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

Ghost and Vacancy Peaks

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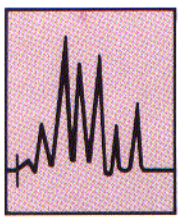

One of the more confusing occurrences in liquid chromatographic (LC) separations is the appearance of artifactual peaks in a chromatogram. These are unexpected peaks that are consistent from run to run. Artifactual peaks giving a positive baseline disturbance are called "ghost" peaks; negative peaks are referred to as "vacancy" peaks. In this month's installment of "LC Troubleshooting," we look at some of the factors that cause these peaks and how to avoid them. Although in some cases it is not possible to eliminate artifactual peaks, a better understanding of their causes will help you make a valid interpretation of the chromatographic data.

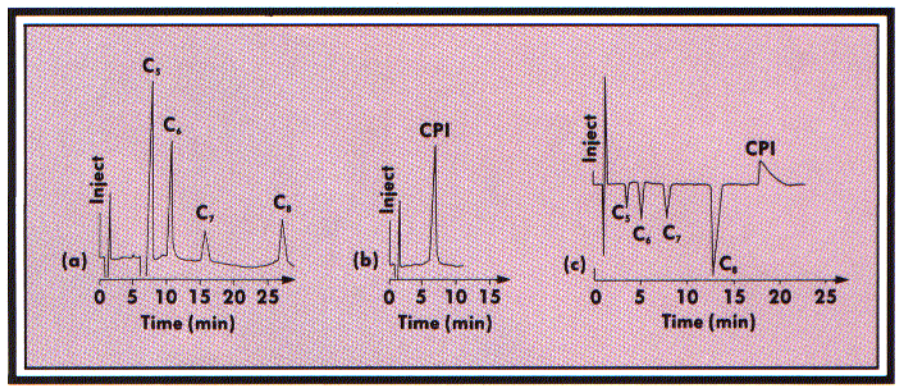

FIGURE 1: Artifactual peaks in ion-pair chromatography. Mobile phase contains a UV-absorbing ion-pair reagent (cetyl pyridinium chloride, CPI); sample is mixture of C_5–C_8 alkyl sulfonates (non–UV-absorbing). (a) Injection of alkyl sulfonates; buffer concentration 0.01 m*M*. (b) Injection of CPI; buffer concentration 0.01 m*M*. (c) Injection of alkyl sulfonates; buffer concentration 10 m*M*. (Reprinted with permission from reference 1.)

ARTIFACTUAL PEAKS

An example of ghost and vacancy peaks is shown in Figure 1a. Here, a sample of four alkyl sulfonates is injected under ion-pairing conditions. Detection is at 254 nm, where the sample compounds do not absorb; thus, a flat baseline (no sample peaks) would be expected. But instead we see one negative peak at 6 min and four normal bands at 7–27 min. Because no peaks should have appeared in the chromatogram, we refer to these anomalous bands as artifactual peaks. In this particular case, the workers intentionally created ghost peaks, but a similar situation could arise unintentionally.

WHAT ARE GHOST AND VACANCY PEAKS?

Sometimes artifactual peaks are produced to detect bands that otherwise would not be seen (as in Figure 1a). In other cases, however, artifactual peaks can cause problems in interpretation and calculations based on the chromatogram.

In the simplest case, we might have a mobile phase that absorbs light at the wavelength used for detection. Injecting a solvent that does not absorb at this wavelength creates a local condition in which the mobile phase absorbance is lower than in the surrounding mobile phase. This can be thought of as a "hole" of lower UV absorbance. Because the injected solvent is unretained, this hole then moves through the column at the same rate as the mobile phase and gives a negative peak at t_0. This negative "solvent peak" is commonly observed in LC.

In a slightly more complicated case, the mobile phase might have a UV-absorbing component (or impurity), M, that has a retention time of t_M. If a solution of M is injected as the sample, a positive peak at t_M min will be observed. If, instead, pure solvent is injected, a hole will again be created in the mobile phase, but now the hole moves through the column at the same speed as the compound M, so a negative peak appears at t_M min. If that is difficult to visualize, think of it as a negative injection of M. That is, whether we add extra M by injection or remove M by injecting a pure solvent, the resulting positive or negative peak will elute at the same time, t_M.

The situation is further complicated if the mobile phase component M interacts with some non–UV-absorbing compound S in the sample. Suppose first that S and M interact to form a complex, S–M. If S is injected onto the column, the complex S–M is formed, depleting the mobile phase of M. The resulting hole now moves through the column, as discussed above, appearing as a negative band at t_M min. Likewise, the complex S–M will have a retention time t_S, and the complex will elute at t_S min as a positive band because an excess of UV-absorbing M is associated with molecules of S as they leave the column (although S alone does not absorb light). In this way we see that both ghost peaks (for S–M) and vacancy peaks (for M) can appear in the same chromatogram.

SOME EXAMPLES OF GHOST AND VACANCY PEAKS

Figure 1 illustrates the examples that were described above. The mobile phase contained a UV-absorbing ion-pairing agent, cetyl pyridinium chloride (CPI). When an alkyl sulfonate $R\text{–}SO_3^-$ is injected onto the column, the following equilibrium can be hypothesized:

$$R\text{–}SO_3^- + CPI^+ \rightleftharpoons (R\text{–}SO_3^-\ CPI^+) \quad [1]$$

where molecules of $R\text{–}SO_3^-$ and CPI^+ in the mobile phase combine to form an ion pair ($R\text{–}SO_3^-CPI^+$) that is retained by the stationary phase. The presence of UV-absorbing CPI in the mobile phase leads to a higher baseline; injecting an alkyl sulfonate sample removes some CPI from the mobile phase, decreasing its absorbance so that a lower baseline is observed. The decrease in concentration of CPI that occurs when a CPI-free sample is injected causes the same reaction as an injection of CPI: The CPI hole moves through the column as a negative peak, appearing in the chromatogram at the same retention time as an injection of CPI. You can see that this is what has happened in Figure

1a, where the CPI vacancy peak at about 6 min corresponds with the elution of an injection of CPI as a sample in Figure 1b. This is analogous to the second example (compound M) in the preceding section.

The CPI that was removed from the mobile phase by reaction 1 then moves through the column in association with each alkyl sulfonate injected. When these non–UV-absorbing compounds leave the column, they carry with them the associated CPI molecules, which yield UV absorbance and a positive peak for each alkyl sulfonate (the four peaks in Figure 1a).

MORE CONFUSION

In the above example, the UV-absorbing mobile phase component (CPI) eluted before the sample components. When the CPI elutes after the sample peaks, the chromatogram looks like the one in Figure 1c. Here, the sample peaks are negative and the CPI peak is positive. So, the appearance of the chromatogram depends on the relative retention of the sample and impurity peaks.

Similar (unintended) effects in a routine LC procedure can result in artifactual peaks that can confuse and complicate the chromatogram. While we normally avoid adding UV-absorbing components to the mobile phase, they might be present as impurities in the various mobile phase components, such as solvents, buffers, ion-pairing reagents, and modifiers to reduce tailing. In fact, artifactual peaks often are caused by impure reagents used to formulate the mobile phase. Artifactual peaks also require components in the sample that either promote or hinder the retention of the UV-absorbing mobile phase impurities (cooperative or competitive sorption). These latter sample components often are impurities or components of the solvent used to dissolve the sample. This means that large volumes of injected sample generally lead to more pronounced artifactual peaks. It is good practice, therefore, to inject a smaller volume of a more concentrated sample solution whenever artifactual peaks are observed or expected (as in ion-pair chromatography).

THE MODEL OF STRANAHAN AND DEMING

An excellent general discussion of these artifactual peaks has been given by Stranahan and Deming (2). We will review this treatment briefly to illustrate the principles of artifactual peaks. Two types of sample–mobile phase (X–M) interaction are possible: cooperative sorption (as in equation 1):

$$X_m + M_m \rightleftharpoons XM_s \qquad [2]$$

and competitive sorption:

$$X_m + M_s \rightleftharpoons X_s + M_m \qquad [3]$$

Here, the subscripts m and s refer to molecules in the mobile phase or stationary phase, respectively. We will assume that the sample molecule X is undetectable (non–UV-absorbing) and that the mobile phase component M is detectable, as is the case in Figure 1. Often, M will be an impurity in the mobile phase; X can be either an actual component of the sample or a component or impurity in the solvent used to dissolve the sample. Stranahan and Deming (2) modeled the cases of equations 2 and 3 by computer simulation; their results are summarized in Figure 2. Figures 2a–c illustrate cooperative sorption (equation 2), with three different possibilities for the relative retention times of X (t_X) and M (t_M) indicated at the top of Figure 2. Consider the case in which the mobile phase component leaves the column first ($t_M < t_X$, in Figure 2c). This is the same situation as in Figure 1a: cooperative sorption of M and X by ion pairing, with earlier elution of M than of different sample compounds X (sulfonates in Figure 1). In Figure 2c (or Figure 1a) we see that M leaves the column as an initial negative band, followed by a positive band for X. If, however, the retention times for M and X are reversed ($t_M > t_X$, as in Figure 2a), X leaves the column first as a negative band, followed later by elution of a positive band for M. This is seen in Figure 1c for the ion-pair separation of these same sulfonates under different conditions, such that CPI leaves the column *after* the sample ions X ($t_M > t_X$). Now the sample bands appear as initial negative peaks, followed by a positive band for CPI.

The pattern of Figure 1 and Figures 2a–c normally will be observed for ion-pair chromatography, which can be regarded as a cooperative sorption process. In reversed-phase or normal-phase separations, retention is competitive rather than cooperative (equation 3). This results in a different pattern for artifactual peaks, as summarized in Figures 2d–f. Now, the first artifactual peak is positive, and the following peaks are negative.

REMEDIES FOR ARTIFACTUAL PEAKS

Once you suspect the presence of artifactual peaks, what can you do to minimize their interference with other (real) bands in the chromatogram? I recommend the following:

Avoid impure mobile phase reagents. In order to avoid artifactual peaks in ion-pairing, high-quality buffers and ion-pair reagents must be used to formulate the mobile phase. Alternatively, try different lots of these reagents to determine which combination of mobile phase reagents minimizes artifactual peaks when injections of the sample solvent are made with no sample dissolved.

Use the mobile phase as a sample solvent. You can avoid impurities in the sample solvent (which also can generate artifactual peaks) by using the mobile phase to dissolve the sample. The use of a sample solvent other than the mobile phase (particularly in ion-pair chromatography) can also cause artifactual peaks.

Inject a minimum volume of the sample solution. Because the size of artifactual peaks is often proportional to the volume of solvent injected, it is a good idea to minimize this volume. For ion-pair chromatography, keep the volume of injected sample solution to less than 50 μL.

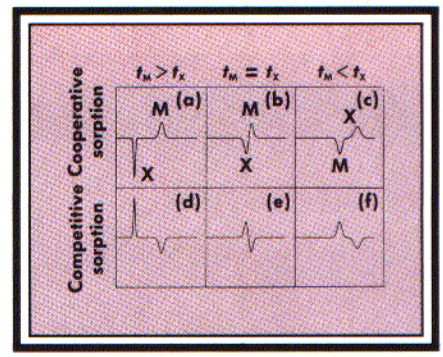

FIGURE 2. Computer simulations of artifactual peaks. (a–c) Cooperative sorption (equation 2); (d–f) competitive sorption (equation 3). (Reprinted with permission from reference 2.)

Pretreat the sample. When impurities or interferences in the sample matrix contribute to artifactual peaks, it is a good idea to remove these interferences.

When artifactual peaks cannot be removed using the above procedures, the problem can be treated in the same manner as for any other interfering peaks. Remember that artifactual peaks correspond (indirectly) to specific compounds and behave just like other bands in the chromatogram. Therefore, chromatographic conditions often can be varied so as to move these interfering artifactual peaks away from peaks of interest.

REFERENCES

(1) B.A. Bidlingmeyer and F.V. Warren, Jr., *Anal. Chem.* **54,** 2351 (1982).
(2) J.J. Stranahan and S.N. Deming, *Anal. Chem.* **54,** 1540 (1982).

"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc., of Lafayette, California, USA, and is a member of the Editorial Advisory Board of LC•GC.

Bulletins

Protein meeting. The Eighth International Symposium on HPLC of Proteins, Peptides, and Polynucleotides will be held at the Hotel Scandinavia in Copenhagen, Denmark, October 31–November 2, 1988. For abstract forms and registration information contact: The Eighth ISPPP, c/o DIS Congress Service, Linde Alle 48, DK-2720 Copenhagen, Denmark, telephone (1) 712244, telex 15476 dis dk.

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