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

# The Highly Protected LC System

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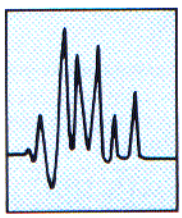


Many reports of devices and methods to prevent problems with liquid chromatographic systems have been published in the literature. One of us (V. Berry) has found that it is possible to combine several protective measures to greatly prolong the trouble-free operation of an LC system. You might choose to use one, several, or all of the methods discussed here to improve the operation of your chromatograph.

To the increasing number of meanings applied to the "HP" in HPLC (high pressure, high performance, and high price), a new meaning of *highly protected* LC system is proposed here. A highly protected LC system incorporates a number of subsystems to greatly prolong the operation of the liquid chromatograph without failure. The concept is to preserve the eluent from chemical or microbial changes, to protect the pump from failing as a result of interference from particles or gas bubbles, and to preserve the analytical column from dissolution of the silica or buildup of particle or chemical contaminants. The eight subsystems used to achieve the highly protected LC will be discussed below.

## ANTIMICROBIAL

Mold or bacterial growth in the aqueous eluent can be a major problem, especially if alkaline buffers are used (1) or if large batches of buffers are prepared to minimize solvent preparation time or batch-to-batch eluent variations. The fibrils from microbes can clog the inlet frits, interfere with ball-valve reproducibility, and produce contaminants that obscure sections of the chromatogram (1). One method to minimize microbial contamination is to add an antimicrobial that is itself unretained in reversed-phase systems. Sodium azide at 0.004% works well with detection at wavelengths down to 210 nm, even though sodium azide is a UV-absorber (1). Because stock solutions of sodium azide decompose slowly, we routinely add 0.400 g of the solid to 10 L of aqueous eluent before the final pH adjustment. Any particles contributed by insoluble azide are removed by the in-line filter described below.

## INERT GAS-SPARGING SYSTEM

The gas-sparging system traditionally has been used to degas the eluent by saturating it with helium. Oxygen and nitrogen from dissolved air diffuse into the helium bubbles and are carried out by the helium (just as sonication produces cavitation pockets into which the air diffuses, forms bubbles, and rises to the surface). It is an experimental observation that helium-saturated "degassed" solvents generally do not produce the bubble problems found with air-saturated solvents (2). Solvents saturated with air can produce bubbles at the pump inlet, especially if the flow is restricted or starved. These bubbles prevent the ball valves in many pumps from operating properly, and flow irregularity will result.

A second bubble problem occurs when bubbles form in the detector (at atmospheric pressure) as a result of air-saturated aqueous eluents being mixed with air-saturated methanol or acetonitrile. This is a problem if a device is not used to raise the pressure of the detector.

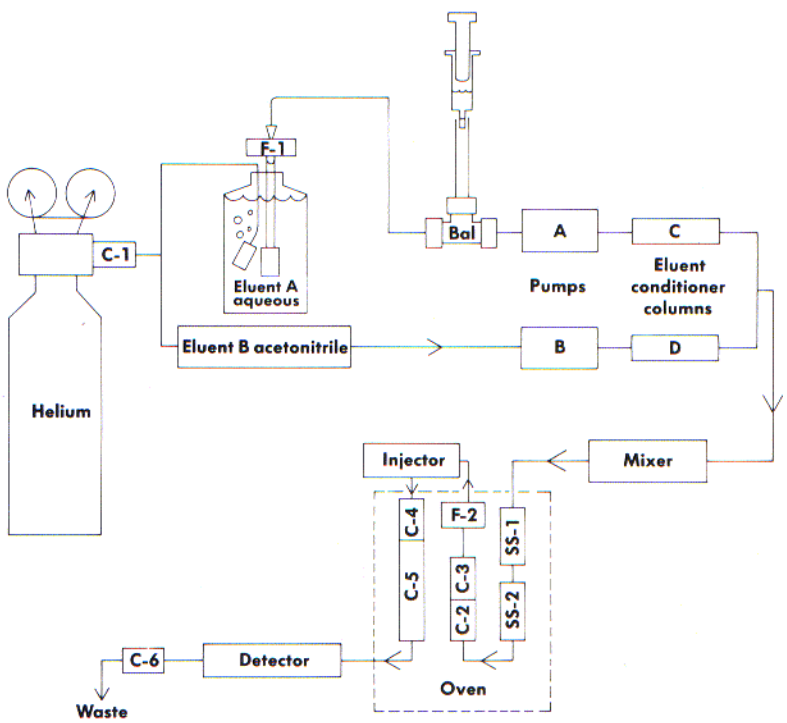
The gas-sparging, degassing, or deoxygenation system has grown to have a number of other functions. To improve reproducibility and to reduce noise, certain detectors require oxygen elimination. The reductive wave of oxygen can interfere with electrochemical detection, oxygen can decrease the system linearity with UV detection, and fluorescence can be quenched by oxygen (1). Oxygen in the mobile phase may also deteriorate some samples and destroy some packings.

Another use of inert gas sparging is to keep atmospheric vapors from contaminating eluents, particularly at night when hoods and air-conditioning systems are shut down. Sparging also will prevent atmospheric carbon dioxide from changing the pH and hence altering retention behavior with weakly buffered alkaline systems (1).

Although helium classically has been used to degas, some recent applications show that the benefits of removing oxygen and protecting the eluent from atmospheric contaminants can be obtained with other gases. In a recent application, mixtures of carbon dioxide (to prepare in situ volatile amine buffers) and nitrous oxide (to adjust the UV absorbance of the aqueous eluent to match the acetonitile eluent) were used (3). The submicron filter system and solvent gas ballast described next are compatible with these systems even though the gas levels in the mobile phase are about two orders of magnitude higher than with helium degassing.

## SUBMICRON FILTER SYSTEM

An alternative to filtering solvent prior to filling the reservoirs is shown in Figure 1. This is accomplished by placing a 0.45-$\mu$m filter in the solvent line immediately before the pump. This filter removes particles that can cause check-valve malfunction and piston-seal wear. This 0.45-$\mu$m *absolute* filter is much finer than the in-




Uncommon and critical components of the system are referenced; other components can be obtained from many LC supply companies. INERT GAS-SPARGE SYSTEM: C-1 is a guard column gas resistor connected directly to a gas regulator via a brass fitting (#B-400-1-4, Cambridge Valve, Billerica, Massachusetts) and by another fitting (#B-400-6-2, Cambridge Valve) to transparent Teflon PFA tubing (#TK-6375-01, 1/8-in. o.d., 1/16-in. i.d., Cole-Parmer Instrument Co., Chicago, Illinois) ending in a sintered metal pump inlet filter with 5-$\mu$m pores, which is used as a bubbler. SUBMICRON FILTER SYSTEM: F-1 is a 0.45-$\mu$m Millex HV filter (SLHV025NS, Millipore Corp., Bedford, Massachusetts) preceded by a 5-$\mu$m sintered metal pump inlet filter connected with transparent Teflon PFA tubing (#TK-6375-02, 1/4-in. o.d., 5/32-in. i.d., Cole-Parmer) and followed with a male luer connector (#47-ML2, Rainin Instruments, Woburn, Massachusetts) to 1/8-in. o.d. PFA tubing. Eluent B is sparged and filtered identically to eluent A except no gas ballast is required. Containers are closed with aluminum foil. GAS BALLAST (also pump prime and bubble trap) (4): A 1/4-in. stainless-steel "T" is connected to the inlet and outlet flow stream with reducers (#SS-200-R-4, Cambridge Valve) and with a "stand tube" of 1/4-in. o.d. Teflon PFA tubing (described above) terminating in a plastic syringe to contain a gas bubble for ballasting pressure before the pump inlet, to prime the pump, and to trap occasional gas bubbles. ELUENT CONDITIONER COLUMNS (1): 25 cm x 0.46 cm or larger steel columns; C = aqueous column to remove ghost peaks, tap-packed with reusable poly(styrenedivinylbenzene) porous polymer (80–100 mesh); D = acetonitrile column to remove midgradient hump impurities, dry tap-packed with Brockman activity I acid alumina (80–200 mesh). SILICA-SATURATOR SYSTEM (1) (contained in the oven at the same temperature as the analytical column): 15 cm x 0.46 cm steel columns SS-1 and SS-2 silica saturator columns, dry tap-packed with silica gel (70–230 mesh) followed by a "polishing" silica-saturator, small-particle guard column (C-2) and a safety guard column of the same material as the analytical column (C-3) followed by a high pressure in-line filter (0.5-$\mu$m pores) (F-2). C-4 is the guard column used to protect the C-5 analytical column. BACK PRESSURE RESTRICTOR: 5-$\mu$m $d_p$ guard column (C-6).

**FIGURE 1: Schematic diagram of the highly protected LC system.**

line, sintered-metal *bed* filter usually used (2-, 5-, or 10-$\mu$m). The Millex-HV filter is compatible (wettable and resistant) and used with both aqueous and organic eluents (although no gas ballast is required with the less viscous organic solvent). In accordance with good filtering practice, the filtering operation takes place immediately (in time and place) before the use of the eluent. Additional time and effort is saved in that eluents are not usually filtered before this operation. The filter is changed when permeability is low, which is assessed by pulling fluid with the syringe into the bubble-trap system. We routinely change the filter with each 9.6-L lot of eluent. Regular maintenance is essential with this system to avoid filter blockage at an inopportune time. Any air in the frit must be sucked through a new, dry Millex filter, because air below a wet filter will completely block the eluent flow.

The successful use of this 0.45-$\mu$m low permeability filter, especially if carbon dioxide or nitrogen deoxygenation is used, depends upon the use of the following device.

## GAS-BALLAST SYSTEM

Figure 1 shows details of a stainless-steel "T" with a syringe assembly that serves three functions. First, when allowed to contain a 1–5 ml volume of gas, the syringe acts as a gas ballast. The moment-to-moment pump suction pulsations act to expand the volume of the gas bubble and greatly diminish the magnitude of the vacuum pulses experienced by the eluent on the inlet to the pump. Thus, bubble formation, even with carbon-dioxide saturated water, is eliminated, as is cavitation with organic solvent systems.

Second, the "T" assembly serves as a priming device. Suction on the syringe will start the flow of solvent from the reservoir. When the syringe is full of eluent, pressure on the syringe will start the flow through

the pump because flow back through the 0.45-μm filter is hindered.

The third function of the "T" assembly is to act as a bubble trap. If temperature changes cause bubble formation in the pump-inlet line, or if gas is in the inlet line when the system is started up initially, the gas is trapped and rises into the vertical portion of the tube.

An important part of this gas-ballast assembly is the use of the smooth-wall, translucent, inert Teflon-PFA tubing for all low-pressure connections. The PFA tubing is denser and less porous to resist air diffusion through the tubing. It does not kink, which prevents nucleating sites from forming bubbles that can be held up in the system, and it is nearly transparent so that gas bubbles can be seen (4).

## ELUENT-CONDITIONER COLUMNS

Two different high-pressure eluent conditioner columns (1) greatly simplify chromatography in the low UV wavelength range (200–214 nm), and can improve reproducibility and prolong the column life at higher wavelengths. As is indicated in Figure 1, the eluent-conditioner columns are for high-pressure mixing liquid chromatographs that use high-pressure mixing of eluents. These columns are inserted after the pump and before the mixing chamber. An eluent-conditioner column of porous polymer in the aqueous line (eluent A) serves to remove organic components that can cause ghost peaks with low wavelength UV-detection systems. This column is regenerated by placing the aqueous inlet in acetonitrile for about 3 min every 2 to 3 days. An eluent-conditioner column of activated alumina in (dry) acetonitrile (eluent B) removes amine-like impurities that can cause a midgradient hump with low wavelength UV detection. This column must be repacked with activated alumina when the midgradient hump recurs (every 2 to 5 days) and must be used with dry acetonitrile. If reasonably clean eluents are used and detection below 254 nm is not used, these columns may be left out of the system.

## SILICA-SATURATOR SYSTEM

This system, which is inserted after the mixer and before the injector, preserves the analytical column. The two silica-saturator columns (5) (made from old 15 cm x 0.46 cm analytical columns) are filled with 63–200 μm $d_p$ silica and cause a pressure loss of less than one atmosphere. About 2–4 cm of packing height was found to dissolve when 10 L of pH 4 eluent at 70°C was passed through the silica-saturator column, which illustrates the importance of using a silica-saturator column. This silica would have disappeared from the analytical system had the saturators not been used. We typically remove the first silica-saturator column, move the second to the position of the first, and replace the second with a new (dry, tap-packed) column.

The silica-saturator columns are followed by two guard columns. The first is a 5-μm $d_p$ silica-guard column, which acts to "polish" the saturation of the eluent in silica. A second guard column of the same material as the analytical column (if available) is intended as a *safety* column. It should be affected by any phenomenon, known or unknown, that would affect the analytical column. The safety column intercepts chemicals or particles that would collect, react with, or be released from the silica-saturator column. Note that for the silica-saturator and safety columns to function properly, they must be at the same temperature as the analytical column, and therefore must be located in the same oven. Remember that these columns are located after the mixer but before the injector; they therefore create a delay in the gradient, but do not affect the peak-spreading found on the analytical column.

The final part of this system is a 0.5-μm in-line filter that usually has a very low capacity for filtering and is intended to protect the injector from particles from the guard column system.

## GUARD COLUMN

A guard column above the analytical column and after the injector serves to intercept chemicals or particles from the samples and to act as another safety column (described above). For routine or research work, the use of a guard column of the identical material (particle size, functionality, manufacturer) as the main analytical column is strongly recommended. Many workers feel that replacing the guard column periodically rather than filtering or centrifuging samples is a great time and materials savings. Other workers prefer guard columns of 30-μm $d_p$ pellicular particles that can be easily replaced by the user and, thus, are less expensive. Although these guard columns may not filter out particles from injected samples as well as small-particle guard columns, they still protect the analytical column from many contaminants and cause only a slight loss in the column plate number.

## BACK-PRESSURE RESTRICTOR

If degassing is not used, or if gassing with carbon dioxide is used (as in some applications referenced above), then gas will bubble out of solution after aqueous and organic eluents are mixed, especially if the columns are heated. These bubbles can lead to spikes in the detector signal, or even to no detector signal at all. A simple solution to this problem is to place a restrictor after the detector outlet. Low-pressure adjustable restrictors are available, or a (used) guard column can be used, as is shown in Figure 1. Either of these devices will prevent bubble problems, but caution should be observed so that the pressure limits of the detector cell are not exceeded.

## SUMMARY

The regular use of the above devices to produce a highly protected LC system can lead to long periods of trouble-free LC operation. With the highly protected system, a wide-pore (300 Å silica) C18 column was used at 70°C and a flow rate of 3 ml/min with continuous operation (7 days a week, 24 hours a day) for over 3 months with more than 2000 injections of unfiltered samples without problems.

## REFERENCES

(1) V.V. Berry, *J. Chromatogr.* **236**, 279 (1982).
(2) S.R. Bakalyar, M.P.T. Bradley, and R. Honganen, *J. Chromatogr.* **158**, 277 (1978).
(3) V.V. Berry, presented at 20th Advances in Chromatography Meeting, Amsterdam, Oct. 3, 1983, *J. Chromatogr.*, submitted.
(4) "Spring Catalog 1983" (Cole-Parmer Instrument Co., Chicago, Illinois), p. 82.
(5) J.G. Atwood, G.J. Schmidt, and W. Slavin, *J. Chromatogr.* **171**, 109 (1979). ■

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