TROUBLESHOOTING

Chromatographic Fittings: Proper Use to Minimize Dead Volume

JOHN W. DOLAN and VERN V. BERRY



There are a number of critical fittings in a liquid chromatograph that require special attention to prevent problems. These problems fall in three categories: leakage, contamination, and dead volume. Leaks can develop wherever a fitting is damaged or improperly assembled in the system. Contamination can occur if the tubing or fittings are not properly cleaned or deburred before use. Dead volume causes problems mainly in parts of the system that contact the sample. The following discussion centers on the need to minimize dead volume in the LC system through the proper use of tubing connectors.

DEAD VOLUME

The term *dead volume* (or *extracolumn volume*) is one that is receiving greater attention from LC users as technology progresses. With modern 3- and 5-μm column packings, particular attention must be paid to the minimization of system dead volume. Most of you know that 0.010-in. or smaller i.d. tubing should be used to connect any component in the liquid chromatograph that will contact the sample. You should also be familiar with the directions on proper assembly of 1/16-in. tube fittings that connect the various components of the liquid chromatographic system. The existence of tiny mixing chambers in the system creates dead volume, which causes broadening of chromatographic peaks, poor column plate counts, and reduced resolution. A discussion of the impact of tubing and fitting dimensions on chromatographic performance as well as a method to experimentally determine the combined effects of extracolumn contributions may be found in an earlier issue of *LC* (1).

You may have noticed that all the fittings in your system do not look alike or that the fittings on one manufacturer's column are not the same as those from another manufacturer. Leaks and peak broadening can result if the wrong fittings are used in the liquid chromatographic system, especially in the areas where they contact the sample. Manufacturer's bulletins often contain valuable information on the proper use of chromatographic fittings. Paul Upchurch of Upchurch Scientific (Oak Harbor, Washington) prepared a useful discussion, the *Interchangeability of HPLC Fittings* (2). He has given the authors permission to extract the following discussion and diagrams from his bulletin.

INTERCHANGEABILITY OF FITTINGS

The distance protruding beyond the ferrule, x, for 1/16-in. o.d. tubing properly swaged into a female port from the same manufacturer is shown in Figure 1. These fittings are usually assembled by bottoming the tube in the female port and tightening the nut onto the ferrule three-quarters of a turn past finger tight with a wrench. Although the ferrules from each manufacturer are shown, similar distances will result if different vendors' ferrules are interchanged in one female port. These distances should be taken as typical; however, some manufacturers use different port depths in different products. For instance, an in-line filter may not have the same port depth as an injection valve from the same vendor. The *key factor* in determining the distance x is the dimensions of the female port used initially to swage the ferrule onto the tubing.

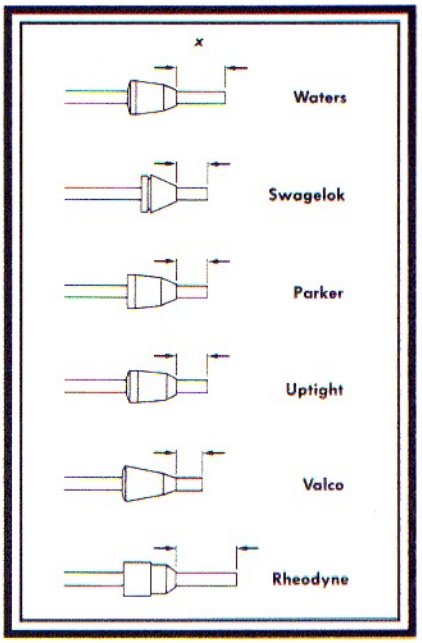


FIGURE 1: Comparison of the distance x between the end of a ferrule and the end of the tubing for fittings from several manufacturers.

If preswaged ferrules are used with the improper female fitting, the result may be leakage or creation of a mixing volume in the fitting. If a ferrule swaged for an Uptight female port (Upchurch Scientific) were inserted into a Waters port (Waters Associates, Milford, Massachusetts), for example, the tubing would not reach the bottom of the port, creating dead volume in the system (Figure 2a). Conversely, if a ferrule swaged for a Waters port were placed in the Uptight port, the ferrule would not make proper contact and the fitting would leak (Figure 2b). It is important that you take care to use the proper pair of fitting components to minimize these problems.

The ferrules from the various manufacturers appear to be interchangeable, but in fact are only interchangeable prior to swaging them onto a piece of tubing (Figure 3). Ferrules are available either as sin-

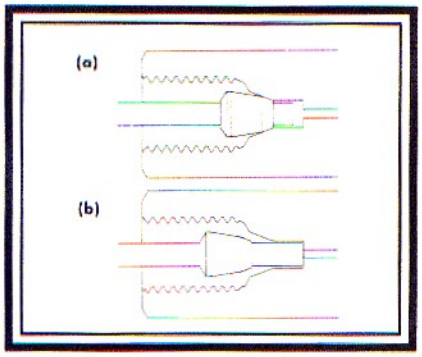


FIGURE 2: Incompatibility of preswaged ferrules and fittings. (a) Dimension *x* is too short and a mixing chamber is created because tubing will not bottom out properly. (b) Dimension *x* is too long, therefore ferrule leaks because it does not seat properly.

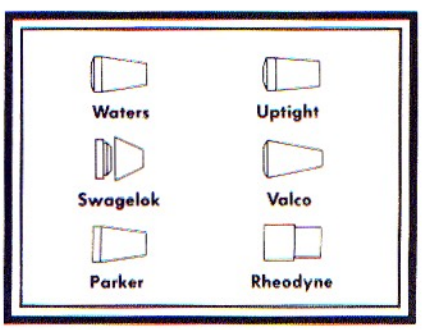


FIGURE 3: Examples of interchangeable ferrules.

gle-piece or two-piece items, with no obvious performance differences between these types. Local availability, storage, handling, and cost may influence your choice of ferrules and fittings.

The various male nuts have the same type of threads (10–32) and are therefore generally compatible (Figure 4); however, the length of the thread varies, making the nuts with extreme dimensions incompatible. For example, the Parker nut (Parker-Hannifin Corp., Instrumentation Connectors Division, Huntsville, Alabama) is too short to thread correctly into a Valco (Valco Instruments, Houston, Texas) or Waters fitting.

In summary, you should be careful when assembling any preswaged fitting in a part of the system where dead volume is critical. Ferrules or male nuts from different sources are not necessarily incompatible; in fact they are often interchangeable. The mismatch between how the ferrule was originally swaged and the depth of the port into which the tube is inserted is the source of ferrule-fitting incompatibility.

ALTERNATIVES TO MINIMIZE FITTING INCOMPATIBILITY

One safe solution to minimize fitting incompatibility is to assemble the critical fittings for each connection and leave them in place or label the fitting to indicate with which type of female port it is compatible.

Another solution is to use polymeric ferrules in the fittings. These offer the advantage of allowing for adjustments in the distance *x* with various fittings, because the ferrules do not become attached permanently to the tubing. Hence, you can slide the tubing to the bottom of the port and the ferrule slides up or down the tubing to seal with the taper in the port. Because the ferrules are relatively loose, they can be reused many times and in different types of fittings. Some of these ferrules will hold very high pressures when finger-tightened. One such finger-tight fitting is a single unit in which the nut and ferrule are one piece and produce a pressure-tight seal to 4000 psi. Another uses a polymeric ferrule that seats over a pair of grooves made in the end of the tubing by the user to provide 6000-psi sealing capacity. You can also use Teflon or nylon ferrules with conventional metal nuts to make movable and resealable connections for critical low pressure seals, such as between the column outlet and detector. Although these ferrules may hold pressures higher than 1000–2000 psi, they may slip under higher pressures, creating hidden dead volumes if they are not assembled with great care.

PROPER TUBING CUTTING

Even if the fittings in a liquid chromatograph are assembled with the utmost care, dead volumes can exist unless proper care is taken to cut the tubing correctly. The easiest way to prevent problems with tubing is to purchase it precut from your LC supplies vendor. If you cut your own tubing, several cutting devices are available that provide a finish superior to that obtainable by cutting the tube with a file and breaking it with pliers. One of these uses an abrasive cutoff wheel to produce a smooth cut and a finishing tool to remove any burr from the tubing. Another is a miniature version of the C-clamp-type circular cutter used for cutting larger tubing and pipe. Carefully clean the cut tubing in a sonicator or pump solvent through the tubing prior to using it in the liquid chromatograph to remove any unwanted substances. Tiny burrs can become dislodged and ruin injectors and detectors. Grease and oil from improperly cleaned tubing can contaminate the LC system and cause chromatographic interferences.

MICROBORE APPLICATIONS

There are even more precautions that need to be taken to prevent problems with extracolumn dead volume when using microbore columns. Tubing needs to be cut perfectly square at the ends to ensure proper seating in the fitting. Buying precut tubing is the safest way to ensure proper preparation. We mentioned earlier the need to prevent mixup of fittings once they have been swaged onto the tubing. In microbore applications a further precaution needs to be taken. Because of manufacturing tolerances, the distance *x* discussed above (which is really the depth of the female port) may vary by several thousandths of an inch between fittings from a given manufacturer. The way to avoid these small mixing volumes is to keep the paired male and female portions of each fitting together and to label each pair when it is disassembled.

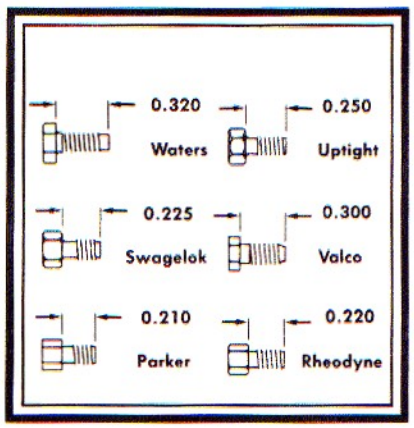


FIGURE 4: Dimensions of available male nuts.

ACKNOWLEDGMENTS

The authors would like to thank Paul Upchurch of Upchurch Scientific for contributing the information concerning fitting interchangeability and Steve Bakalyar of Rheodyne Incorporated for technical input about microbore systems.

REFERENCES

(1) Ronald E. Majors, *LC* **1**, 464 (1983).

(2) Paul Upchurch, *Interchangeability of HPLC Fittings* (Upchurch Scientific, Inc., Oak Harbor, Washington, 1983). ■

John Dolan is LC technical support manager for IBM Instruments, San Jose, California, and Vern Berry is a chromatography consultant and an assistant professor of chemistry at Salem State College, Salem, Massachusetts. Both are consulting editors for LC.