LC TROUBLESHOOTING

LC Problems — Past, Present, and Future

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Over the next decade, chromatographers will be able to take advantage of improved hardware and intelligent software to increase their productivity.

Ten years ago, if workers performing liquid chromatographic (LC) separations had been asked to identify their profession, I think a sizable portion would have called themselves chromatographers. Today, however, I believe a much smaller proportion of the workers would identify themselves in this manner. Instead, we would hear answers such as organic chemist, biochemist, clinical chemist, and so forth. During this period, LC has progressed from a rapidly growing technology to one that shows signs of maturity; that is, the technique has moved from being an end in itself to being just another analytical tool. As this has happened, the chromatographic knowledge base and technical skill level of the typical worker has dropped. This is not a discredit to these workers but is a natural progression of LC from a research-and-development focus to an applications focus. When such changes take place, the frequency of hardware and separations problems tends to increase. The reverse has been true for the most part, however, primarily because manufacturers have done a fine job of improving the reliability of LC products.

When we look at various LC problems that have plagued chromatographers over the years, we see that some have been eliminated, some have stayed about the same, and some have arisen that were nonexistent 10 years ago. In this column, I look at some of these problems and comment on the key elements of change. I also gaze into my crystal ball and dream a little bit about what may be in store for us in the next 10 years.

WHEN I WAS A KID . . .

Ten years ago, LC was in the early part of its second decade of popular use. During the first decade the technique was commonly referred to as high pressure liquid chromatography. By the early 1980s, this title was largely replaced with high performance liquid chromatography. The 1970s had brought much experimentation with basic equipment design. Pneumatically driven pumps came and went. New detectors were developed in research environments but were too unreliable for commercial production. Gradient equipment improved. The first electronic integrator–chart recorder was commercialized. Companies came and went.

During the 1980s, equipment design focused primarily on improvements in reliability and performance. Unlike in the 1970s, we saw few instrument changes that were giant steps during the past decade. A couple of notable exceptions were the PC-based data system and the diode-array detector. A lot of experimentation was done on hyphenated techniques, especially coupling liquid chromatographs with mass spectrometers (LC–MS), facilitated by the innovation of thermospray and other vaporization techniques. Refinement of column production during the 1980s moved us from an environment in which we needed to check each new column for performance degradation caused by shipping to one in which new columns rarely fail to meet specifications.

What were the major LC problems 10 years ago, and what improvements have been made? Five areas are of particular significance: pump seals, air bubble–related problems, detector lamps, column failure, and fittings.

Pump seals: I vividly remember the frustrations of working with the early Altex 110A pumps. It seemed like all I ever did was change pump seals. When using strongly buffered mobile phases, the seals typically had to be replaced once a week. Even under the best conditions, seals would rarely last longer than three months. This problem was not unique to the Altex pumps. As indicated by a survey in 1985 (1), 25% of LC users considered pump-seal problems to be the most common pump problem they encountered. Today, pump seals are of little concern to most workers. In an informal survey I conducted last December, only one out of about 200 workers considered pump seals to be a major problem. In fact, some manufacturers guarantee that their seals will last at least a year.

What has improved pump-seal reliability? Improvements have been made in three areas: seal materials, pump design, and user knowledge. Improved seal materials mean seals are less wear-prone. In the early 1980s, many pumps had pistons that were firmly fixed to the driving rod, which often caused minor misalignment with the pump seal. Misalignment coupled with poor seal material resulted in accelerated wear. Today, nearly all pumps use a flexible mount for the piston so it is self-aligning and correctly centered in the seal and wear is reduced. Finally, in the early 1980s most of us didn't know that buffers weren't good for LC pumps. We didn't flush buffers out at the end of the day, and buffer crystals built up on the piston just behind the seal. Each time the pump was started up, these crystals would abrade the seal and accelerate wear. Today, most workers flush the buffers from the system when the pump is shut down, and in many cases they take advantage of pump-design innovations that enable users to wash the piston behind the pump seal to remove buffer deposits.

Air bubbles: In the same survey mentioned above, air bubbles were listed as the primary LC problem for 33% of the respondents. Another survey (2) indicated that bubble-related problems were a primary concern in detector problems as well. Bubbles trapped in the pump cause pressure and flow problems; bubbles in the detector flow cell cause noise spikes. Although engineering advances have been unable to eliminate these problems, the widespread use of degassing has. Some LC systems require helium-sparged mobile phase to operate reliably, but all systems will give improved reliability when degassed mobile phases are used. Many LC systems have built-in helium degassing systems, so workers are more likely to use this technique today than they were 10 years ago.

Detector lamps: Deuterium lamps in UV detectors have always been a source of problems. Ten years ago, manufacturers suggested expected lifetimes of ~500 h. Few analysts felt they could get 500 h of use from a lamp, however, although little hard evidence was available to prove it. Deuterium-lamp failure is a result of leakage, on–off cycles, total time on, and a combination of other factors. With older detectors, full power was sent to the lamp as soon as the start button was pushed, giving the lamp an abrupt starting cycle. Today's detectors warm up the lamp before igniting it and reduce the on–off shock. Lamp construction seems to have improved, so aging caused by leakage has been reduced. Most of today's lamps have a meter that records total on-time built into one of the

electrical leads. I think that improved technology and user pressure on manufacturers have combined to result in today's reliable lamps. Deuterium lamps commonly are useful beyond the 1000-h mark on the meter. It is wise, however, to order a replacement lamp when the lamp has been in service for 700–800 h.

Column failure: Analytical columns have been the primary source of LC problems since the technique was first used. This trend continues; some causes of column failure, however, have been improved or eliminated in the past decade. For example, improved techniques for the synthesis and sizing of packing particles enable production of more-uniform particles of narrower size distributions. When columns are packed properly with these particles, problems of bed settling and high back pressure largely are eliminated. In contrast, columns 10 years ago occasionally settled with use and required additional packing material to fill the void. Voids are still a problem today, but usually they result from chemical dissolution of the packing rather than physical disturbances. With today's more-uniform particles, column pressure is lower, so the column has a longer useful life before it exceeds system pressure limits. A decade ago, it was quite common to have a method that generated 3500-psi back pressure using 10-μm d_p particles; today most analysts try to keep the pressure below 2500 psi with 5-μm spherical packing materials.

Batch-to-batch column reproducibility has improved over the past decade because of an improved understanding and control of the important packing-synthesis and column-preparation parameters. Batch blending and preparation of larger batches of packing are other techniques used by today's column manufacturers to ensure reproducible products.

Retention reproducibility and peak tailing are better understood today than a decade ago. We now know that the addition of low molecular weight acids and bases such as acetic acid and triethylamine serves as a kind of magic bullet to reduce peak tailing and improve column-to-column retention reproducibility when analytes with acidic or basic functions are to be analyzed.

The widespread use of guard columns over the last decade has also increased analytical column life. The innovation of cartridge columns has reduced hardware costs somewhat and greatly improved column-handling convenience.

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With these advances, it seems that all the easy samples have been provided for, leaving today's workers to face challenging separations. These challenges include chiral separations and separations of proteins, peptides, and other biological materials. These new samples introduce new problems, so even though we may have solutions for many separation problems, new problems seem to come at an accelerating pace.

Fittings: One of the most sweeping innovations of the past decade is the development of finger-tightened fittings. Ten years ago, if I had three brands of columns, then I had to have three sets of adapters to connect the columns to the LC system. Today, one set of finger-tightened fittings is all that is required. These fittings allow adjustment of the ferrule position and have eliminated many problems related to improper fitting assembly, which is a pitfall for the uninitiated chromatographer. These fittings are used on most LC systems today. Early concerns about pressure limits and inertness have been addressed. The introduction of PEEK tubing has been very important because of PEEK's convenience and biocompatibility. Traditional stainless steel tubing and fittings are still the workhorses of LC, but plastic materials have made life much easier.

TODAY

Chromatographers often ask me to recommend a particular LC system for purchase. Although I do have my favorites, I am reluctant to make blanket recommendations because I believe there are no bad systems available to-

day. Sure, some have features others don't, but you will probably be able to do your job with the equivalent system from any manufacturer. I could not have said that a decade ago.

Manufacturers have made great improvements in system reliability and design. When making purchasing decisions, I emphasize support and service. The current economic situation requires that we make every dollar count, so it is especially important that the LC system you choose is backed by a competent, responsive support and service staff. Excellence in these areas tends to be regional rather than the domain of a particular manufacturer, so ask around to find out who provides the best support in your area.

THE CRYSTAL BALL

It is fun to speculate about what could be in store for LC hardware in the next decade. Because of the need for minimum downtime and an increasing emphasis on good laboratory practice (GLP), I think we will see some interesting developments. Some of these developments have already been pioneered by a few manufacturers. Here are my predictions — or perhaps my wish list — for the next 10 years.

Replacement components: I think we'll see more modular replacement components. We've seen the introduction of cartridge check valves and cartridge columns in the past decade. These replacement components increase operator convenience and reduce replacement costs. I expect to see quick-disconnect pump heads in common use (at least one manufacturer has this option now). A quick-disconnect pump head would enable users to snap a new pump-head assembly onto the pump and be back at work in a few minutes if a seal, piston, or check valve fails. The bad unit could be returned to the manufacturer for refurbishing or be repaired in the lab at the operator's leisure. Other modular parts that could be replaced with a minimum of downtime would facilitate problem isolation and correction.

Built-in diagnostics: A few systems currently have built-in diagnostic capabilities. An expansion of this innovation in scope and industry usage will provide an improved basis for preventive maintenance. Some currently available pumps count the total number of pump strokes; if these systems were programmed so that diagnostic messages were printed at the beginning or end of each day (or anytime, if the messages are urgent), users would be able to perform system maintenance more effectively. If it could tell users that at the current rate of use the pump seals would need to be changed in two weeks, then spare parts could be checked or ordered, and the maintenance could be scheduled. If columns had a bar-code label, the system could be designed to keep track of the volume of solvent pumped through each column, the number of sample injections, and the pressure history. The system could facilitate planning by providing analysts with firm numbers related to column life and per-sample cost of analysis.

Remote servicing: We all recognize the increased information content of a chromatogram as compared with a table of retention times and areas ("a picture is worth a thousand words"). For people like me, who diagnose problems over the telephone, the fax machine has revolutionized troubleshooting. I can solve problems much more quickly if the user faxes me a copy of the chromatogram rather than trying to describe it. Similarly, the manufacturer's service engineer can isolate problems much more quickly if certain internal electronic measurements are made. I think every LC system will have a built-in modem that will enable the user to connect the instrument directly to a service engineer's diagnostic machine through a telephone line. In this manner, the service engineer will be able to remotely operate and test the LC system without leaving the service center. Remote diagnosis and customer-replaceable subassemblies should minimize downtime and reduce service costs.

Push-button experts: Many times a problem could be resolved quickly if only an expert were available for questioning. I believe that improved expert-system software will become a standard part of many LC systems of the future. These systems would provide technical assistance for all kinds of LC problems,

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ranging from instrument maintenance to method development. Closely coupled to the LC system, such software could interact with the instrument and gather important data about a separation. With these data as background, the program could provide intelligent advice to help solve problems that arise.

It's all possible: The above developments are not just fantasy. All of the technology exists today, and, in one form or another, these features are commercially available today. As we seek more intelligent use of our time and other resources, these instrument improvements are logical additions to today's automated instrumentation. Read the March 2002 issue of *LC•GC* to see how many of my predictions have come true!

REFERENCES

(1) *LC Mag. Liq. Chromatogr. HPLC*, User Survey VI, Aster Publishing Co., Metuchen, New Jersey, 1985.

(2) *LC Mag. Liq. Chromatogr. HPLC*, User Survey VII, Aster Publishing Co., Metuchen, New Jersey, 1985.

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