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

Equipment Usage

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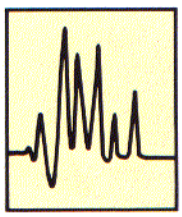

Recently, *LC Magazine* compiled the results of a survey that contained questions on sample preparation, pumps, filters, and solvents. The survey was distributed to 1500 HPLC users; 546 people (36.4%) responded. The results of the survey are useful for troubleshooting purposes because they point out common problems and possible misconceptions. As I have emphasized before, economics plays a very important role in motivating any troubleshooting regime: one must invest time and money wisely to prevent problems, and, when they do occur, they also must be dealt with in an economical and efficient manner.

TABLE I: COMMON PUMP PROBLEMS

Type of Problem	Respondents (%)
Bubbles	32.8
Pump seals	25.4
Check valves	23.9
Leakage from fittings	13.1
Inaccurate flow rate	3.7
Other	1.1

TABLE II: FILTER USE

Type of Filter	Respondents (%)*
Guard column	85.7
High-pressure filter	39.3
–before injector	(10.6)
–after injector	(28.7)
Low-pressure filter	33.0

*of the total number of respondents, 91.3% indicated they use filters in their LC systems

PUMP PROBLEMS

Respondents were asked to indicate the most common pump problem they encounter; the results are summarized in Table I. Gas **bubbles** in the solvent was the problem most often reported. Bubble problems can be severe with low-pressure mixing (40% of the respondents indicated that they preferred low-pressure mixing), but most bubble problems can be eliminated by degassing all solvents before use, making sure that the solvent inlet line is not constricted, and keeping all low-pressure tube connections leak-free. These problems have been discussed in earlier Troubleshooting columns (1–4).

Difficulties with **pump seals** were indicated as the second most common problem. There are two procedures that will greatly reduce the number of pump-seal problems. First, flush all buffers, ion-pair reagents, and other salts from the pump at the end of each working day so that abrasive crystals do not form behind the seal. Second, replace the pump seals on a regular basis — once every three months is usually sufficient. Early seal replacement eliminates system wear resulting from seal leakage, reduces problems caused by flaking of particulates from the seals, and saves money because seal failure often results in other, more costly, problems. See references 1, 5, and 6 for additional information.

Check-valve problems — encountered by nearly 24% of the respondents — can be minimized if you practice preventive maintenance in the two areas described above. Bubbles cause a large number of check-valve problems; solvent degassing eliminates most of these. Mobile phase contamination is also responsible for check-valve failure. In order to minimize contamination, flush mobile phase salts from the system every working day, use filtered or LC-grade mobile phases, and replace the pump seals before they disintegrate (1).

Approximately 13% of the respondents had trouble with **leaky fittings.** Leakage can be a special problem if low-pressure fittings are not properly tightened. When plastic fittings are used, overtightening probably will be more of a problem than undertightening because overtightened fittings distort the sealing surface and can strip the threads on the fittings. Be sure to follow the manufacturer's recommendations when assembling and tightening low-pressure fittings. If you have a problem with fittings becoming loose during use, you can obtain lock nuts from one of several vendors for securing them. For additional information, see references 4 and 7.

I was surprised at the percentage (3.7%) of respondents who complained of **flow rate** inaccuracy. In general, flow rate accuracy is not important as long as the flow rate is nominally the same as the setting. It is important, however, that the flow rate be constant and reproducible. In other words, it doesn't matter that the flow rate is 0.95 mL/min when the setting is 1.0 mL/min, as long as the flow rate is 0.95 mL/min every time 1.0 mL/min is selected on the dial. If flow rate inaccuracy is truly a problem, the flow rate on most pumps can be adjusted using the compressibility compensation adjustment (see the operator's manual for instructions).

SYSTEM BACK PRESSURE

More than 90% of the respondents operate their chromatographs at less than 3000 psi, and about half of them typically use pressures less than 1500 psi (most LC systems have a 6000-psi upper limit). The practice of using reduced pressure will extend the life of pump seals, minimize leakage, and reduce mechanical wear. If you routinely run your chromatograph at pressures greater than 3000 psi, you should consider making changes so that a lower maximum pressure can be used (8).

FILTER USE

More than 90% of the respondents use some form of filter in their LC systems; the various types used are listed in Table II. Clearly, most users take advantage of **guard columns** to extend the life of more expensive analytical columns. The results of this survey demonstrate a nearly threefold increase in usage as compared with the period 1982–1983 (9,10).

About 40% of the respondents use **high-pressure filters** to protect the system from problems associated with particulate matter. The results indicate, however, that slightly more than a quarter of those using high-pressure filters place the filter ahead of the injector. A preinjector filter will stop particles that originate in the mobile phase or pump but will have no effect on particles resulting from valve-seal wear. Valve-seal wear can be a significant source of column problems, especially as the seal nears the end of its useful life. Unless you have a need for protecting the valve from a specific source of particulates (for example, from a precolumn or saturator column), the in-line filter should be placed between the injector and the column. Be sure to use one of the "zero-dead-volume" models that adds negligible extracolumn volume to the system (3).

I was surprised that only one-third of the respondents use **low-pressure filters.** "Sinker" frits on solvent inlet lines should be used for nearly every LC application. These frits should be removed only when they cause a problem with system performance, such as under certain ion-pairing conditions. Regular use of inlet-line filters is the least expensive and most effective way to prevent system problems that result from the presence of particulate matter in the mobile phase. If you find that 5-μm-porosity inlet filters become blocked too quickly or cause excessive flow restriction, change to 10-μm filters (2).

Sample filtration: Seventy-one percent of the respondents indicated that they filter some or all of their samples prior to injection. I suspect that this is an area in which cost savings could be realized. Disposable sample filters cost a dollar or more each; hence, unnecessary use can be expensive. You should carefully examine your need to filter samples — don't filter them "just for good measure." As a rule, a sample that is cloudy, opalescent, or that contains visible particles should be filtered, but others may not need filtration. You may miss an occasional sample that should have been filtered, but if you use a guard column it will remove the particulates before they reach the analytical column. Routine sample filtration should be reserved for cases in which it is really needed.

Solvent filtration: About three-fourths of the respondents filter their solvents and mobile phases before use. Those of you using HPLC-grade solvents (~95% of the respondents indicated that they do) may be performing unnecessary filtrations — and may be *increasing* the risk of contamination. HPLC-grade solvents typically are filtered and bottled under much cleaner conditions than those found in most LC labs. Hence, each additional graduated cylinder, flask, and/or filter funnel used in mobile phase preparation increases the risk of chemical and particulate contamination of the mobile phase. When online mobile phase blending is used, the original solvent bottle is often the best reservoir because there are no additional solvent-handling steps that could introduce contaminants. Nevertheless, mobile phases containing buffers or other salts should *always* be filtered before use. Only 32% of the respondents reported adding buffers to the mobile phase, however. When mobile phases consist entirely of HPLC-grade solvents, filtration is not necessary (2).

SOLVENT SAVINGS

Proponents of some of the newer LC techniques, such as microbore or fast LC, cite solvent savings as an important reason for choosing these methods (11,12). In this survey, 60% of the respondents reported annual budgets for solvents of $2500 or less. Even complete elimination of solvent costs would have a minimal effect on most laboratory budgets. When looking for ways to trim budgets, concentrate on reducing *labor* costs through automation and increased laboratory

efficiency. Evaluate the need for new equipment purchases carefully — don't buy into a new technology for the wrong reasons!

LAB WORKLOAD

On average, weekly sample analysis load reported was quite low, as indicated in Table III. More than half of the labs reported analyzing 60 or fewer samples each week. This workload is comfortably within the capacity of a single LC system. If your lab falls in this category and is considering the purchase of another chromatograph, consider alternatives that will make more efficient use of your present equipment. Adding an autosampler or data system, for example, could result in a significant reduction in labor and an increase in sample throughput. It may seem surprising to suggest delaying the purchase of another LC system, but I firmly believe that there are fewer problems when a single chromatograph is used regularly than when several are available and are only used occasionally. Why? If a chromatograph is not used daily, preventive maintenance tends to be ignored; contamination, microbial growth, and corrosion are more likely; and the likelihood of anticipating failure decreases because less attention is paid to the "feel" of normal operation.

REFERENCES

(1) J.W. Dolan and V.V. Berry, *LC, Liq. Chromatogr. HPLC Mag.* **2,** 78–82 (1984).

(2) J.W. Dolan, *LC, Liq. Chromatogr. HPLC Mag.* **2,** 582–587 (1984).

(3) J.W. Dolan, *LC, Liq. Chromatogr. HPLC Mag.* **3,** 234–238 (1985).

(4) J.W. Dolan, *LC, Liq. Chromatogr. HPLC Mag.* **3,** 312–316 (1985).

(5) J.W. Dolan, *LC, Liq. Chromatogr. HPLC Mag.* **3,** 576–577 (1985).

(6) J.W. Dolan, *LC, Liq. Chromatogr. HPLC Mag.* **3,** 868–874 (1985).

(7) J.W. Dolan and V.V. Berry, *LC, Liq. Chromatogr. HPLC Mag.* **2,** 20–21 (1984).

(8) J.W. Dolan, *LC, Liq. Chromatogr. HPLC Mag.* **3,** 782–785 (1985).

(9) R.E. Majors, H.G. Barth, and C.H. Lochmüller, *Anal. Chem.* **54,** 300R–349R (1984).

(10) J.W. Dolan and V.V. Berry, *LC, Liq. Chromatogr. HPLC Mag.* **1,** 542–544 (1983).

(11) D.R. Baker, *LC, Liq. Chromatogr. HPLC Mag.* **2,** 38–41 (1984).

(12) M.W. Dong and J.R. Gant, *LC, Liq. Chromatogr. HPLC Mag.* **2,** 294–303 (1984).

TABLE III: AVERAGE NUMBER OF SAMPLES RUN PER LAB PER WEEK

Number of Samples	Respondents (%)*
0–30	34.8
31–60	22.6
61–90	14.0
91–160	14.0
161–230	5.3
>230	9.4

*total does not equal 100% because of rounding

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