LC TROUBLESHOOTING

Pumps: Operation and Maintenance

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Like the body's heart, the LC pump will provide years of reliable service if treated properly.

Several years have passed since "LC Troubleshooting" discussed the general function and maintenance of major liquid chromatography (LC) system components. Over the next several months, the column will focus on how these components work and how to keep them working with a minimum of effort. This installment examines pumps.

The LC pump resembles the heart in many ways: It pumps vital fluids through the system and maintains the pressure and flow within desired limits. And, like the heart, an LC pump lasts a long time given minimum care, but we must follow some simple guidelines to prevent present oversights from causing future problems.

A COMMON DESIGN

Nearly all LC pumps in use today are based on some variation of the reciprocating-piston pump (Figure 1). A cam and connecting rod transform the rotational movement of a motor into the linear movement of a piston. With the help of check valves, the piston movement draws mobile phase from a reservoir at atmospheric pressure and delivers it to the column at pressures as high as 6000 psi (41 MPa). Commercially available pumps represent variations of this design. Innovations such as shaped cams, variable-speed motors, and multiple pistons are designed to minimize pressure pulsation. However, the pumps all function similarly, and understanding the general principles of their operation can simplify troubleshooting.

Check valves: The pump head contains two sets of moving parts: the check valves and the seal–piston assembly. Figure 2 illustrates a simplified check valve, which includes a stainless steel body, a plastic or ceramic seat, and a ruby ball. Pressure applied on one side of the ball raises the ball from its seat (Figure 2a) and allows liquid to pass around it (a screen or retainer prevents the ball from being washed out of the valve body). When pressure is applied in the opposite direction, the ball drops onto the seat (Figure 2b), and the seal between the ball and the seat prevents liquid from flowing around the ball. Obviously, a precise fit between the ball and seat is required to prevent leakage. In most check valves, the ball is loose inside the body, and gravity alone is responsible for its movement. Some designs, however, use a spring to ensure that the ball seats properly. Other designs include two balls and seats in each valve. The proponents of the two-is-better-than-one philosophy believe that these valves are less likely to leak than single-ball valves. Conversely, those favoring single-ball valves assert that valves with two balls are twice as likely to have problems.

The materials that a valve is made of depend on the valve's application. Titanium may be used instead of stainless steel to improve the system's biocompatibility. To simplify maintenance and reduce costs, some manufacturers use a cartridge check valve — a disposable plastic unit that drops into the stainless steel body and contains the valve's functional parts.

Piston and seal: The piston moves back and forth in the pump head to draw mobile phase into and force it out of the pump head. The piston typically is made of sapphire, but stainless steel and other materials may be used for special applications. The pump seal prevents liquid from leaking around the piston. Figure 3 shows the piston's action.

During the fill stroke (Figure 3a), the piston is pulled out of the head to create a low-pressure area. Because the pressure on the column side of the pump is now higher than that inside the pump head, the outlet check valve drops onto its seat. The inlet check valve rises from its seat because the incoming solvent is at or near atmospheric pressure, which is greater than the pressure inside the liquid chamber of the pump head.

During the delivery stroke (Figure 3b), the piston moves into the pump head and pressurizes the mobile phase. As the pressure increases, the inlet check valve drops onto its seat. When the pressure inside the pump head exceeds that on the column side, the outlet check valve opens, and the mobile phase flows to the column.

The pump seal consists of a plastic matrix, such as PTFE, filled with carbon fiber or other fillers to improve performance. As a cross-sectional view of the seal installed in the pump head shows (Figure 4), the seal contacts the piston along a narrow region. The seal does not prevent all liquid from passing; in fact, the mobile phase wets the surface of the piston and acts as a lubricant to prevent excessive seal wear.

COMMON PROBLEMS

Just as many commercial pumps share the same essential parts, most pump problems observed in the laboratory share the same causes. The three major problem sources are air bubbles, particulate matter, and pump-seal wear. Other areas may cause problems from time to time, but if you control these three problem sources, reliable pump operation should be the norm.

Air bubbles: The most common source of pump problems is excessive air in the mobile phase. Air can enter en masse when a reservoir is pumped dry, but more often the cause is poorly degassed mobile phase. When re-

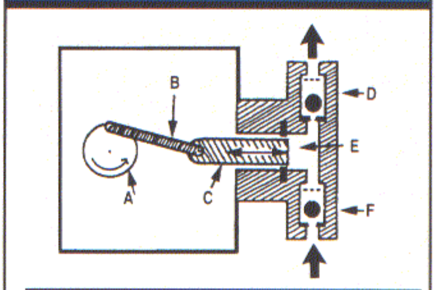

FIGURE 1: The reciprocating-piston pump. Legend: A = cam, B = connecting rod, C = piston, D = outlet check valve, E = cylinder, F = inlet check valve. (Reprinted with permission from reference 1.)

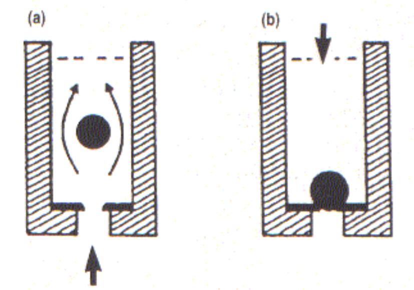

FIGURE 2: Check-valve operation. The check-valve ball is shown in (a) open and (b) closed positions. (Reprinted with permission from reference 1.)

versed-phase solvents (for example, methanol and water) are combined, the mixture generally is supersaturated with air. As a result, air comes out of solution. When the solvents are mixed upstream from the pump, as with low-pressure mixing, or inside the pump head, as in some models, the released air can reach the pump. Even hand-mixed mobile phases can release air in the pump because the pressure inside the pump is below atmospheric pressure during the fill stroke. Diffusion through PTFE pump-inlet tubing is another cause of air in the mobile phase.

Air in the pump can cause three problems. First, if it is large enough, the air bubble can fill the liquid chamber completely and rob the pump of its prime; the piston moves back and forth trying to pump air, but no liquid enters or leaves the pump, and zero flow results. More common, however, is the entrapment of smaller bubbles that do not fully fill the pump. When a bubble is trapped in the pump chamber, it is compressed as the piston enters the delivery stroke but then expands if it remains in the pump during the next fill stroke. As a result, the delivery volume is lower than expected because the pump delivers only the volume of mobile phase that can fit in the part of the pump chamber unoccupied by the bubble. Finally, a bubble lodged in a check valve can interfere with the ball's seating and can cause valve leakage, manifested as lower-than-expected flows or pressure pulsations. Microbubbles, such as those that diffuse through the pump-inlet tubing, can become trapped inside the pump wherever its inner surface is rough or dirty. These bubbles tend to coalesce into larger bubbles that pass through the pump and cause a momentary pressure pulse or stay in the pump and produce the symptoms discussed above. (Some pumps are better than others at purging trapped bubbles.) A simple way to remove trapped air bubbles is to open the purge valve and increase the flow rate until the air is forced out through the waste line.

The best way to prevent air bubble problems in the pump is to prevent air from entering it. The most effective method is degassing the mobile phase by helium sparging. Alternatively, many workers successfully degas their mobile phases using a vacuum or a vacuum in conjunction with sonication. Batchwise degassing may be sufficient, but the magnitude of the bubble problem may necessitate continuous degassing. Another advantage of using degassed mobile phase is that it tends to redissolve any bubbles that may inadvertently enter the system. Elevating the reservoir above the pump heads provides some siphon pressure at the pump and improves operation of the inlet check valve. Be sure that the mobile phase flow to the pump is unrestricted. A blocked inlet-line frit or a too-tight reservoir seal can force the pump to draw a vacuum in the tubing or reservoir and prevent mobile phase from reaching the pump.

Many workers find that maintaining a slight (4–5 psi) head pressure on the solvent in the reservoirs improves pump reliability. To maintain this pressure, you can use a homemade apparatus, but commercial degassing systems are safer and more convenient. These devices allow normal helium sparging for degassing and then seal the reservoirs; helium is added only as mobile phase is pumped from the reservoir. This method conserves helium and prevents air from reentering the mobile phase. If you thoroughly degas the mobile phase, microbubbles diffusing through the PTFE pump-inlet tubing generally will cause no problems.

Although many pumps are designed to reduce degassing requirements, nearly every LC system will work more reliably using degassed mobile phases.

Particulate matter: Another cause of pump problems is particulate matter. When microscopic particles of dust or other contaminants in the mobile phase become lodged on the ball or the seat of the check valve, they can prevent adequate sealing. The result is erratic pressure or, in extreme cases, no flow. You can remove these particles from the mobile phase by filtering all mobile phase components through a 0.5-μm (or smaller) membrane filter. You can omit the filtering if you are using only HPLC-grade solvents because they typically are filtered through a 0.2-μm or 0.5-μm filter during manufacture. Using a

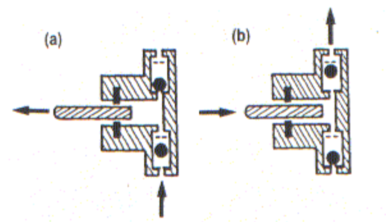

FIGURE 3: Pump operation, including the (a) intake or fill stroke and (b) delivery stroke. (Reprinted with permission from reference 1.)

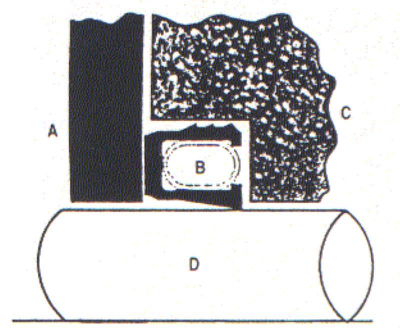

FIGURE 4: Cross-sectional view of the pump head. Liquid end of the pump is to the right. Legend: A = backup washer, B = piston seal, C = pump head, D = piston. (Reprinted with permission from reference 2, courtesy of Bal Seal Engineering.)

5-µm or 10-µm–porosity sinker frit on the pump-inlet tubing can prevent dust particles from entering the pump, but the porosity of the sinker frit is inadequate for general mobile-phase filtering.

A second source of particulate matter is buffer precipitation in the pump. Buffer precipitates can cause the same problems as other particulate matter that enters the pump. Under normal operating conditions, keep the buffer concentrations low (≤50 mM) for most applications. When using stronger buffers, take extra care to prevent buffer precipitation in the system. Adding 100% organic solvent, as when flushing the system at the end of the day, can cause buffers to precipitate. To flush the system, wash it using first unbuffered mobile phase and then a strong solvent rather than going directly to a strong solvent (for example, methanol or acetonitrile).

PUMP-SEAL WEAR

A more common buffer-related problem is increased pump-seal wear. As it moves, the piston makes contact with the seal; so gradual seal wear is normal. Materials currently used in seal construction and pump designs have maximized pump-seal life. In many cases, pump seals last a year or more in regular service. As mentioned above, the mobile phase lubricates the piston and thus minimizes friction between the seal and the piston. Problems arise, however, when buffered mobile phases dry on the piston after the pump is shut off. The dried buffer creates an abrasive deposit on the piston's surface that can damage the pump seal when the pump is first started. As soon as the piston is rewetted, the deposit dissolves, and the pump operates normally. Repeated cycles of drying and abrasion can greatly reduce pump-seal life and can cause fluid leakage and pressure problems.

To minimize buffer deposits, wash the buffers from the system before shutting it off at the end of the day. As described above, switch first to unbuffered mobile phase and then to the desired wash solvent. Unbuffered mobile phase should dilute or replace the buffered mobile phase that wets the piston, thus minimizing problems caused by buffer deposits. Once you have removed the buffer, increase the system pressure by increasing the flow rate to encourage leakage of buffer-free mobile phase under the seal and speed the cleaning process.

Some LC pumps are designed to enable flushing behind the piston seal to facilitate the removal of buffer residues (Figure 5). Water can be flushed behind the main pump seal to remove buffer (in C and out E in Figure 5); a second seal prevents the wash solvent from entering the rest of the pump. To remove the water, follow it with a syringe full of isopropyl alcohol when flushing is complete. When using methods that require very high buffer concentrations (>200 mM), it may be helpful to continuously pump wash solvent (water) through the flushing channel. This procedure allows you to flush nearly any pump that has a hole above and below the piston behind the seal. (If your pump is not designed for such flushing, visually inspect the cavity before flushing to avoid inadvertently flushing water where it can do damage.)

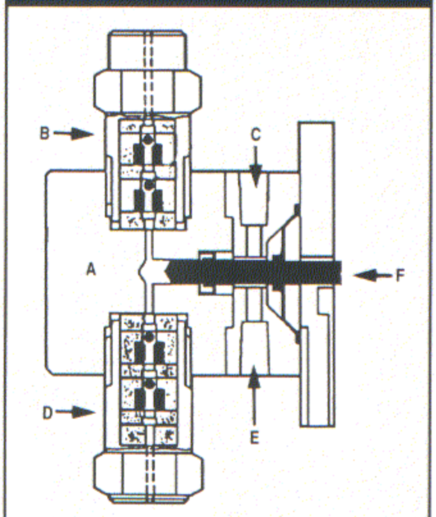

FIGURE 5: Pump design enabling flushing behind the pump seal. Legend: A = pump head, B = outlet check valve, C = inlet to flushing passage, D = inlet check valve, E = outlet from flushing passage, F = piston. (Reprinted with permission from reference 2, courtesy of Pharmacia LKB Instruments.)

SUMMARY

LC pumps work quite simply, and we can trace the cause of most regularly encountered problems to just a few sources. Three simple preventive maintenance procedures can minimize the problems caused by these sources. First, filter the mobile phase to prevent particulate matter from entering the system and interfering with check-valve operation. Second, degas the mobile phase to prevent bubbles from causing check-valve and pressure problems. Third, flush buffers from the system at the end of each working day to prevent the buildup of abrasive residues and thereby extend pump-seal life.

The next "LC Troubleshooting" column will discuss innovations designed to improve the performance of the simple single-piston pump described here.

REFERENCES

(1) L.R. Snyder and J.W. Dolan, *Getting Started in HPLC, User's Manual* (LC Resources, Lafayette, California, 1985).

(2) J.W. Dolan and L.R. Snyder, *Troubleshooting LC Systems* (Humana, Clifton, New Jersey, 1989).

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