision, a volume of sample two to three times the loop volume should be flushed through the loop to ensure that the loop is completely full (see below for discussion of filling characteristics). Now, leave the syringe in place and turn the valve to inject the sample. Remove the syringe after injecting the sample to prevent bending the needle accidentally. Because the loop volume controls the amount of sample injected, the amount of sample in the syringe is not critical. For the best injection precision, however, it is good practice to (over) fill the loop with about the same amount of sample (±10%) each time. To change the sample size with the filled-loop technique, it is necessary to change sample loops.

When using the **partial loop** injection technique, a volume of sample less than loop capacity is injected. If, for example, a 23-μL injection is desired, inject 23 μL of sample into a larger loop (for example, a 50-μL loop); the rest of the loop remains filled with the solvent from the previous injection (usually mobile phase). Now rotate the valve to the *inject* position, and the sample will be placed on the column. Because in this case the injection volume is determined by the syringe, care

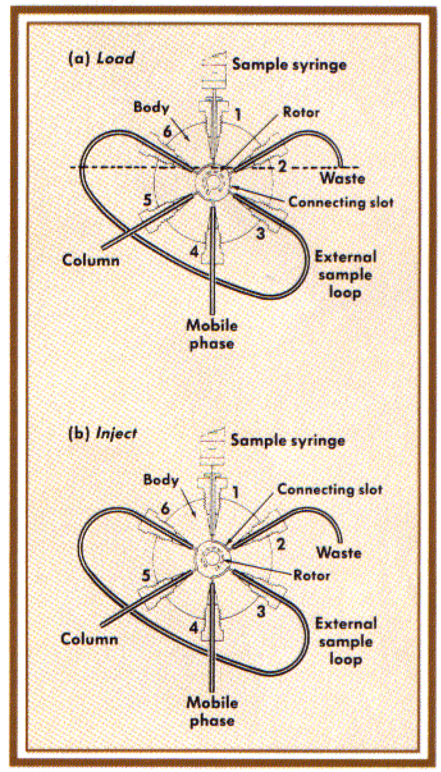

FIGURE 1: Six-port sample injection valve (courtesy of Valco Instruments, Houston, Texas).

must be taken when measuring the sample, and sample size should be limited to 50% of loop capacity to obtain the best precision possible (see below for discussion of filling characteristics). The partial-fill method is less precise than the filled-loop technique, but it is much more convenient when the injection volume must be varied because any volume (up to loop capacity) can be injected.

The **moving injection** technique can also be used for precise injections of partial loop volumes. With this method, the valve is momentarily switched to the *inject* position and then back to the *load* position before the loop is fully flushed (1,2). This technique is especially suited for small-volume (that is, <2 μL) injections such as those required in microbore LC (3).

Filling characteristics: As sample is loaded into the loop, it pushes the mobile phase ahead of it out of the loop. In this process, the front of the sample becomes diluted because a fluid traveling through a tube has a parabolic flow profile that is caused by laminar flow. In laminar flow, the center of the fluid stream travels faster than portions near the tubing walls. The practical result is that the diluted sample occupies about 2 μL of loop for every 1 μL of sample loaded from the syringe. This means that for filled-loop injection, all diluted sample must be removed before the loop can be filled homogeneously. Generally, two to three loop volumes of sample are sufficient to load the loop and flush all solvent from the previous injection. When partial-loop injection is used, sample size needs to be kept below about 50% of loop capacity to prevent the diluted front of the sample band from leaving the loop during filling.

Figure 2 illustrates the relationship between sample loaded into the loop and sample injected onto the column (as determined by peak-area measurements). When <10 μL of sample is placed in a 20-μL loop, there is a linear relationship between volume placed in the loop and amount of sample injected; when >40 μL is used, the injected volume is ±5% of loop volume. In the 10–40 μL range, however, a nonlinear relationship is in evidence (4). This is the basis for recommending use of less than 50% of loop capacity (partial-loop injection) or more than two times loop capacity (filled-loop injection) for maximum injection precision.

Laminar flow also causes dilution of the sample band as it is flushed from the loop during injection. Five to ten loop volumes are required to completely displace the sample from the loop (5). For this reason, it is wise to leave the injector in the *inject* position to allow for sufficient time to fully flush the loop.

The **leading bubble** technique, an alternative method of partially filling the loop, allows very precise injection of any fraction of the loop volume (6). In this technique, the syringe is filled with sample, then the plunger is drawn back to create a small bubble at the syringe tip (~0.2 μL). When the sample is pushed into the loop, the bubble is injected first and forms a "seal" between the previous loop contents (generally mobile phase) and the sample. Because the bubble prevents the sample from mixing with mobile phase, problems caused by laminar flow are eliminated and sample volumes up to loop capacity can be precisely injected. When the valve is rotated to the *inject* position, the bubble will go into solution in the mobile phase and not cause problems.

Enhancing injection: To achieve maximum column plate number, the sample should be injected in an infinitely small volume. This is obviously impossible, however, so the sample volume should be kept as small as is practical. With partial-loop injections, the effective injection volume is minimized by backflushing the loop onto the column. This is especially important when small injections are made from a large-volume loop (as is the case with the Waters injector). When 20 μL is injected from a 2-mL loop, the sample becomes greatly diluted (because of laminar flow) if it is required to travel through the entire 2-mL loop before reaching the column. This travel also results in a considerable delay between the time when the valve is turned and the time when the sample actually reaches the column. On the other hand, if the loop is backflushed, the 20-μL sample is immediately flushed onto the column — with little dilution and no delay. The valve in Figure 1 is plumbed so that the loop is backflushed onto the column. (If the connections at ports 4 and 5 were interchanged, the loop would not be backflushed.) For filled-loop injections, the direction of flow through the loop is not important.

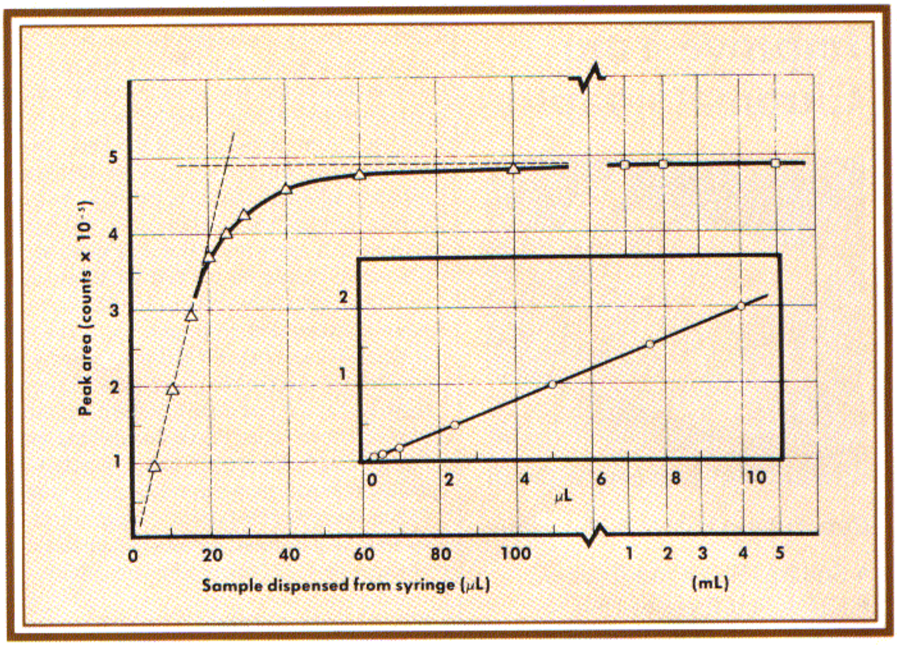

FIGURE 2: Linearity of filling using partial-loop injection technique. (Courtesy of Rheodyne, Cotati, California.)

PROBLEM PREVENTION AND VALVE MAINTENANCE

Proper adjustment and cleanliness are keys to extended injection-valve life. Follow the manufacturer's instructions when adjusting the tension on the rotor seal: too much pressure on the seal will increase seal wear and too little pressure will make the valve prone to leaks. Be sure to flush buffered mobile phases from the valve daily; abrasive crystals can form from buffer residues and scratch the rotor seal. If you use a precolumn or suspect another source of particulate contamination upstream from the injector, install an in-line filter just before the valve to trap these particles. Particles can scratch the valve seal and cause *cross-port leakage*, which means that the valve leaks between adjacent ports. For example, a scratch on the seal between ports 3 and 4 in Figure 1 would cause the pump to leak into the sample loop in the load position, making accurate sample injection impossible. An injection valve that is properly adjusted and kept clean should last 10,000 cycles or more before seal replacement is required.

Valve rotor-seal damage can also be caused by using an improper type or length of syringe needle. If, for example, a gas chromatography syringe with a pointed needle were used in a valve like that shown in Figure 1, the sharp tip of the needle could penetrate too far and damage the rotor seal. Rotor damage by the syringe is less frequent today because most valves are constructed so that the needle cannot contact the seal; nevertheless, many injectors with larger passages are still in use.

The Teflon sleeve in the injection port should be adjusted occasionally (approximately once a month) or whenever any leakage around the needle is noticed.

Valve disassembly and seal replacement: Valve disassembly should be performed only when cleaning blocked ports or replacing worn parts (some internal-loop valves must be disassembled to change loops). Improper adjustment or inadvertent contamination by particulates when reassembling the valve can create problems. First, be sure that you have a new rotor seal, an exploded diagram of the valve, and the manufacturer's rebuilding instructions. Second, disassemble the valve. Match the parts with the exploded diagram as you take the valve apart; lay them out in order on a paper towel if you are unsure of the order of reassembly. Third, clean all parts. Place them in a beaker filled with methanol or a mild laboratory detergent solution and sonicate for several minutes. *Do not* clean in solutions having a pH above 9 because the rotor is commonly made of Vespel, which is not stable above pH 10. Rinse the parts in water, then in methanol, and allow them to air-dry. Inspect the sealing surfaces with a hand lens. If the valve body is scratched, it may need to be returned to the factory for reconditioning. Fourth, reassemble the valve, replacing any worn parts (such as the rotor seal). Follow the manufacturer's instructions carefully, paying attention to orientation of the rotor seal and adjustment of the seal tension. If the seal is not correctly oriented, the holes or grooves will not align properly with the valve ports and the valve will not work. If the seal is not properly adjusted, the valve can leak, be hard to turn, or wear too quickly.

Spares: A spare rotor seal or a spare injection valve should be stocked for emergency use. Although a valve can last 10,000 cycles or more between seal replacements, it can fail within 500 injections if it is contaminated with particulates. Keep a variety of sizes of sample loops on hand if you expect to make injections of different volumes. Make sure that the spare fittings match the valve type; they may be a different brand than the connecting fittings used in the rest of the system. A spare injection syringe is essential; syringes can become blocked, bent, or broken without warning.

PROBLEMS AND SOLUTIONS

Fittings problems: Fittings problems at the injection valve show the same symptoms as fittings problems elsewhere in the system: leakage and/or band broadening. Fittings problems are discussed in detail in an earlier article (7).

Blockages: Blockage of the valve can cause symptoms ranging from high system pressure to difficulty in filling the loop. Isolate the blockage by loosening first the outlet to the column (port 5, Figure 1) and then the pump input line (port 4). If the pressure is still high after the column has been disconnected, but is low with the pump disconnected, the injector is the cause of the pressure rise. A blocked loop will be difficult to fill in the *load* position and will cause a pressure increase in the *inject* position. If the valve is difficult to flush using a syringe in the *inject* position, loosen the waste line (port 2). At this point, if flushing becomes easy, the waste line is blocked. If it is still hard to flush the valve, however, the valve body or rotor is blocked. Note that a blocked (or misaligned) rotor seal can give the same symptoms as a blocked loop or port.

Blocked ports sometimes can be cleared by backflushing. If you are unable to clear the ports, disassemble the valve and sonicate it in a cleaning solution (see above). If the valve is still blocked, return it to the manufacturer for reconditioning. *Do not* attempt to clean the valve ports with a fine wire. You can permanently damage the valve by scratching the sealing surfaces or by breaking the cleaning wire off in the port.

A blocked loop often can be cleared by backflushing. Replace the loop if this is not successful.

A dirty syringe can masquerade as a valve problem. Before disassembling a valve for clearing, confirm that the same symptoms appear by using a second syringe. Clean the dirty syringe by flushing thoroughly with methylene chloride or detergent or by clearing the needle with a needle-cleaning wire.

Injection-port problems: Leakage around the syringe needle during injection and leakage out of the injection port when the syringe is removed are symptoms that appear at the injection port. Leakage during injection can be caused by a blockage or resistance downstream (for example, a blocked loop or waste line), improper adjustment of the seal (injection-port liner) around the syringe, or a syringe needle with a diameter that is too small for the system setup.

Leakage from the injection port when no syringe is in place can be caused by a siphoning waste line or cross-port leakage. To prevent waste-line siphoning, be sure that the waste line is not below the surface of the waste reservoir and, if necessary, add a restrictor to the waste line (use 1 m of 0.010-in. i.d. Teflon tubing or crimp the waste line with a pair of pliers). Cross-port leakage will generally occur in only one position (that is, either *load* or *inject*); rotor replacement is necessary to correct this problem.

Sample carryover: Sample carryover is indicated when peaks from a previous injection appear in a chromatogram of a blank injection. Sample carryover is caused by poor loop flushing, which itself has three causes. First, if the loop is not left long enough in the *inject* position, the mobile phase cannot fully wash the sample onto the column, so be sure to keep the valve in the *inject* position for flushing for a period that will accommodate at least 5–10 loop volumes of mobile phase. Second, if residual sample is left in the injection port (or in the syringe), or if it is siphoned back from the waste line, contamination of the next injection can result. Rinsing the injection port between each injection may be necessary: flush 5–10 sample volumes of injection solvent (or mobile phase) through the injection port between injections. If backsiphoning is a problem, either add a restrictor or reposition the line to break the siphon action. Third, if the fittings on the loop are not assembled properly, the added dead volume can retain a small amount of sample that can be difficult to flush from the loop. Check the fittings for proper assembly and replace them if necessary.

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