Connections

When Good Columns Go Bad

All gas chromatography columns will go bad eventually, but gas chromatographers often find that it happens sooner than they expect. In this month's "GC Connections," John Hinshaw takes a look at some causes and modes of premature column failure and at good practices that help ensure the longest possible column life.

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Despite all the care that gas chromatography (GC) column manufacturers exercise to ensure that their products meet user expectations right out of the box, often columns never reach that level of performance or they do so only for a brief time. The manufacturers rarely are at fault; it's improper column installation and use that are to blame in most situations. Chromatographers can expect to match or exceed documented performance levels from column to column over many new installations of the same product from any single manufacturer. Not only should multiple columns' dimensions, plate counts, tailing factors, and bleed levels be consistent, but column polarity — as measured by the relative retentions of several reference peaks — also should remain virtually constant. With use over time, all of these column metrics can change significantly. If GC operators don't perform setup and installation steps properly, a column's life may be as short as the time it takes to install and test it. Chromatographers who consistently exceed a column's maximum operating parameters or subject it to chemical attacks may experience a more gradual performance loss. In either case, users sometimes can recover part or all of the lost column performance.

Common Problems

Gas chromatographers routinely encounter diverse column problems such as column bleed, excessively broad peaks, tailing peaks, adsorption, and polarity shifts. To assist in identifying these problems, it's best to keep a record of column performance that includes the manufacturer's test chromatogram and periodic test runs obtained after column installation. Precipitous changes are easy to identify, but gradual performance shifts may not be obvious unless an operator compares current test chromatograms with earlier ones. For a more quantitative assessment, chromatographers can use minimum performance requirements for individual column types and analytical methods to help establish the point at which a column's metrics are no longer sufficient for a particular separation. For example, if the number of theoretical plates for a particular peak falls below a minimum level, then the column should be revived or replaced. Setting minimum levels requires additional work, of course, but in my experience it is worth the effort.

Profiles in column bleed: Column bleed — the rise in detector background signal that occurs with increasing column temperature — is a natural consequence of the presence of volatile chemical substances inside the column. Their vapor pressures increase exponentially with temperature and cause a larger signal as the column heats. Volatile substances also can form during column heating from thermolytic or catalytic breakdown of the stationary phase. These two sources produce baseline profiles of an exponentially increasing signal that levels off in the temperature program's final hold time.

Figure 1 illustrates the appearance of column bleed as column temperature rises. At the final temperature, the detector signal reaches an offset level of 8 mA at the recorder, which corresponds to 32 pA (32 $\times 10^{-12}$ A) in the flame ionization detector at the range of 1 mV/pA and attenuation of 4. This particular column has a high bleed level because of a relatively thick Carbowax stationary-phase coating and the large stationary-phase volume associated with its 0.53-mm inner diameter. Not only does Carbowax have a lower average molecular weight than most of the silicone stationary phases, but it also is particularly sensitive to destruction from oxygen in the carrier gas. It's a good example of a stationary phase to which chromatographers should pay particular attention with regard to carrier-gas purity.

An astute observer will notice that some small peaks are imposed on the baseline as it rises. External sources such as contaminated carrier gas or a dirty inlet system can introduce materials with relatively low molecular weights into the column. In the case of temperature programming, these materi-

als are trapped at the head of the column during extended intrarun periods at the initial column temperature. During a subsequent temperature program, they are separated as if they were injected solutes, and, thus, they appear as peaks at the detector. In addition, especially with Carbowax phases, when the beginning of the column is heated inside the inlet system — to 220 °C in this case — stationary-phase oxidation products will be trapped at the beginning of the cooled portion of the column that rests inside the column oven. With subsequent temperature programming, these extra materials also will appear as peaks.

Some available silicone phases are designed to produce low column bleed. These materials are particularly suitable for use with highly sensitive devices such as mass spectrometers and electron-capture detectors, but their overheating or extended use with contaminated carrier gas or dirty inlet systems will degrade the apparent bleed performance. When using a mass spectrometer, analysts sometimes can tell the difference between column bleed that is attributable to the stationary phase and bleed that comes from external sources by examining the background signal for the presence of silicon-containing molecular fragments. Septa also are made from silicone polymers. With temperature programming, septum contamination usually appears in the form of peaks, and stationary-phase breakdown products tend to appear as a baseline offset that increases with higher oven temperatures.

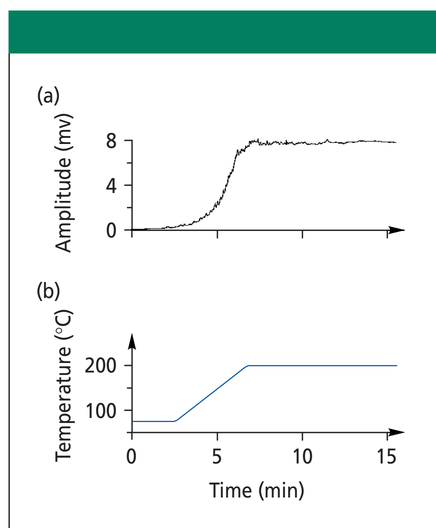

Figure 1: Column bleed and column temperature. Shown are (a) the detector response and (b) the temperature program. Column: 30 m × 530 µm, 1.0-µm d_f bonded Carbowax; temperature program: 75 °C, hold 2.5 min, 30–200 °C, hold until end; carrier gas: helium at 43 kPa and 6 mL/min; inlet: split–splitless; temperature: 220 °C; vent flow: 50 mL/min; flame ionization detector temperature: 250°C; detector range: 1×10^{-12} A/mV; detector attenuation: 4.

For analyses in which column bleed is a concern, analysts should determine a reference bleed level for newly installed and baked out columns that they can use for later evaluation. Column manufacturers may specify a bleed level for some of their products, but in most cases it's a good idea to measure the actual bleed level after installation rather than to rely on a manufacturer's example. Establish a set of bleed determination conditions that includes temperature programming to the highest temperature applied in operation — limited, of course, to the manufacturer's specified maximum allowable operating temperature. After installation and thorough leak checking, perform a series of bakeout runs until the bleed level has settled to a constant value at the end of each run. Record a chromatogram and measure the bleed level in picoamperes. Later, if column bleed becomes an issue, this information forms a basis for comparison.

When bleed is problematic, several bakeout runs may help to reduce it. Keep the temperature within limits, of course, and make sure the carrier gas is free of oxygen and leaks. Recovery sometimes is possible by removing the first turn or two of the column or by rinsing the column with a suitable solvent in the reverse direction from detector to inlet end. Refer to column manufacturers' recommendations about column recovery for more information about these procedures. Avoid column bleed problems in the first place by operating the column and inlet within recommended temperature limits, keeping the oxygen concentration under control, and injecting only suitably prepared clean samples.

Theoretical plates: The number of theoretical plates obtained from a column provides a fundamental measurement of column performance and the quality of installation. After first installing a column, chromatographers should expect to measure theoretical plate numbers close to the manufacturer's specification or close to the maximum level that can be expected for the column. This performance figure — when measured at close to the optimum carrier-gas linear velocity — depends upon both the column inner diameter and the stationary-phase film thickness. If the inner diameter alone is considered, the maximum number of theoretical plates (N_{max}) approaches the value calculated from equation 1 for peaks with retention factors (k) of 10 or higher:

$$N_{max} \approx 1.1\frac{L}{d_c} \quad [1]$$

In this equation, L is the column length in centimeters and d_c is the column inner diameter, also expressed in centimeters. For example, a 25 m × 0.25 mm column should produce approximately 110,000 theoretical plates in the best case for well-retained peaks. The maximum plate number increases by roughly 25% for earlier-eluted peaks with retention factors as low as 3. I don't advise using peaks at retention factors less than 3 because the theoretical plate number tends to exaggerate column performance for weakly retained peaks.

If the column contains a significantly thick stationary-phase film, then the maximum theoretical plate number will decline relative to the number calculated in equation 1. This effect is attributable to resistance to solute mass transfer into and out of the stationary phase as well as to solute diffusion in the stationary phase, both of which cause peak profiles to broaden in addition to the gas-phase-only effects that are implied in equation 1. A film thickness of less than 0.25 µm generally will not contribute significantly to peak broadening. As stationary-phase film thicknesses approach 1 µm or 5 µm, then theoretical plate number losses of 50% or more relative to thin-film columns can result. This effect is most notable at retention factors ranging from 1 to 10; losses are lower at higher retention factors.

Determine the number of theoretical plates experienced by several peaks of interest on their way through a column by measuring the retention time (t_R) and width at half-height (w_h) under isothermal conditions. Calculate the measured theoretical plate number (N_{meas}) from equation 2:

$$N_{meas} = 5.545\left(\frac{t_R}{w_h}\right)^2 \quad [2]$$

Operators can compare measured theoretical plate numbers with maximum theoretical plate numbers to obtain a good indication of how well GC systems and columns are performing. If a thin-film column doesn't come close to its potential after initial installation in a split–splitless inlet system, first check the carrier-gas linear velocity and then look to see how the

first chromatograms compare with the manufacturer's test chromatogram. The average linear velocity should fall between 30 and 50 cm/s. Higher values in this range are more suitable for hydrogen carrier gas, and lower values are better for helium carrier gas. Avoid nitrogen carrier gas if possible, but if it is necessary, then carrier-gas velocities of approximately 25–30 cm/s are suitable. If the results still don't match expectations, then check that the split flow rate is 50 mL/min or greater, the injection volume is 1 μL or less, and the column entrance is unobstructed and correctly positioned in the inlet. Try to duplicate the manufacturer's test conditions as closely as possible and use the same or similar substances for this test.

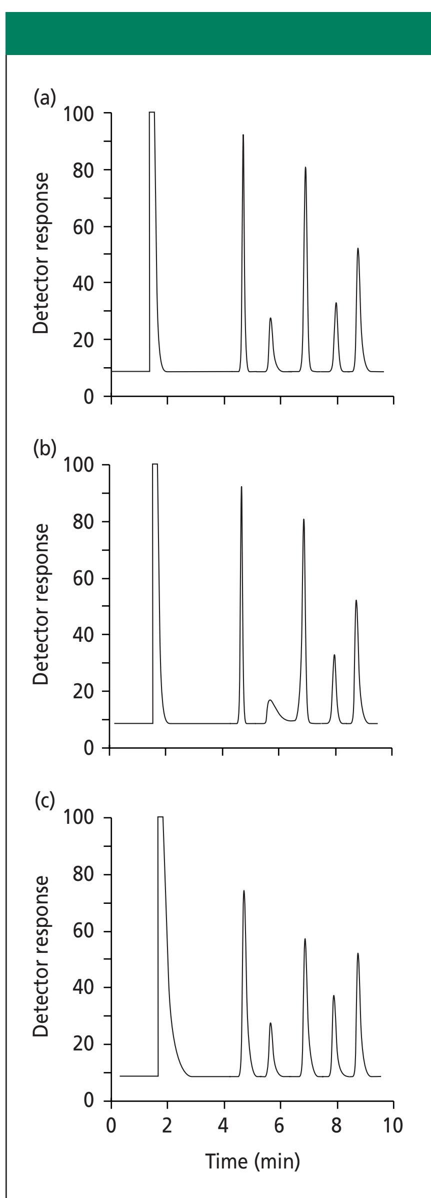

Figure 2: Examples of peak tailing in which (a) two polar peaks tail, (b) one polar peak tails excessively, and (c) all peaks tail.

The degree of additional peak broadening caused by thicker stationary phases depends upon the column temperature and the nature of the solute, so the best course with these columns is to determine theoretical plate numbers for several critical peaks immediately after column installation and to periodically check column performance against its initial measured theoretical plate level. A thick-film column can deliver significantly different performance than that demonstrated in the manufacturer's chromatogram if conditions are slightly different, so it's best to use the provided test chromatogram as a guideline and not a fixed specification.

Chromatographers may observe a gradual loss in measured theoretical plate numbers with normal column use. The net effect on peak resolution is related to the square root of the theoretical plate number, so a drop to 75% of the initial theoretical plate number — from 100,000 to 75,000 plates, for example — will reduce resolution to roughly 87% of its initial level. The accumulation of nonvolatile residue at the column head and, sometimes, the disruption of the stationary-phase film by chemical attack cause theoretical plate losses. Rinsing a column and removing the first meter of its length may help to recover theoretical plates and reduce bleed levels.

Polarity shifts: Chromatographers shouldn't rely solely on plate number measurements for column evaluation. The real objective is peak resolution, which depends not only upon the theoretical plate count but also upon peak retention and separation. Column temperature and stationary-phase polarity, in relation to the peaks of interest, both affect peak separation and resolution in ways that can be complex. As column temperatures or temperature programs change, some peaks may be eluted closer together and some may leave the column farther apart. To complicate this situation further, changes in the carrier-gas flow have a similar effect in temperature-programmed GC runs. For example, switching from constant-pressure to constant-flow operation with electronic pressure control can cause closely eluted peaks to merge or to separate better.

Other effects can occur during a column's lifetime. Chemical damage and the deposition of nonvolatile materials at a column's head can cause shifts in column polarity for both isothermal and programmed operation. Stationary-phase losses caused by overheating or by chemical damage will reduce column retentiveness, which can cause relative shifts in temperature-programmed retention times and retention-time reduction for isothermal analyses. Even the loss of column length over the course of multiple column installations — if greater than roughly 10% — can cause a noticeable shift in apparent column polarity with temperature programming.

Polarity shifts that better separate peaks are of little concern, but when closely eluted peaks move even closer together their resolution can be compromised. Chromatographers can largely prevent these shifts by controlling the oxygen level of the carrier gas with appropriate filtration and stringent leak checking, by sticking to recommended maximum temperatures, and by avoiding chemical stress in the form of reactive species, dirty samples, and uncontrolled solvent incursion into the column.

The recovery of much of the original column polarity is possible when residue accumulation at the column head causes polarity shifts. Rinsing the column in a reverse direction often will remove the guilty contaminants. Small shifts caused by dimensional or pneumatic changes are reversible by modifying the temperature program accordingly. A free calculator for this purpose is available on Agilent Technologies' web site (http://www.chem.agilent.com/cag/servsup/usersoft/main.html). However, if polarity shifts are the result of column overheating or stationary-phase degradation from oxygen exposure or solvent flooding throughout the length of the column, then performance recovery may be impossible.

Peak tailing: Tailing peaks can provide an early indication of a potentially serious problem. The resolution from adjacent peaks is degraded as trailing peak edges broaden, and the overall effect is similar to a loss of theoretical plates. A small degree of peak tailing may be normal for more-polar solutes, but watch for tailing that increases following multiple GC runs. Figure 2 shows three grades of peak tailing. In Figure 2a, only two of the peaks tail noticeably. This appearance is typical of minor peak tailing for polar components. The slight shape distortion doesn't affect resolution with adjacent peaks, and accurate peak area integration still is possible. Figure 2b shows more serious tailing of a polar component, so much that resolution and area counts definitely are affected. For some components — notably free acids and bases — it may be difficult to obtain

perfectly symmetrical peaks in any case. Here again, evaluate newly installed columns for tailing and record a good example chromatogram for later reference. Rinsing the column with solvent or removing the first meter at the column inlet may aid recovery from tailing polar peak shapes.

Poor column installation, inlets or detectors that are unsuitable for capillary columns, or incorrect flow settings also can cause noticeable peak tailing. The difference in this case is the appearance of a tailing peak profile across all the peaks in the chromatogram instead of for only the polar components. Figure 2c illustrates this situation. When all peaks tail, examine the column entrance for small particles and be sure that the split flow is at least 50 mL/min. Check the column outlet, too, and set the detector makeup gas flow appropriately, if required.

Summary

Chromatographers should expect the best possible performance from their columns. After an initial evaluation, document the test chromatograms and measurements that demonstrate the performance level after column installation. By comparing subsequent test chromatograms with these initial results, chromatographers can monitor and diagnose column problems as they occur. Recovery may be possible when column bleed, loss of measured theoretical plates, shifts in polarity, or peak tailing become significant problems. Evaluate the column installation. Check for contaminated carrier gas, leaks, and a dirty inlet system. Be sure that the sample is free of significant nonvolatile residues and chemically reactive materials. If column bleed is unacceptable, then try several column bakeouts.

For other problems, baking the column may only force contaminants deeper into the column. Instead, remove 1 m or so of length from the inlet end and reevaluate. If the problem persists, try rinsing the column from the detector end to the inlet end with an appropriate solvent. If all of the above solutions fail to restore column performance to an acceptable level, then it's time to get a new column and improve the operating environment to minimize the chance of these problems recurring.

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For an ongoing discussion of GC issues with John Hinshaw and other chromatographers, visit the Chromatography Forum discussion group at http://www.chromforum.com.