Non-linearity in a Chromatographic System

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Obtaining accurate peak areas and correctly identifying the peaks of interest require attention for the best results. Chromatographic speed, peak shapes and baseline profiles affect how well a data-handling system can measure and identify analytes. The relationships between injected analyte quantities and measured peak areas cannot be expected to be linear, especially for very small or very large peaks.

This month in "GC Connections," John Hinshaw discusses strategies that help minimize these non-linearities in a gas chromatography system.

In last month's "GC Connections" I discussed chromatographic peak integration as applied to the unique challenges of fast chromatography.[1] By examining two partially merged peaks at different concentrations, I concluded that successful peak detection and identification do not necessarily guarantee peak integration results that accurately reflect the amounts of analytes present in a sample. A number of effects, especially at lower solute levels, combine to produce non-linear relationships between the apparent and actual concentrations of analytes as they traverse a chromatographic system through injection, separation, detection, identification and quantification. For the purposes of this discussion, I will ignore sample acquisition and preparation, although they, too, can skew apparent chromatographic results.

When examined across an extended range of amounts or concentrations, integrated peak areas are not necessarily linear with respect to known solute amounts. Linearity, if it is expected at all, might appear only in a limited range, within which increasing solute amounts produce proportionately higher area counts. Extrapolating outside this range will simply produce erroneous results without additional measurements to confirm an extended linear operating range. At solute levels that approach the minimum detectable quantity of a chromatographic system, the uncertainty of the level of results can become as large as the result values themselves.

Fortunately for practising chromatographers, several strategies can help bring these effects under some control. Chromatographers should always take steps to ensure the most inert and discrimination-free chromatographic system. Although data-handling systems can provide ways to compensate for non-linear chromatographic behaviour, laboratories should not rely solely on a data system to remedy obvious chromatographic problems; instead they should view data systems as tools that can compensate for unavoidable system effects.

Sources of Non-linearity

Non-linear behaviour only becomes evident after quantifying a series of separations across a range of solute amounts. Chromatographers often observe some evidence of non-linearity as tailing or lost peaks at low levels, but quantitative measurements are necessary to ascertain the severity of the non-linear effects. Although non-linearity could be observed at the detector and data-handling end of a separation, any of the upstream components that engage the sample could be a problem source.

Inlet system: Gas chromatography (GC) inlet systems are a well-recognized source of non-linearity. Ideally, an inlet transfers a representative sample into a column; it neither adds nor subtracts components, partially or completely. In reality, however, inlet systems often modify relative solute amounts, so their ratios as they enter the column are different from those in the sample itself. These effects are most pronounced at lower solute levels and larger amounts tend to mask them. Some techniques — conventional splitless and on-column injection in particular — are prone to side effects caused by an injection that is too large, and chromatographers occasionally observe non-linearity with larger injection volumes, even at higher solute levels. Several of these effects, taken separately or in combination, engender less-than-ideal injection. Solutions to these problems involve keeping inlet systems clean and operating them within normal temperature and flow constraints. Sometimes, switching to an alternative injection system will cure a problem. Table 1 summarizes some of the major injection artefacts and suggests common remedies.

Column effects: A column can produce effects similar to those of the inlet, and sometimes it's hard to tell the difference, especially when decomposition or adsorption are involved. When in doubt, it's good practice to switch to a different but verifiably good column with the same specifications to discern if the column is the problem source. In some instances, solute amounts might be so low or solutes might be so sensitive that chromatographers can't find a suitable column. Increasing the injected solute level by lowering the split ratio or switching to splitless or on-column injection could be a viable solution.

The narrow-bore, thin-film columns used for fast GC can accentuate adsorption and decomposition effects. These columns don't tolerate large solute amounts as well

Table 1: Major sources of chromatographic system non-linearity.

Effect	Symptoms	Remedy
Adsorption in inlet or column	Peak tailing; area losses at low levels	In the inlet, use a deactivated inlet liner and quartz wool; clean the inlet system; derivatize the polar solutes; and switch to cold on-column or temperature-programmed split–splitless injection. In the column, reinstall the column and make a clean column cut; remove the first 1 m portion; and bake out or rinse the column.
Mass discrimination in inlet	Enhanced or depleted peak areas that correlate with solute molecular weight	Use the fastest autosampler speed; check glass wool in the liner; check the column position in the inlet; inject less sample; increase the inlet temperature; maintain a split flow-rate greater than 40 mL/min; and switch to programmed-temperature split–splitless or on-column injection.
Thermal or catalytic decomposition in inlet or column	Peak tailing; area losses; appearance of extraneous early eluted peaks; unusual rising baseline profile	Check carrier-gas pneumatics for leaks that allow oxygen incursion and maintain carrier-gas oxygen filters. In the inlet, clean the inlet; install a deactivated liner and quartz wool; use the fastest autosampler speed; reduce the inlet temperature; and derivatize polar solutes. In the column, reinstall the column and make a clean column cut; remove first 1 m portion; bake out or rinse column; swap it with a verifiably good column; increase the linear velocity; reduce the temperature; and derivatize polar solutes.
Non-linear detector response	Response falls off at high levels or is flat at low levels	See "Adsorption" and "Thermal or catalytic decomposition" listings above. In the data system, verify the data-handling method parameters. In the detector, check the detector gas, temperature and electronic settings, and leak-check the detector. In the inlet, adjust the split ratio to bring injected amounts to within detector linear range; switch to splitless or on-column injection for low-level samples; and use large-volume injection techniques for sample volumes greater than 2 mL.

as wider bore or thicker film columns do; the threshold of peak shape distortion is lower as solute amounts increase. One study determined a sample capacity — defined as the amount of solute above which at least a 20% theoretical plate loss occurs — of approximately 115 ng of *n*-undecane for a 0.26 µm d_f, 0.32 mm i.d. methylsilicone column, which fell to roughly 53 ng on a 0.25 µm d_f, 0.25 mm i.d. column and to only approximately 1 ng on a 0.1 µm d_f, 0.10 mm i.d. column.[2] Table 2 summarizes these results. These columns are more prone to exhibit adsorption and decomposition at low solute levels because of the increased exposure of the columns' inner walls because of the thinner stationary-phase films. Thus, both the upper and lower ends of the useful solute amount range are restricted in comparison with more-conventional columns. Table 1 also lists some of the major column contributions to non-linearity.

Table 2: Measured sample capacities for several columns.[2]

Column Inner Diameter (mm)	Film Thickness (µm)	Sample Capacity (ng)	Effective System Dynamic Range†
0.10	0.25	0.9	20:1
0.25	0.25	52.9	1000:1
0.32	1.00	114.5	2200:1

* The solute amounts were those necessary to cause a 20% reduction in theoretical plate number. These data are for a *n*-undecane solute and a 100% methylsilicone stationary phase. The column length was 25 m and the temperature was 110 °C.
† With a method detection limit of 50 pg.

Detector response: Aside from easily fixed side effects caused by incorrect temperatures and flows, detectors inherently have a limited range of response to changing solute amounts. Changing the solute amount produces less of a change in area counts at the low and high ends of a detector's working range than it does in the middle of the range.

Figure 1 illustrates this effect for a hypothetical detector in a log–log plot of area counts as a function of solute amount. At low and at high solute levels, the area counts don't change very much with changing solute amounts, but the middle of the range exhibits an approximately linear relationship between them. The minimum detectable amount — the solute amount sufficient to produce a response of two times the noise level[3] — lies at the bottom end of the detector's dynamic range. In this instance, the minimum detectable amount is approximately 10 pg. The upper limit of this detector's dynamic range — beyond which increasing solute levels yielded no further increase in signal — was at approximately 1 µg, so the dynamic range spans roughly five orders of magnitude.

A detector's linear range is defined as the working range across which the detector response factors, in reaction to changing solute amounts, remain constant within a ±5% band.[3] The linear range is visualized more easily by plotting detector response factors — that is, the area counts divided by the solute amount — as a function of the solute amount. Figure 2(a) shows a log–linear plot of response factors and the boundary lines that represent the ±5%

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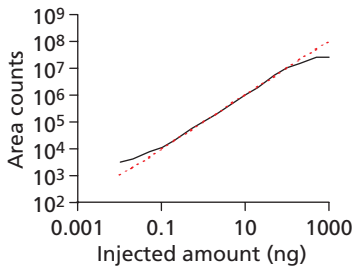
linear response factor band. In this instance, the linear range runs from approximately 0.1 to 100 ng and spans three decades.

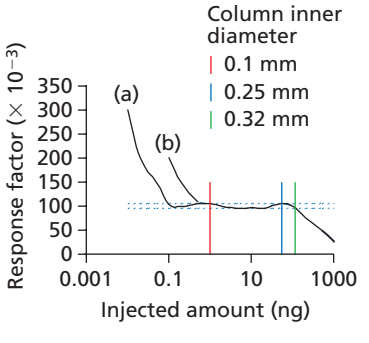
Instrument manufacturers evaluate a detector's dynamic range and linearity in the absence of inlet and column effects; for example, by using an exponential dilution system tied directly to the detector. In the real world, however, column and inlet effects combine at lower levels to produce an apparently higher minimum detectable amount than the manufacturers' specifications and thereby reduce the usable dynamic range. For example, the complete adsorption of some polar solutes on a column or inlet can occur at levels less than 50 pg. Even though the detector's minimum detectable amount is five times less than that value, no response can be measured at solute levels less than 50 pg because nothing reaches the detector. This level sometimes is called the method detection limit to differentiate it from the minimum detectable amount. At solute amounts greater than 50 pg, some of the solute molecules still don't reach the detector. At 100 pg, this loss might represent 50% of the injected solute, and the losses at 1 ng would amount only to approximately 5%. At even higher levels, the losses can be ignored with little consequence. As a result, the system's apparent linear range becomes truncated at the low end, as Figure 2(b) shows. At this point, the chromatographic system falls outside of the ±5% linear response factor band at 1 ng and less, and the effective linear range is reduced by an order of magnitude, even though the detector alone is capable of much lower minimum response levels.

Figure 1: Log–log plot of area counts as a function of injected amount. The red dashed line represents an ideal linear response.



The limitation on higher solute levels imposed by capillary columns' sample capacities constrains the upper end of the solute amounts that a chromatographic system can handle and still deliver the highest possible column efficiency. Figure 2 shows this cap on solute amounts for the 0.32, 0.25 and 0.1 mm i.d. columns mentioned above, which are represented as three vertical lines labelled with the columns' internal diameters. This added restriction is very significant for the 0.1 mm i.d. fast GC column because it reduces the effective system dynamic range from approximately 20 000:1, with the detector's upper working limit of 1 µg, to 20:1, with the 1 ng upper limit imposed by the column's thin film and narrow internal diameter. The system dynamic range could be even smaller if adsorption is significant at greater than the 50 ng level, which is easily possible for a column with such a thin stationary-phase film. The conventional larger internal-diameter columns impose less extreme limits, but they are still significant relative to the full dynamic range of the detector alone. Of course, chromatographers should accommodate these restrictions when specifying an analytical method and the equipment to be used with it so they can obtain the best results.

Figure 2: Log–linear plot of response factor as a function of injected amount. The red dashed lines show a ±5% response linear dynamic range. (a) Detector response in the absence of column or inlet effects. (b) Apparent detector response with a 50 pg solute loss in the column and inlet. The vertical lines show restrictions imposed by limited column sample capacities.



Data handling: To add insult to injury, after transiting the inlet, column and detector, solute peaks are subject to many artefacts introduced by the peak-detection, integration and quantification processes that occur in a data-handling system. Fortunately, modern data systems include some processes that help alleviate non-linear chromatographic behaviour. I'll delve into this area in a future "GC Connections" column.

Conclusion

A chromatographic system comprises several well-defined processes in series. The inlet, column, detector and data-handling components can contribute to non-ideal behaviours such as adsorption, decomposition and an overall non-linear relationship between the injected and measured solute amounts. By taking steps to minimize the contributions of each component to such problems and by understanding the restrictions imposed by the interactions of each component with the others, chromatographers can reduce quantitative errors and obtain more-defensible results. Common-sense procedures such as keeping the inlet and column free of contamination; ensuring high-purity carrier gas; operating the inlet, column and detector within their known dynamic ranges; and choosing optimum temperatures and flows can greatly improve the reliability and quality of results from a chromatographic system.

References

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