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

# Connecting Tubing

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Connecting tubing can be easily overlooked in the maintenance and troubleshooting of a liquid chromatograph. Fortunately, the improper use of tubing usually is not a problem, but given the current trend toward the use of columns that produce smaller peak volumes, you need to be aware of when to use certain types of tubing. This article will describe the various types of tubing that are available and when to use them, and will give some tips on handling tubing to minimize problems. The discussion is limited to the most common size of LC tubing (1/16-in. o.d.) used for connecting various components of the system. It should be noted that tube sizing in liquid chromatography has not, for the most part, been affected by the metric system; tubing is manufactured to English-unit specifications. Table I lists the nominal metric equivalent sizes.

## TYPES OF TUBING

Table II lists the types of tubing available for LC work. Stainless-steel tubing should be used if pressures above about 100 psi are encountered. Stainless-steel tubing is most commonly available in type 316 or type 304, which differ primarily in the amounts of chromium, nickel, and molybdenum in the alloy. Type 316 is more common and also more corrosion-resistant than 304, but type 304 is a little softer and easier to cut and bend. For most LC applications, either type of tubing is satisfactory.

Polymeric tubing is commonly used wherever stainless steel is not required. Because it does not have as high a resistance to pressure as does stainless steel, polymeric tubing is limited in its use to supply lines connecting the mobile-phase reservoirs with the pump, to the outlet side of the detector, and to lines such as the injector waste line. Polymeric tubing is easy to cut, and its flexibility facilitates the routing of liquids to waste containers or to collection vessels. Fluorocarbon tubing is often the first choice, but polyethylene or polypropylene tubing is considerably less expensive. These types, however, should not be used if the tubing is unstable in the presence of the solvents to be used in the chromatograph.

Stainless-steel tubing is available in six internal diameters (Table II), although the smallest four are the most common. The 0.007-in. and 0.010-in. i.d. tubing are used for connecting the injector, the column, and the detector. Larger sizes are used elsewhere for connections and for sample injection loops. Polymeric tubing is available in the same general size range, but because it is generally not used where sample integrity is important, a single diameter suffices. The 0.030-in. i.d. plastic tubing is a good choice because it can be connected to components either with stainless-steel fittings or with fittings specially designed for plastic tubing.

Three grades of stainless-steel tubing are readily available. Industrial-grade tubing can be purchased from local tubing suppliers (see "Tubing" in the Yellow Pages). This grade is the least expensive of the three, but tolerances on diameters and concentricity may not be as fine. Industrial-grade tubing is also quite dirty — from a chromatographer's viewpoint — and should be carefully flushed with methylene chloride or tetrahydrofuran and then a detergent solution to remove grease and oil residues prior to use. LC-grade tubing is widely available from LC supply houses and from instrument and fittings manufacturers. LC-grade tubing is clean and ready to use for most LC applications, although it is more expensive than industrial-grade tubing. In selecting this tubing for sale, the vendor takes care to ensure concentricity, which means that the tubing bore is centered. This is especially important in small-diameter tubing so that the tubing bore will align properly with the hole in connecting fittings. Finally, if the application requires extra cleanliness, it is possible to purchase premium-grade tubing, which is LC-grade tubing that has been through five to ten extra cleaning steps. Of course, it costs more, too.

## CUTTING

Stainless-steel tubing can be purchased either in precut lengths or in coils from 5 ft to 100 ft long. To some, buying precut tubing may seem like a luxury, but the quality, convenience, and savings in time are often worth the extra expense. Commonly available are kits of precut lengths of tubing (5 cm, 10 cm, and 20 cm), which are convenient lengths for connecting the column to the rest of the LC system. Precut tubing has a very good finish, so you are assured of a squarely cut end that has been carefully deburred and cleaned. Because proprietary electropolishing techniques are often used, you may be unable to match the quality of precut tubing. If precut tubing is used, there is little question that, when made up into fittings, the tube end will properly meet the fitting.

Precut tubing may be several times more expensive than bulk tubing, is not available (except by custom order) in every length that may be required, and may not be available for an immediate need. For these reasons, nearly all chromatographers will sometimes need to cut tubing. Three general techniques are used for cutting small-bore tubing. The most difficult step to master in all three is to prevent the hole in the center from closing while at the same time cutting the end squarely. Use of an abrasive-wheel cut-off machine (for example, from Scientific Systems, Inc., State College, Pennsylvania) will produce squarely cut tubing every time. The tubing is clamped in a guide and is then cut by an abrasive cutting wheel. Next, a deburring tool is used to remove the burr from the inside and outside of the tubing. When used properly, this system gives very good results; however, some individuals find it difficult to use the deburring tool, especially for smaller tubing, without breaking off the tip of the tool.

Another cutting option, which does not give as good a finish as the abrasive wheel but is easy to use and less expensive, is the Terry Tool cutter (Scientific Marketing, Georgetown, Texas). This is a miniature version of the C-clamp cutter used by plumbers for copper tubing. The tubing is clipped into the cutter; as the cutter is rotated, a blade scores the tubing. Next, the tubing is removed and snapped at the score line. This generally leaves the tubing without burrs, but with a slightly irregular end. The final option is to use a file to score the tubing. Holders that facilitate the filing operation are available from several vendors, or the tubing may be held with a pair of pliers. A small triangular file or a knife-edge file is used to file a groove about one-third of the way through the tubing; then the tubing is held on each side of the cut with a pair of pliers and snapped apart. Filing the outside edge to deburr the tube is usually necessary, and the end of the tube is seldom squarely cut. For all cutting methods, be sure to clean the tubing before using it in order to remove any filings or burrs that may dislodge and cause problems elsewhere in the system. Generally, it is sufficient to pump several milliliters of methanol through the tubing to flush it.

## LABELING

It is important to label tubing after it is cut so that sizes will not become confused. The most important tubing dimension to note is the internal diameter, because it is difficult to distinguish visually between 0.007-, 0.010-, and 0.020-in. i.d. tubing. One supplier of precut tubing (Upchurch Scientific, Inc., Oak Harbor, Washington) codes each piece of tubing with a colored band so that the sizes will not be confused. While you are noting the internal diameter of the tubing on the label, include the length and fitting types for convenience. If you confuse the different fitting types and brands, they may be assembled improperly and cause leaks or add dead volume to the system. An earlier Troubleshooting column described fitting assembly in detail (1).

**TABLE I: TUBING CONVERSIONS**

| Internal Diameter (in.) | Internal Diameter (mm)* | Volume (μl/cm)** |
|---|---|---|
| 0.007 | 0.18 | 0.25 |
| 0.010 | 0.25 | 0.51 |
| 0.020 | 0.50 | 2.03 |
| 0.030 | 0.75 | 4.56 |
| 0.040 | 1.00 | 8.11 |
| 0.046 | 1.20 | 10.72 |

* nominal value
** calculated from English internal diameter measurements

## SELECTING TUBING DIAMETER

As was mentioned above, in the parts of the system that the sample does not contact, the choice of tubing diameter is relatively unimportant. In fact, 0.020-in. and larger internal diameters are beneficial because they are less prone than narrower tubes to blockage by particulate matter. One should keep in mind, however, that 0.040- and 0.046-in. i.d. tubing add significant volume to the system (Table I), which can affect the gradient-delay volume of the system when gradient elution is used.

Another use of 0.040- and 0.046-in. i.d. tubing is for the construction of injection-valve sample loops. It is wise to purchase precut sample loops with a volume of about 50 μl (or less) because of the superior finish and fit obtained from precut tubing. When small injection volumes are involved, small dead volumes created by poorly cut tubing may contribute to poor washout and to sample carryover. For injection volumes above 100 μl, however, these extravolume contributions are insignificant when compared with sample size and generally do not cause problems. When injections of 500 μl to several milliliters are needed, cut the loop to the desired size from a length of 0.040- or 0.046-in. i.d. tubing. When loops are cut, it is only necessary to approximate the volume because standardization should be performed using the same loop as is used for sample analysis. A nominal 500-μl loop, for example, may actually measure 490–510 μl, with no significant impact on the analysis. If exact loop volumes need to be determined, the new loop should be calibrated using a loop of known volume. Table I compares the volume per unit length of the various diameters of tubing to aid you in selecting the proper dimensions for a sample loop.

**TABLE II: TUBING CHOICES**

| | |
|---|---|
| Type | Stainless steel: type 316, type 304 |
| | Polymeric: fluorocarbon, polyethylene, polypropylene |
| Internal diameter (stainless steel) | 0.007 in. |
| | 0.010 in. |
| | 0.020 in. |
| | 0.030 in. |
| | 0.040 in. |
| | 0.046 in. |
| Grade | Industrial |
| | LC |
| | Premium |

Tubing with 0.020- or 0.030-in. i.d. is most commonly used to connect the pump to the injector and in other portions of the LC system that are not exposed to sample. Tubing of these sizes will degrade the separation in most cases if used to connect the column to the rest of the system.

The column should be connected to the injection valve and detector using 0.010- or 0.007-in. i.d. tubing. If 0.010-in. i.d. tubing is satisfactory, it is better to use than the smaller diameter tubing, because 0.007-in. i.d. tubing is much more prone to blockage by particulate matter.

## HOW MUCH TO USE

Table III can be used as a guide for choosing the diameter and length of tubing to use for column connections. Several of the most common column dimensions are listed, along with the maximum length of tubing that can be used and still hold the increase in band width to 5%. For example, Table III shows that if a 250 mm × 4.6 mm column packed with 5-μm particles is used, up to 114 cm of 0.010-in. i.d. tubing can be used for connections, but that any length of 0.020-in. i.d. tubing will cause an increase in band width greater than 5% for a peak with a capacity factor of 1 and plate number of 20,000. The column plate number (*N*) shown corresponds to a reduced plate height (*h*) of 2.5, which means that the detector cell and time constant need to be properly matched to the column to provide this level of performance. Remember, too, that tubing is required at both ends of the column for connections. Each fitting requires about 2 cm of tubing for the nut and ferrule, so a minimum of 8 cm of tubing is needed to connect the column to the rest of the system (assuming that there are two fittings at each end). For this reason, lengths of less than 8 cm are not listed in Table III.

**TABLE III: GUIDE TO TUBING LENGTH AND INNER DIAMETER**

| Column Characteristics | | | | Maximum Length (cm) for 5% Increase in Band Width | | |
|---|---|---|---|---|---|---|
| $L$ (mm) | $d_c$ (mm) | $d_p$ (μm) | $N$ | 0.007 in. | 0.010 in. | 0.020 in. |
| 33 | 4.6 | 3 | 4400 | 22 | 9 | * |
| 50 | 4.6 | 3 | 6677 | 33 | 14 | * |
| 100 | 4.6 | 3 | 13333 | 67 | 27 | * |
| 150 | 4.6 | 5 | 12000 | 167 | 68 | * |
| 250 | 4.6 | 10 | 10000 | 556 | 228 | 14 |
| 250 | 4.6 | 5 | 20000 | 278 | 114 | * |
| 250† | 2.0 | 5 | 20000 | 50 | 20 | * |
| 250‡ | 1.0 | 5 | 20000 | 12 | * | * |

Based on references 2 and 3. $L$ = column length, $d_c$ = column internal diameter, $d_p$ = particle diameter, $N$ = column plate number; flow rate = 1 ml/min, except: † 0.2 ml/min, ‡ 0.05 ml/min
* less than 8 cm

Except where noted, a flow rate of 1 ml/min was used in the calculation of the values in Table III. The length of tubing is inversely proportional to the flow rate, so a flow rate of 3 ml/min will permit only one-third as much connecting tubing in the system. If columns of larger internal diameter are used, even at increased flow rates, more connecting tubing can be used because larger peak volumes are generated. This effect can be seen by comparing the entries in Table III for the 250-mm columns with internal diameters of 1.0, 2.0, and 4.6 mm.

What does all of this mean in terms of chromatographic performance? If the important peaks in the analysis are baseline-separated and each has a $k'$ value greater than 1, then 5% to 10% increases in band width will generally be insignificant. In this case, you can take the guidelines in Table III with a grain of salt. If, however, peaks of interest elute before $k' = 1$ and resolution is $\leq 1.2$, you will see a degradation in the separation with a 10% increase in band width. If narrow-bore columns or short, fast columns are used, additional restrictions may be placed on overall chromatographic performance by other system components. For instance, using a nonoptimal detector-cell volume or time constant with one of these columns can limit tubing to lengths less than that shown in Table III.

## TUBING PROBLEMS

Fortunately for troubleshooting purposes, chromatographic tubing does not spontaneously change and degrade system performance. Except in the rare case when a ferrule slips in a fitting under high pressure, the operator must intervene to cause a change in tubing. If you label the internal diameter of each piece of tubing and record any changes in tubing in the system logbook, it will be easy to pinpoint tubing problems. A sudden decrease in column plate number or resolution after a piece of tubing has been replaced indicates either that tubing with too large an internal diameter was used or that too long a piece was substituted. Increases in system pressure immediately or soon after a new piece of tubing has been added may indicate a poorly cut and deburred tube. Additional peaks in the chromatogram may mean that the tubing was not cleaned carefully before it was used. It is possible to inject inadvertently a small volume of immiscible solvent if a piece of tubing is inserted that was last used for another LC analysis. If a column has been replaced and does not perform to the manufacturer's specifications, it is wise to double check the connecting-tubing diameter before exchanging the column for a new one, because column performance is measured by the manufacturer on an LC system in which extracolumn effects are minimized.

Tubing blockage usually shows up as an increase in the system pressure and is most common with 0.007-in. i.d. tubing. To locate a blocked tube, leave the system pressurized and loosen the connecting fittings, starting at the detector and moving toward the pump (be sure to wear eye protection). A sudden drop in system pressure will be seen when the fitting is loosened immediately upstream of the blocked tube or component. Clear the blockage or replace the component, retighten all fittings, and resume operation. User-packed columns frequently cause tubing blockage at the column outlet if any column packing is left in the outlet side of the fitting. If you experience problems with 0.007-in. i.d. tubing, a zero-volume filter can be added to prevent particles from reaching the tubing. You may find, however, that the added volume of the filter cancels out the advantage of 0.007-in. over 0.010-in. i.d. tubing. In this case, change back to 0.010-in. tubing, which is much less prone to blockage.

## CONCLUSION

The connecting tubing in an LC system generally does not cause problems in system performance if care is taken in selecting, cutting, and cleaning the tubing and in assembling the fittings. The purchase of precut lengths of tubing assures you of a well-cut tube and is an added convenience when fittings need to be assembled. User-cut tubing can also produce high-quality tube ends if care is taken to make a square cut and the tube is then carefully deburred. It is necessary to choose the proper diameter and length of tubing to maintain optimum chromatographic performance. Careful labeling of cut tubing will help reduce problems that arise from selecting the wrong size tubing.

## REFERENCES

(1) J.W. Dolan and V.V. Berry, *LC, Liq. Chromatogr. HPLC Mag.* **2**, 20–21 (1984).
(2) L.R. Snyder and J.J. Kirkland, *Introduction to Modern Liquid Chromatography*, 2nd Ed. (Wiley-Interscience, New York, 1979).
(3) R.P.W. Scott and P. Kucera, *J. Chromatogr. Sci.* **9**, 641 (1971).

Readers are invited to contribute their troubleshooting tips to this column or to submit topics or questions for discussion in future articles. Write to: The Editor, *LC Magazine*, P.O. Box 50, Springfield, OR 97477.

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Erratum: Please note the follow correction in the article "Liquid Chromatographic Separation of Rhenium Analogues of Technetium Radiopharmaceuticals" by Richard E. Needham and Michael F. Delaney, which appeared in the November 1984 issue of *LC* (Volume 2, Number 10), p. 760. References 1–5, which are cited in the first paragraph of the Introduction, should have appeared as follows: "With the advent of the combined techniques of HPLC and gamma-ray detection of the $^{99m}Tc$ label, multiple radiopharmaceutical species in these agents have been determined and reported (1–5)."