Column Diameter, Linear Velocity, and Column Efficiency

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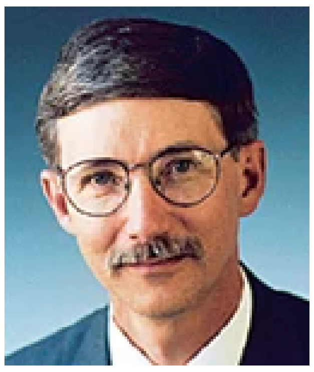
John Dolan examines some questions he recently received concerning the changes that took place in the 2009 edition of the United States Pharmacopoeia.

I recently had a question regarding some changes that took place in the 2009 edition of the United States Pharmacopoeia. In USP 32, General Chapter 621 (1), the adjustments allowed to meet system suitability for liquid chromatography (LC) methods include a change in column internal diameter of ±25%. A change was made in USP 32 Supplement 2 (2).
This stated that column internal diameter "can be adjusted provided that the linear velocity is kept constant."
The person was not sure how this adjustment of linear velocity should be made and wondered about what effect it would have on column efficiency.



John W. Dolan

Adjustment of Flow Rate

Much of the band broadening that takes place within an LC column is a result of slow diffusion of the analyte in and out of the pores in the column packing. This means that the mobile phase flow rate can have an affect on the volumetric width of a peak. By volumetric width, I mean the peak width in units of volume (for example, milliliters or microliters), not time. In the days when 10-µm diameter particles were used as column packing, a noticeable reduction in column efficiency could be observed when the flow rate was increased. The smaller the particle diameter, the less the dependence of column efficiency upon flow rate. Although it is easy to demonstrate a loss in efficiency with an increase in flow rate for very well-behaved analytes with 3- and 5-µm diameter particles, a twofold change in flow rate is rarely noticed with real applications. With sub-3-µm particles, flow rate has little influence on column efficiency, even with well-behaved compounds.

In this discussion, I have referred to flow rate, but the important variable is not flow rate, but linear velocity. Linear velocity is the speed at which the mobile phase travels through the column, for example, in millimeters per second. For comparison of equivalent conditions between columns of different internal diameters, the linear velocity should be kept constant. To keep linear velocity constant, the flow rate should be adjusted in proportion to the column cross-sectional area, which is directly proportional to the square of the ratio of column diameters.

For the current discussion, let’s consider two cases. The first is a change from a 4.6-mm i.d. column to a 2.1-mm i.d. column $(4.6/2.1) = 4.8 \approx 5$

and the change from 4.6 mm to 1.0 mm:

$(4.6/1.0) = 21.2 \approx 20$

In both cases, we'll consider the approximations as close enough for practical work, and certainly easier to remember and use for mental calculations.

This means that for equivalent linear velocities, a change from a 4.6-mm i.d. column operated at 1 mL/min to a 2.1-mm i.d. column would require a flow rate adjustment of fivefold, to 1.0/5 = 0.2 mL/min. A change to a 1.0-mm i.d. column would mean a new flow rate of 1.0/20 = 50 µL/min.

The Old Allowance

USP 32 (1) allowed a change in column diameter of ±25%. This meant that a 4.6-mm i.d. column could be exchanged for any column in the 3.45–5.75 mm i.d. range. I suspect that this specification was originally written to allow the old Waters µBondapak 300 mm × 3.9 mm column to be used instead of the 4.6-mm i.d. versions. USP 32 also allowed a ±50% change in flow rate. The 3.9-mm i.d. column would require a flow-rate reduction of 30% for constant linear velocity, so the necessary flow-rate change was covered. Note, though, that the flow-rate change was not required by USP, so a significant change in linear velocity was allowed. Today, however, the most popular column internal diameters are 4.6, 2.1, and 1.0 mm, so the ±25% diameter-change allowance eliminated the option of using smaller-internal-diameter columns. It is clear that a change in the USP was needed.

The New Allowance

The change in USP 32, Supplement 2 (2) at first seems more complicated by introducing the linear velocity, but actually, it gives more flexibility and ensures a more equivalent result than the old allowance. Now any column diameter can be used, so the 2.1- and 1.0-mm i.d. columns can be used in addition to the 3.9-mm i.d. ones. In addition, the adjustment in linear velocity means that the columns will be operated under more similar chromatographic conditions than before. By properly adjusting the linear velocity, the column pressure and analyte retention times should be the same when column diameter is changed.

Column Efficiency

What wasn't addressed in the original question was why one would want to use a column of different diameter. There are two primary advantages, decreased mobile-phase consumption and a reduction in peak volume. If the flow rate is adjusted for constant linear velocity, as discussed earlier, the retention times should be the same when column diameter is changed, so the sample run time will be unchanged. If the run time is the same and the flow rate is reduced, less mobile phase will be used. This is attractive, especially in these days of high acetonitrile prices.

The peak volume (peak width in volumetric terms) drops with the reduction of the cross-sectional area of the column, or square of the change in diameter.

This translates into proportionally taller peaks, assuming that the same mass of sample can be loaded onto the column, which may or may not be true. Thus, moving from a 4.6-mm i.d. column to a 2.1-mm column should reduce peak width by fivefold and increase peak height by the same amount (assuming the same mass of sample is injected). This leads to the second part of the original question, regarding how a change in column diameter affects column efficiency. In theory, there should be no dependency of efficiency upon column diameter, but from a practical standpoint, narrower columns usually are less efficient than larger-diameter ones.

The primary factor in determining the column efficiency is the number of particle diameters in the length of the column.

$$N = L/H \qquad [1]$$

where L is the column length. For a 5-μm particle (d_p), two particle diameters would give H = 10 μm = 0.01 mm. Packed in a 150-mm-long column, this would give

N = 150/0.01 = 15,000

You might be able to get 15,000 plates for a 150 mm × 4.6 mm column mounted on an LC system optimized for column testing and using a well-behaved test compound such as toluene or methyl benzoate. However, for real samples on a typical laboratory's LC system, most of us would feel very good to get 10,000 plates for this column. Even if we used toluene or methyl benzoate, we might get only 12,000 plates on the system in the laboratory.

Extracolumn Effects

What is the reason for the reduced plate number on a real LC system? Part of it has to do with extracolumn peak broadening. Here's how it works. The width of a peak in a chromatogram can be expressed as

$$W_T = (W_c^2 + W_{ec}^2)^{0.5} \qquad [2]$$

where W_T is the observed peak width at baseline, drawn between tangents to the sides of the peak, W_c is the peak broadening that takes place as the analyte travels through the column, and W_{ec} is the peak broadening outside the column, often referred to as extracolumn effects.

We have all observed peak broadening within the column W_C. For example, with an isocratic separation, the early-eluted peaks are narrower than later-eluted ones. This is because the longer the compound stays on the column, the broader it is. Some other factors related to on-column peak broadening include the particle diameter, column temperature, mobile phase viscosity, and analyte diffusion coefficient.

Extracolumn effects include all sources of peak broadening excluding the column itself. These include such things as the injection volume and solvent, the length and diameter of connecting tubing between the autosampler and column and between the column and detector, the detector cell volume and time constant, and the data rate of the data system. For the present discussion, we'll consider these as a composite influence. For a conventional LC system, such as the ones most of us use, set up for routine analysis with 150 mm × 4.6 mm i.d. columns packed with 5-μm diameter particles, W_{EC} ≈15 μL is a typical value.

Impact of W_{EC} on N and R_S

It is an interesting exercise to assess the degradation of the column plate number N and resolution, R_S, for columns of different diameter. In Table I I have summarized data for a peak with the retention factor, k= 1. The assumptions in both tables are a column length L= 150 mm; column internal diameter, d_c = 4.6 mm (or as noted); column void volume of 60% of the column volume; flow rate F= 2.0 mL/min for the 4.6-mm column; 5-μm particles for a column plate number, N= 15,000 in absence of extracolumn effects; and an extracolumn volume of 15 μL. Data are shown for 4.6-, 2.1-, and 1.0-mm i.d. columns.

I have rounded the displayed values for clarity, so if you are trying to repeat my calculations in a spreadsheet, keep the maximum number of significant figures for all calculations.

d_c (mm)	V_M (mL)	F (mL/min)	t_R (k = 1)	W_T (s) for ec =		N	R_s loss
				0 µL	15 µL		
4.6	1.5	2.0	1.5	2.9	3.0	14,655	1%
2.1	0.31	0.42	1.5	2.9	3.6	9723	19%
1.0	0.071	0.095	1.5	2.9	10	1298	71%

Table I: Extracolumn effects for k = 1

In the second and third columns of Table I, you can see the column volume *V* calculated for each column diameter and the fl ow rate adjustments required to maintain a constant linear velocity. For an early-eluted peak with *k* = 1 and a 4.6-mm i.d. column, there is a very small increase in peak width resulting from the extracolumn volume. However, the 15 µL of extracolumn volume increases the peak width by more than threefold for the 1.0-mm i.d. column (2.9 versus 10 s). This reduces the plate number by more than an order of magnitude, and because resolution is proportional to the square root of the plate number, resolution drops by 71%! Most of us consider a drop in resolution of 5% or less to be of little consequence, but it is obvious from the data in Table I that use of a 2.1- or 1.0-mm i.d. column with an early-eluted peak will not produce satisfactory results with this LC system.

Table II: Extracolumn effects for k = 2, 5, and 10

d_c (mm)	k = 2		k = 5		k = 10	
	N	R_s loss	N	R_s loss	N	R_s loss
4.6	14,844	1%	14,961	0%	14,988	0%
2.1	12,085	10%	14,147	3%	14,736	1%
1.0	2636	58%	6904	32%	11,120	14%

Table II: Extracolumn effects for k = 2, 5, and 10

Table II summarizes the impact of 15 µL of extracolumn volume on peaks with larger *k*-values. It can be seen that more retained peaks are influenced less by extracolumn volume for all column diameters. This is expected, because peak width within the column W_C increases with retention, so with constant W_{EC}, the observed peak width W_T will be less affected. However, for the chosen chromatographic conditions, the 2.1-mm i.d. column is acceptable (*R* -loss < ≈5%) only for well-retained peaks. The 1.0-mm i.d. column does not produce satisfactory results at any retention time.

A disclaimer should be included here, as on the advertisements for new cars: your mileage may vary. Tables I and II were calculated based upon the assumption that the 5-µm particle diameter column generated *N* = 15,000. As mentioned earlier, this represents the performance of a well-packed column operating under ideal conditions. It is likely that such a column will generate plate numbers in the 10,000 range for real compounds under real analytical conditions. This means that the peaks will be broader, even under the best circumstances, so the impact of extra column volume will be less than that shown in the tables. However, the general conclusion is the same: you will have to minimize extracolumn effects if you want to get acceptable results with 2.1-mm i.d. columns on a conventional LC system. Furthermore, it is unlikely that you will get satisfactory performance from 1.0-mm i.d. columns unless you take great care. This will mean limiting injection volume, replacing the detector with one with a smaller-volume flow cell, and making other changes to reduce extracolumn effects.

Conclusions

It is fairly simple to make the necessary changes in flow rate to maintain constant linear velocity when changes in column diameter are made. A reduction in column diameter can be useful to conserve solvent and to improve detection limits. However, a drop in system performance, as measured by resolution, might be observed for smaller-diameter columns unless special care is taken to reduce extracolumn effects.
From a troubleshooting standpoint, it is tempting to blame poor system performance on the column itself, when the real culprit may be the extracolumn volume of the LC system.

A relative insensitivity to extracolumn effects is one reason why the 150 mm × 4.6 mm column packed with 5-µm diameter particles is likely to remain the workhorse column for routine LC work for many years to come.

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For an ongoing discussion of LC troubleshooting with John Dolan and other chromatographers, visit the Chromatography Forum discussion group at http://www.chromforum.org.

References

(1) United States Pharmacopoeia **32**, General Chapter 621 (2009) 237.

(2) United States Pharmacopoeia **32** (Supplement 2), General Chapter 621 (2009) 4149.

Evolution of LC Troubleshooting: Degassing

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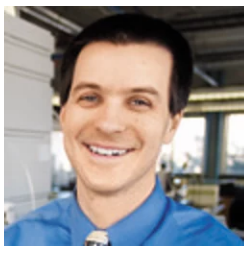

The Evolution of LC Troubleshooting: Degassing

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As chromatography technologies change, our approaches to using them in ways that value reliability, as well as the ways we should approach troubleshooting and fixing problems, should also change.
In this installment, I continue the discussion started last month about how some specific troubleshooting topics have evolved over time, this time focusing on mobile phase degassing.
Understanding how approaches to degassing have evolved is helpful for troubleshooting problems with pumps and detection, designing reliability into new methods, and when considering updates to legacy methods that have been in use for decades.

In recent months, I've been hearing from several readers of this column that they've appreciated the more educational, "back-to-basics" flavor of some of the "LC Troubleshooting" installments in the last year.
So, last month I decided to dip a little further into that theme and provide some perspective on the evolution of various troubleshooting topics over the last few decades.
My view is that this kind of perspective is particularly valuable to those who are relatively new to the field.
There are some aspects of "the way we do things" that may seem peculiar on the surface, but are in fact very important to the reliability of high-performing liquid chromatography (LC) methods.
On the other hand, some aspects of certain methods and ways of doing things are simply unnecessary in 2023 because LC technology has evolved in such ways that the old tricks aren't needed anymore.
Sometimes implementing the old tricks with new technology – though they provide no benefit – doesn't do any harm, but they do add cost to analyses because they take time and resources to implement.
Thus, we really ought to let them go if they are not adding any value to the method.
In other words, let's be smart about the methods we deploy. If we can't come up with a better explanation for why something about the method is the way it is, then it's time to let it go. In this installment, I will discuss the evolution of degassing in liquid chromatography.
In general, degassing has become much more convenient, to the extent that most users probably rarely think about it anymore.
However, some of the older techniques are still useful, and even with modern techniques it is useful to have a broad sense for how things have changed, as this can impact proper instrument operation when working with different generations of equipment.
Readers interested in learning more details about the degassing topic are referred to previous installments of "LC Troubleshooting" (1,2), Dolan's book on LC troubleshooting (3), and a very old, but very rich, paper by Bakalyar and coworkers (4).

Why Bother with Degassing at All ?

In most applications, the biggest problem with gas bubbles inside of an LC system is that pumps generally don't deal with the bubbles very well (see the last section below for other problems that can be significant in some applications). In the worst cases, a gas bubble can cause one or both of the check valves in a high-pressure pump to fail, causing a highly erratic flow or no flow at all. Where, then, do these bubbles come from? Although there are multiple mechanisms that can lead to bubble formation, the most practically relevant one in LC is the situation where two solvents are brought together to make a mixture (here, the mobile phase) that has a lower gas solubility than either of the individual solvents alone. This is most problematic with pumps that use the "low-pressure mixing" design. For a refresher on the differences between low-pressure and high-pressure mixing designs used in LC pumps, readers are referred to previous articles in this magazine (5). In the case of low-pressure mixing, the individual solvent components are brought together under nominally atmospheric pressure conditions before a high-pressure pump provides the force needed to push the mixture through the rest of the LC system.

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An example is valuable for illustrating the point here. The solubilities of oxygen gas at room temperature and atmospheric pressure in water or ethanol are about 0.3 × 10 and 5.7 × 10, respectively (4) (albeit on a mole fraction basis, meaning moles of oxygen relative to the total moles of oxygen and solvent). In other words, when saturated with oxygen, ethanol carries about 20 times more oxygen dissolved in the solvent compared to water. If we mix the two solvents in equal parts, then the amount of oxygen present in the mixture initially will be about 3.0 × 10 (mole fraction). However, the solubility of oxygen in a 0.5 (mole fraction) mixture of water and ethanol is only about 1.8 × 10 (mole fraction). This difference of 1.2 × 10 is the amount of oxygen in excess of the carrying capacity of the mixed solvent at saturation, and this excess will manifest in the formation of significant bubbles. In a low-pressure mixing pump, these bubbles will be drawn into the pump, and may cause failure of one or both check valves. So, in this example, avoiding the problem requires that the concentration of gas dissolved in each of the individual solvents is decreased prior to mixing such that the gas concentration in the mixed solvent (mobile phase) is lower than the solubility limit of the gas in that solvent. If this is achieved, then bubble formation will not be nearly as serious. A different way of thinking about the same problem is to consider the volume of gas that will be evolved as bubbles upon mixing of two solvents. Figure 1 shows data along these lines from the work of Bakalyar and coworkers (who physically measured the volumes of bubbles formed using an apparatus for trapping the bubbles) for mixtures of methanol or acetonitrile with water (4). We see that the volumes can be quite remarkable – 60 µL of gas per milliliter of mixed solvent! – and that these volumes roughly maximize around 50:50 mixtures of the organic solvent and water.

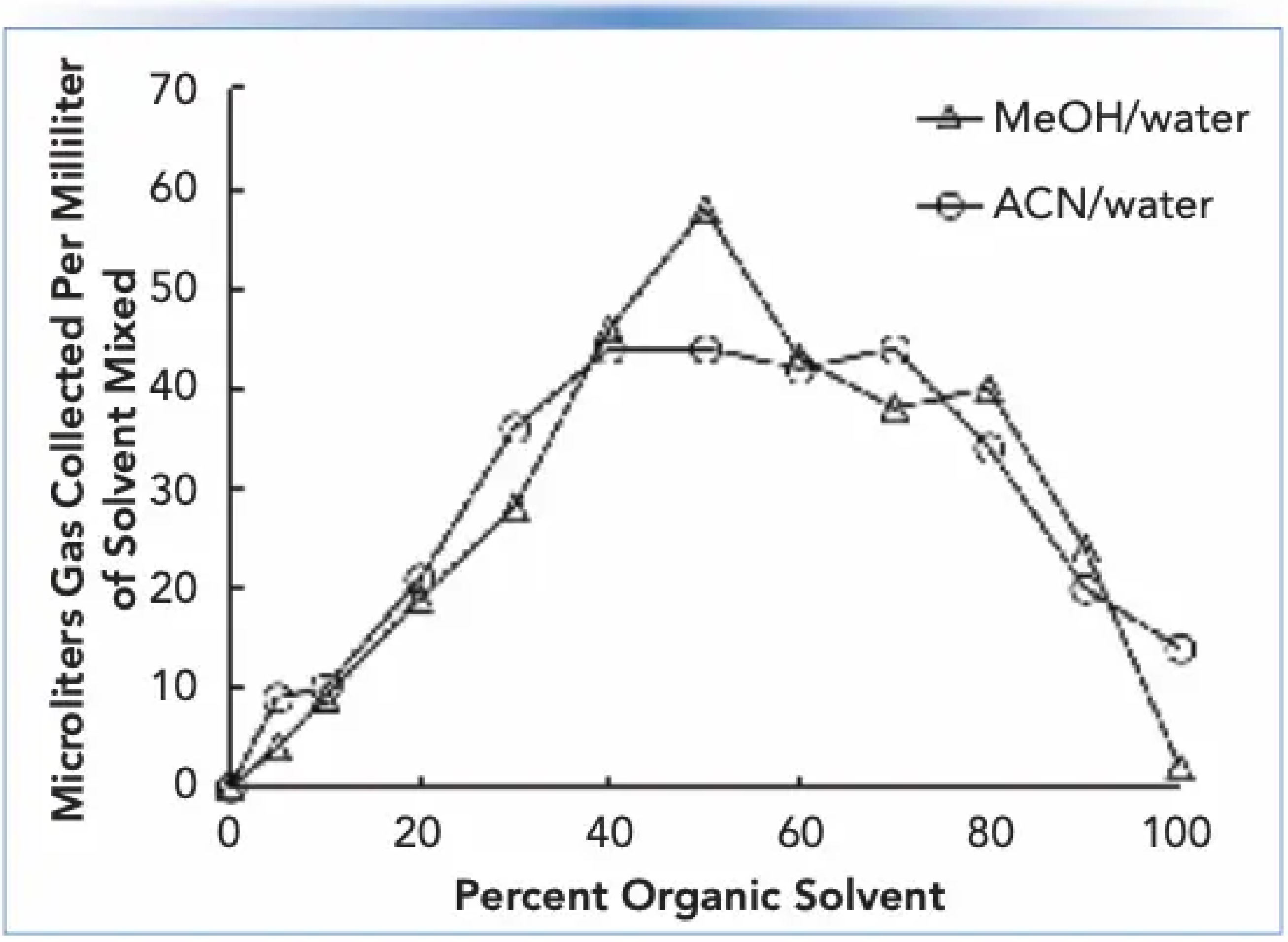

FIGURE 1: Gas evolved from different mixtures of solvents commonly used in reversed-phase LC. Adapted from (4).

The situation with pumps that use a high-pressure mixing design is quite different. In this case the mixing point for the mobile phase components is downstream from the high-pressure pump heads, and the pressure at which the mixing occurs will be nominally the same as the column inlet pressure (that is, well above atmospheric pressure). Under these conditions the gas solubility in the mobile phase will be much higher and bubbles will not form while the liquid is under pressure. However, as the pressure drops toward atmospheric pressure at detector, bubbles may form when the gas concentration reaches the solubility limit in the mixed solvent. If bubbles form in an optical flow cell (for example, ultraviolet-visible
(UV-vis) or fluorescence), this can lead to unstable baselines, including spiking patterns.

Survey of Degassing Techniques, Old and New

Including older discussions of degassing techniques used in LC, the list includes: heating, sparging, refluxing, sonication, offline vacuum degassing, and inline vacuum degassing. I personally am not aware of anyone currently routinely using heating or refluxing for degassing, and I won't discuss those approaches further here.

I like to think of sparging as a technique that "scrubs" dissolved gases from a liquid. The principal fact in play here is that helium is much less soluble in LC solvents than other gases, including oxygen and nitrogen. In methanol, which is particularly problematic as discussed above, the solubilities of these three gases at room temperature and atmospheric pressure are about 0.7 × 10 , 2 × 10 , and 4 × 10 (mole fraction), respectively (4). To use the sparging approach in practice, helium is deliberately bubbled into a LC solvent. Other gases dissolved in the liquid diffuse into the helium bubbles and are carried out of the liquid as the helium bubbles rise to the surface. After an initial scrubbing period (15 minutes of sparging will remove about 80% of the oxygen dissolved in methanol [6]), the "gas-free" solvent can be maintained on the instrument with a very low flow of helium into the solvent bottle. Helium sparging has fallen out of favor due to the introduction of other approaches (mostly inline degassing, discussed below), practical inconvenience (nobody wants to deal with compressed gas cylinders if they don't have to), and the cost of helium, meaning it is not commonly used today. That said, I do still have LC instruments in my laboratory that have the necessary plumbing and sparging stones to support this approach.

Sonication

A very simple approach to degassing is to simply place a bottle of solvent to be used on the LC instrument in an ultrasonic bath for several minutes before use. Unfortunately, this approach is not very effective; fifteen minutes of sonication will only remove about 30% of the dissolved oxygen dissolve in methanol (6).

Sparging

Offline Vacuum Degassing

In the offline vacuum degassing approach, a vacuum is applied to a bottle containing the solvent that will ultimately be used on the LC. The most convenient and safe source of vacuum would be a house vacuum system, as found in many laboratories. The fundamental idea here is that reducing the pressure in the bottle decreases the solubility of gases dissolved in liquid, and any gas present above the solubility limit will spontaneously bubble out of the liquid. The vacuum can be applied using a sidearm type of flask with a hosebarb connection, or a plastic bottletop adapter with a hosebarb fitting that can be used to connect to the bottle to the vacuum source. When using this approach, great care should be taken to ensure that the container placed under vacuum is not physically compromised with cracks or other defects, as this could lead to a dangerous implosion of the container. Using a safety shield when the bottle is under vacuum is a good idea. Users should also be careful to use an explosion-proof vacuum pump if using a pump as the vacuum source, particularly when working with solvents that produce explosive vapors.

In my laboratory, we have found it most effective to combine offline degassing and sonication. The additive effects of the two approaches are both visibly obvious and unforgettable. As a demonstration, I suggest preparing a 50:50 mixture of acetonitrile and water, and placing the mixture under vacuum until no bubbles are observed. Then, while the bottle is still under vacuum, place the bottle in an ultrasonic bath. A vigorous rush of bubbles will be observed, and then completed within seconds. Although we only use inline degassing (see below) for routine degassing of solvents on LC instruments in my lab, the combination of offline vacuum degassing and sonication is still very useful in situations where a complementary degassing approach is needed. For example, if one suspects that the inline degasser in a pump is not working, and leading to bubble formation, degassing the solvent offline can enable a very informative test of the rest of the system to rule out the possibility that the inline degasser is the source of the problem.

Inline Vacuum Degassing

A simple block diagram illustrating the use of inline vacuum degassing in LC pumps is shown in Figure 2. In this approach, the solvent is drawn from the bottle through a polymeric tube situated inside of a vacuum chamber on its way to the inlet check valve of the pump (in the case of a high pressure mixing design) or a proportioning valve (in the case of a low-pressure mixing design). The polymer chosen for the tubing inside the vacuum chamber is one that is permeable to the major gases dissolved in the solvent that we need to remove (mainly nitrogen and oxygen), such that gas molecules are drawn across the tube and removed from the liquid selectively. Following the discovery in the early 2000s of particular polymers with enhanced permeability for these gases (7), inline vacuum degassing has become the dominant means of degassing used in LC instruments today. In general, it is easy to use (no manipulation by the analyst) and quite robust (except for an

vacuum pump. When using pre-mixed solvents (that is, mixed in the solvent bottle so that a mixture such as methanol/water enters the degasser), this means that the composition of the solvent exiting the degasser module will be different from the composition of the solvent entering the module, due to preferential loss of the more volatile component of the solvent. For example, an acetonitrile/water mixture will contain a little less acetonitrile exiting the degasser than it did entering it. For most applications these changes will not be large enough to affect method performance, but some applications that are more sensitive to solvent composition may be affected. Similar effects on the solvent composition occur with other degassing methods, including sparging and offline degassing, so this is not a problem unique to inline degassing.

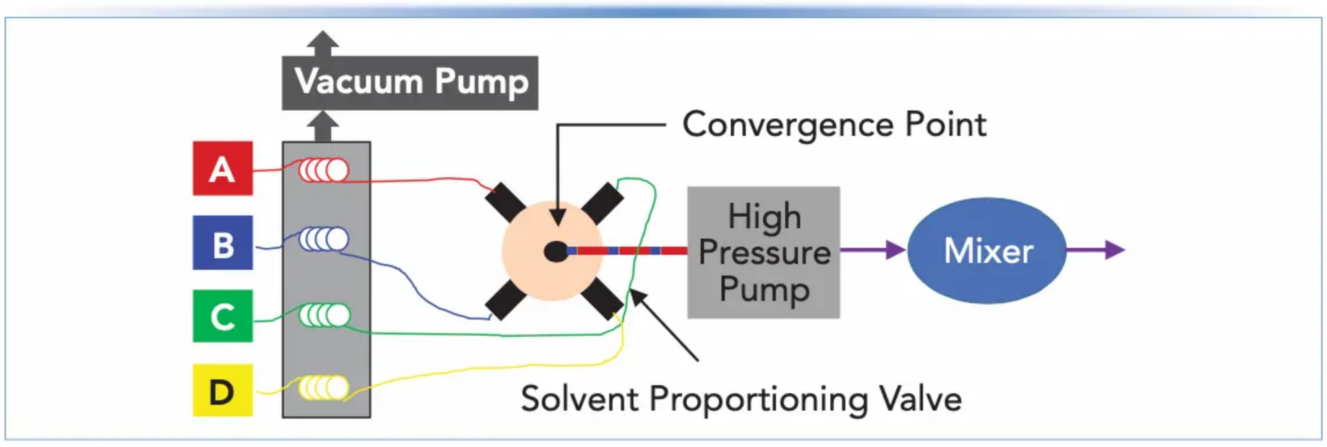

FIGURE 2: Block diagrams for a LC pump with a low pressure mixing design. The inline vacuum degasser is placed between the solvent bottles and the proportioning valve.

Tips and Other Details to Consider

Degasser Holdup Volumes

Older models of inline vacuum degassers relied on long lengths of less permeable tubing compared to the polymers used in newer models. A practical consequence of this is that the volumes of liquid inside older degasser modules (sometimes referred to as the holdup volume of the degasser) were much larger than they are now. This can be important for two reasons: 1) when working with a newer instrument, the analyst may not need to flush the degasser as long as they had to with an older module when changing solvents; and 2) conversely, when moving to work with an older instrument or an instrument from a different vendor, the degasser flushout time used when changing solvents may need to be adjusted to account for different holdup times. In every case, users should either consult the user manual for the particular model of degasser they are using to find out what the degasser holdup volume is, or a find a recommendation for the flushout volume for the degasser when changing solvents.

In addition to the effect of dissolved gases on bubble formation and pump performance, these gases can also affect detection in LC. For example, Brown and coworkers showed that there can be a 400 mAU difference in the absorbance of methanol at 210 nm when the solvent is fully degassed compared to when it is saturated with air (6). Thus, if degassing efficiency varies over time, this variation can lead to detector baseline drift (long-term changes) or waves in the baseline (short-term changes). Moreover, Bakalyar and coworkers showed that changes in the level of dissolved oxygen in the mobile phase significantly affected the fluorescence signal for some analytes in an application focused on the quantitation of polyaromatic hydrocarbons (4). Here, variations in degassing efficiency could have significant effects on the quantitative performance of methods using fluorescence detection.

Effects of Dissolved Gases on Detection

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Summary

In this installment of "LC Troubleshooting," I've discussed the importance of degassing solvents used for liquid chromatography, as well as older and newer approaches to the task. The primary problem that can be avoided with proper solvent degassing is bubble formation, which can cause erratic or even no flow from the LC pump. While inline vacuum degassing is currently the dominant approach used in commercially available instruments, there certainly are situations where older approaches, including sonication and offline degassing, are still useful. Understanding the history of these practices, and how they have evolved over time, is a useful facet of knowledge for aspiring LC troubleshooters.