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

Chromatographic Theory as a Problem-Isolation Aid: Part II

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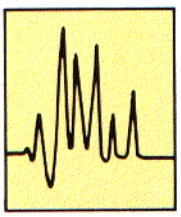

Last month's column described column plate number or efficiency (N), capacity factor (k'), and column selectivity (α) as tools for determining whether or not a chromatographic system is operating properly. This article focuses on the use of a fourth parameter, resolution (R_s), for the same purpose. Resolution is a combination of the other three parameters and enables quantification of the "goodness" of a separation. The quality of a separation can usually be determined visually; however, a quantitative measure of the separation is useful for comparative purposes.

DETERMINING RESOLUTION

There are three ways to determine the resolution of a separation. The first two methods are most useful when a quick determination of R_s is needed. The third method requires more calculation; however, close examination of the information it provides will help you improve a separation more readily than will the other two methods.

Many chromatographers need only to look at a chromatogram to determine if its resolution is adequate. Snyder and Kirkland have put together a set of model chromatograms to enable rapid, semiquantitative estimation of R_s (1). To demonstrate this method, consider Figure 1c to be a chromatogram that you have generated. To determine its peak resolution quickly, scan through model chromatograms until you find two that resemble yours (for example, Figures 1a and 1b). Use the relative peak heights and the depth of the valleys between them to select the model chromatograms. You can see that the valley between the peaks in Figure 1c is about midway in depth between those in Figures 1a and 1b. You can therefore estimate that the resolution of the peaks in Figure 1a is about 1.0. This quick and effective method for estimating resolution is explained in more detail in reference 1, which also contains several pages of model chromatograms.

SELECTIVITY NOT ENOUGH

In last month's column, the use of column selectivity (α) as a means of measuring the separation of two peaks was discussed. Inspection of Figures 1c and 1d, however, reveals that determining selectivity alone is not sufficient for judging the quality of a separation: band width is also an important factor. In this example, the retention times, capacity factors, and column selectivity values of the peak pairs in Figures 1c and 1d are identical, yet 1c is clearly the superior separation. The only difference between these two chromatograms is that the column efficiency (N) in Figure 1d has dropped to 20% of the efficiency of the column used in 1c, perhaps as the result of a column void.

The second method that is used to determine resolution takes band width into account. The difference in retention times for two peaks is divided by the average band width:

$$R_s = \frac{t_2 - t_1}{0.5(w_1 + w_2)} \qquad [1]$$

where t_1 and t_2 are the retention times of peaks 1 and 2, and w_1 and w_2 are the corresponding peak widths at baseline (Figure 2). This method of determining resolution is simple, rapid, and quantitative. It is preferred over the visual estimation method if a more quantitative measure of resolution is desired. This method is difficult to apply, however, if resolution is below about 1, because peak width with this degree of resolution is hard to estimate.

COMBINING PLATE NUMBER, CAPACITY FACTOR, AND SELECTIVITY

The third method of determining resolution provides the most information about a separation. An understanding of the influence of N, k', and α on resolution can help you determine how to improve a separation during methods development or how to isolate the cause of degradation in a separation.

Column plate number, capacity factor, and selectivity are combined in Equation 2 to determine resolution:

$$R_s = 0.25\,(\sqrt{N})\left(\frac{k'}{1+k'}\right)(\alpha - 1) \qquad [2]$$

Equation 2 is more instructive than convenient; calculating R_s on a routine basis is faster and easier with the two methods described earlier. Each term in Equation 2 will be considered briefly with respect to its effect on a separation.

Column plate number (N) dramatically affects resolution in Figure 1; however, resolution varies as the square root of N. Thus, a fourfold increase in N is required to double resolution; or, from a more practical viewpoint, a column can lose three-quarters of its efficiency before resolution is cut in half. In Figures 1c and 1d, for example, there was a fivefold change in efficiency, but resolution changed by only a factor of 2.2.

When resolution is too low, increasing efficiency is the method of choice in two cases. First, if the initial separation conditions generate only a few thousand plates, such as when short (5 cm) or severely degraded columns are used, then increasing efficiency is easy. Remember: it takes a fourfold increase in efficiency to double resolution. This is a simple task if a 5-cm column is replaced with a 25-cm column. Similarly, a 15-cm column that has dropped in efficiency to 3000 plates can be replaced with a new 13,000-plate column. In other cases, increasing efficiency may not be the best choice for improving resolution.

The influence of the second element of Equation 2 on resolution is seen in Figure 3, where k' is plotted against R_s. Clearly, resolution increases rapidly for very low k' values; but if k' is greater than 10, changes in the capacity factor have very little effect on resolution. A useful rule of thumb, therefore, is that the value of k' should be between about 2 and 10 for optimum performance. Values of k' of less than 2 tend to result in poor resolution, whereas k' values of more than 10 just increase analysis time. From Equation 2 it can be seen that if the value of k' is below 2, an adjustment in experimental conditions should be made to

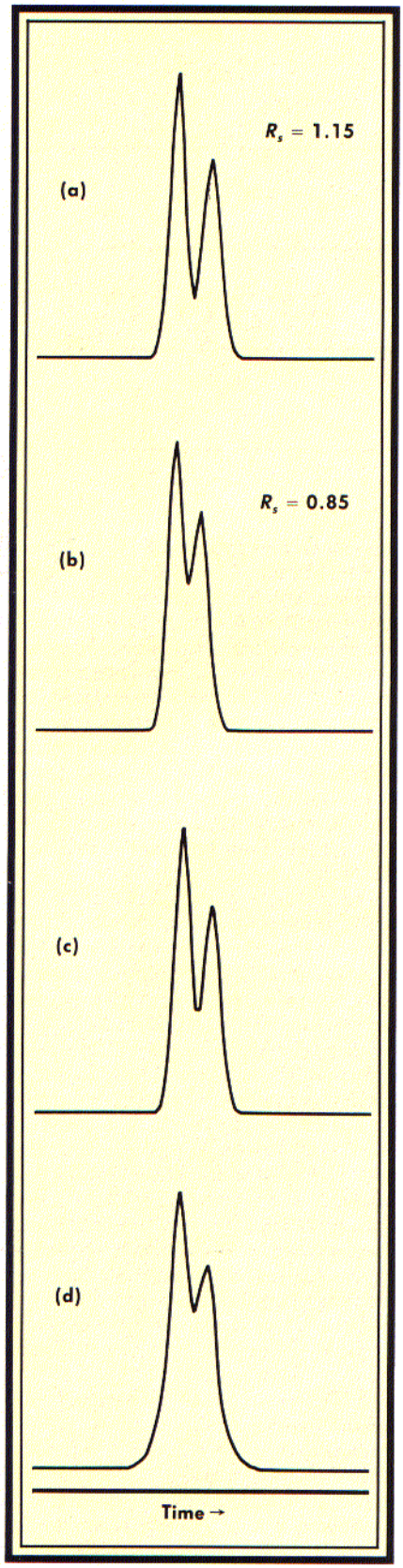

FIGURE 1: (a) and (b) Model chromatograms for estimation of R_s, (c) sample chromatogram, and (d) same conditions as (c) except for lower efficiency.

bring k' into the 2–10 range to improve resolution. Otherwise, changing the capacity factor is probably not the best method for improving a poor separation.

The final variable of Equation 2 is column selectivity (α), which has the greatest effect on resolution of the three parameters under discussion. Unfortunately, selectivity is the least understood parameter. The effect of selectivity on resolution is seen in Figure 1, where the value of α changes from 1.27 to 1.2 to 1.23 in the chromatograms of Figures 1a, 1b, and 1c, respectively, while the value of N remains constant. Efficiency can be changed in a predictable manner by changing column length; the capacity factor can be adjusted predictably by making the mobile phase stronger or weaker; selectivity, however, involves specific chemical interactions among solute molecules and the mobile and stationary phases. Changes in stationary-phase type or mobile-phase components can dramatically affect selectivity and thus resolution, but these effects are generally not predictable. For this reason, to improve resolution it is best to optimize efficiency and the capacity factor before attempting to change selectivity.

CONCLUSIONS

For most analyses, in order to facilitate quantitation or the collection of peaks of interest, it is desirable that the valley between two peaks return to the baseline. This corresponds to an R_s value of about 1.5 or more. Resolution can be estimated by using the visual method illustrated in Figure 1 or calculated by using the method illustrated in Figure 2. If resolution is inadequate, the k' value should be adjusted to fall within the 2–10 range by varying mobile-phase strength. Next, the column can be replaced if efficiency is too low. (Efficiency is usually addressed first if the separation quality has deteriorated from one that was previously acceptable.) Financial and system-pressure limitations generally restrict the maximum plate count to that generated by a 25-cm column packed with 3-μm or 5-μm particles. Finally, adjustments in column selectivity should be made to obtain the desired separation. Optimization techniques such as that provided by the Sentinel system (Du Pont Co., Wilmington, Delaware) can be helpful when it is necessary to change selectivity to improve a separation.

An understanding of the parameters that affect resolution is a valuable troubleshooting aid for separations that have deteriorated in quality and is useful in establishing new separation conditions.

REFERENCE

(1) L.R. Snyder and J.J. Kirkland, *Introduction to Modern Liquid Chromatography*, 2nd Ed. (Wiley-Interscience, New York, 1979).

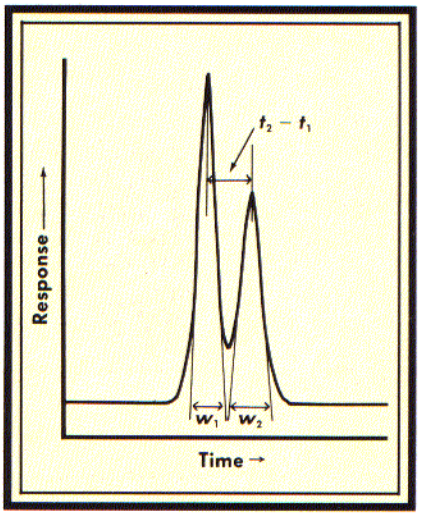

FIGURE 2: Illustration of method for calculating resolution.

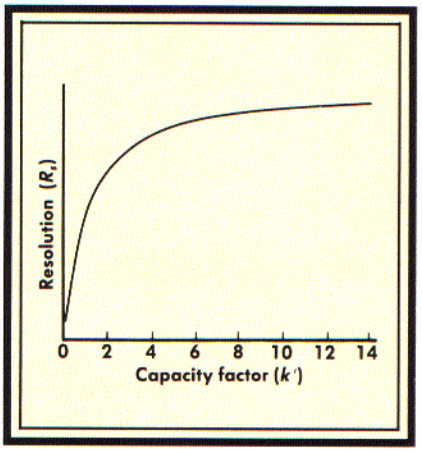

FIGURE 3: Relationship between capacity factor and resolution.

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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