LC TROUBLESHOOTING

How Does Temperature Affect Selectivity?

Is it more than meets the eye?

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LC Troubleshooting Editor

Column temperature plays an important role in controlling peak spacing (selectivity) in reversed-phase liquid chromatography (LC) separations. Temperature has long been known to affect retention time, and more recently, its use in adjusting selectivity has gained popularity (see reference 1 for a review of temperature selectivity). In preparation of a paper (2) for the most recent Pittsburgh Conference, I had an opportunity to reexamine some data that compare temperature selectivity with other variables used to control selectivity in LC separation. This month's installment of "LC Troubleshooting" examines temperature selectivity and its relationship to pH selectivity.

Selectivity and Temperature

One common way to plot changes in peak spacing with a change in a variable is to use a resolution map. This is illustrated in Figure 1a for the effect of temperature on resolution for a mixture of 11 weak acids and bases. The resolution map plots the resolution for the least-resolved pair of peaks in the separation for different column temperatures. The best resolution is seen for the high points on the plot, at approximately 34 °C and 41 °C, whereas two peaks overlap completely whenever the plot dips to the zero resolution baseline. The resolution at 34 °C is illustrated in Figure 2a, where all 11 peaks are resolved from each other. Moving to lower temperatures causes peaks 5 and 6 (4-*n*-butyl benzoic acid and 4-*n*-hexyl aniline) to move together; higher temperatures cause peaks 3 and 4 (4-*n*-pentyl aniline and diflunisal) to move together — 34 °C is the balance point where both peak pairs have the same resolution. In a similar manner, the resolution at 41 °C (Figure 3a) between peaks 6 and 7 (4-*n*-hexyl aniline and diclofenac acid) is balanced with that of peaks 1 and 2 (2-phenyl pyridine and ketoprofen).

Resolution maps are powerful tools for the quick identification of resolution maxima and can be useful to help estimate method robustness to changes in the variable under consideration. Such maps are key features of resolution modeling software, such as DryLab (Molnar Institute, Berlin, Germany). Resolution maps that have many peak reversals (dips to zero resolution), such as the one in Figure 1a, are more common with samples containing ionic compounds than for neutral compounds.

Selectivity and pH

When I examined the resolution map for temperature selectivity for the mixture of acids and bases used here, it struck me that it looked very much like resolution maps that I see for pH as the variable. So I plotted a map of resolution versus pH for the same sample, based upon the data of references 3 and 4. In Figure 1b, I have shown just a portion of the pH map normalized to approximately the same layout as the section of the temperature map of Figure 1a. They are strikingly similar. They have the same peaks forming the least resolved pairs in each region of the resolution map, and the overall maximum resolution is approximately the same in both cases. So just as we had maxima at 34 °C and 41 °C in Figure 1a, we have corresponding maxima at pH 2.785 and 2.910 in Figure 1b. Compare the chromatograms for these temperature and pH conditions in Fig-

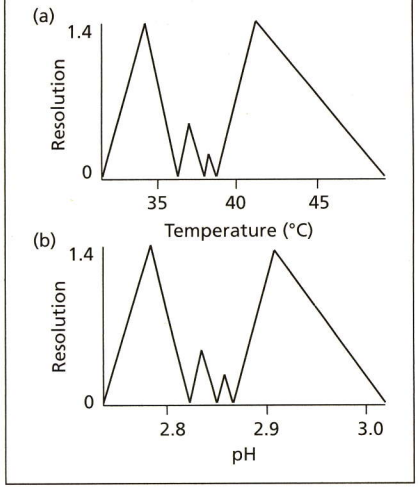

Figure 1: Resolution maps for sample of acids and bases: (a) resolution versus temperature; (b) resolution versus pH. Data of references 3 and 4.

ures 2a and 2b (34 °C versus pH 2.785) and 3a and 3b (41 °C versus pH 2.910). For each pair of chromatograms, the retention order is identical and the peak spacing is almost the same. Also note that the higher temperature of the 41 °C run reduced the retention times of all the peaks, as expected for an increase in temperature.

But Is It Real?

Resolution maps represent predicted separations based upon input data from two (or more) "calibration" runs. In the present case, the temperature map was based upon a run at 35 °C and one at 45 °C, with both runs at pH 2.80. The pH map was made at pH 2.80 and 3.00 for 35 °C. You might suspect that once one interpolates or extrapolates new separations at points other than the input data points, errors are likely. Yes, this is true, and the "garbage-in-garbage-out" axiom of computer programs applies here, as well. However, in my experience, if the data are gathered under carefully controlled conditions (these were), the predictive accuracy is in the ±1–5% region. The comparative results of Figures 2a, 2b, 3a, and 3b certainly support this. But these are predicted separations and you say, "Show me the beef" for real runs.

We can compare the results of real experiments by comparing the input

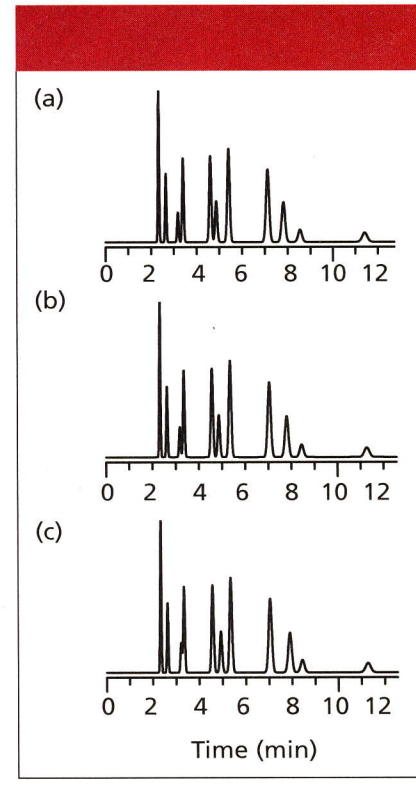

Figure 2: Simulated chromatograms from resolution maps of Figure 1. Reference conditions are a C18 column with a 50:50 acetonitrile–buffer mobile phase. (a) 34 °C and pH 2.80, (b) 35 °C and pH 2.785, (c) 35 °C and pH 2.80. Peaks (in retention order): 2-phenyl pyridine, ketoprofen, 4-*n*-pentyl aniline, diflunisal, 4-*n*-butyl benzoic acid, 4-*n*-hexyl aniline, diclofenac acid, 4-*n*-pentyl benzoic acid, 4-*n*-heptyl aniline, mefenamic acid, and 4-*n*-hexyl benzoic acid.

runs used to generate the resolution maps of Figure 1. In both cases, the input data were suboptimal on the right side of the two resolution maxima in Figures 1a and 1b. Both maps used a common point of 35 °C and pH 2.80, as shown in Figure 2c. A 45 °C, pH 2.80 run was used as a second point to generate the resolution map of Figure 1a. Similarly, a 35 °C, pH 3.00 run was used for the second point of the resolution map of Figure 1b. These two runs are shown in Figures 3c and 3d, respectively. They are not a perfect match. First, we notice that the last peak of Figure 3c is eluted at approximately 10 min, whereas the last peak of Figure 3d is closer to 12 min. Remember that the rule of thumb for a change in retention with temperature says that a 1 °C increase in temperature will decrease retention by approximately 2%. So a 10 °C increase in temperature reduced the retention time by approximately 20%, as expected. Second, note

A Request for Readers

Sticking pump check valves is a problem that many workers encounter when working with acetonitrile. As I have talked to instrument manufacturers, check-valve designers, and laboratory workers, I have discovered that the problem is widespread, not unique to one brand of equipment, and may have more than one solution. One problem I have in pinpointing the cause and solution is that the data are so diffuse. So, I would like to encourage you to share your experiences with me so that I can get some more data on the subject to share with the readership. If you have experience with check valves sticking with acetonitrile, please drop me an e-mail at John.Dolan@LCResources.com. Mark the subject line "check valves" so I can readily distinguish these responses. I will keep the sources anonymous and will not share brand names in the column I write, but I would like to know the brand and model of pump you use, the frequency of check-valve problems (in terms of hours of operation before failure), the mobile phase you use (including solvent brand), flow rate, and the way you solve the problem (replacement, sonication, washing, and so forth). When I have sufficient data, I'll share the trends in a future column. Thanks.

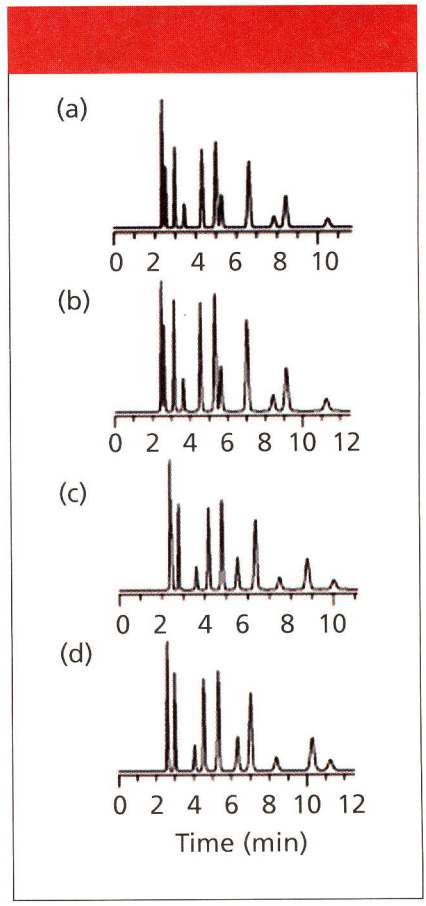

Figure 3: Simulated chromatograms from resolution maps of Figure 1: (a) 41 °C and pH 2.80, (b) 35 °C and pH 2.91, (c) 45 °C and pH 2.80, (d) 35 °C and pH 3.00. Same peaks as in Figure 2, except peak pairs 3 and 4, 6 and 7, and 9 and 10 have reversed retention order.

that the first two peaks in Figure 3c can be distinguished as a doublet, whereas they are merged into a single peak in Figure 3d. This agrees with the resolution map data, too — note that the 45 °C point in Figure 1a is about halfway up the side of the resolution plot with a maximum at 41 °C, whereas the pH 3.00 point is near the bottom of the corresponding plot of Figure 1b.

So What's Happening?

One more way to look at the data is shown in Figure 4. Figure 4a is the reference run at 35 °C and pH 2.80 (same as Figure 2c). When the temperature was increased to 45 °C, the run of Figure 4b resulted (same as Figure 3c). Note that the dotted lines indicate that there were three peak reversals that occurred with the temperature change. Figure 4c shows the results for a change in pH from 2.80 to 3.00, both at 35 °C (same as Figure 3d). The dotted lines show that the peak order for the 45 °C and pH 3.00 runs is identical. That is, the exact same crossovers occurred when going from 35 °C to 45 °C as when changing from pH 2.80 to pH 3.00.

These data strongly suggest that a change in column temperature has exactly the same effect as a change in pH (at least for this sample). If we think about this, though, it is not too surprising — we know that buffer pH changes with temperature, that sample ionization changes with temperature, and that the ionization of the silanols on the column changes with temperature. Some combination of these changes is likely in the present case.

However, these results are more than a novelty — they have very practical implications. Note that it takes a 10 °C change in temperature to make the same change as a 0.2-unit change in pH. If

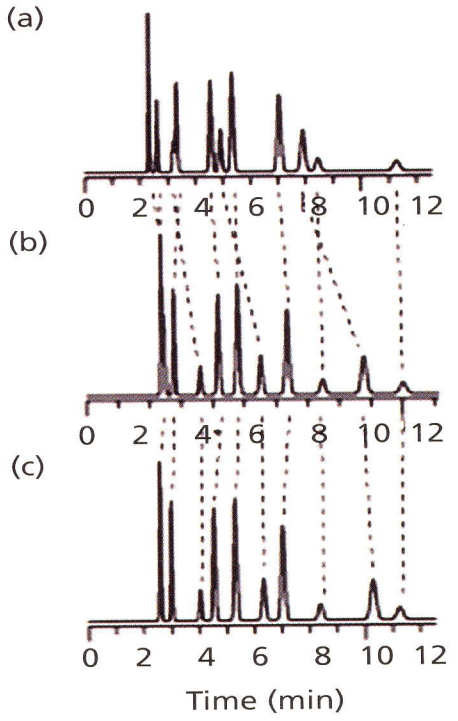

Figure 4: Simulated chromatograms: (a) reference conditions of 35 °C and pH 2.80 (same as Figure 2c), (b) 45 °C and pH 2.80 (same as Figure 3c), (c) 35 °C and pH 3.00 (same as Figure 3d). Dotted lines track peak positions between runs.

you use the pH adjustment technique of titrating the buffer to a desired pH with a pH meter, the normal variation of the actual pH is in the range of ±0.1 pH unit. It is much easier to control the temperature within a degree or two than it is to control the pH within <0.1 pH units. Furthermore, we can "tweak" the temperature a degree or two to adjust a separation much easier than adjusting the pH of the buffer.

What are the take-home lessons? First, temperature is a powerful variable to use to adjust selectivity when ionic compounds are present, so it should be considered when developing a method. Second, because temperature is such a powerful variable, it is very important to use temperature control for your column and to use a buffered mobile phase if you want to obtain reproducible separations with ionic compounds. Finally, a small 1–2 °C change in temperature might be sufficient to make minor adjustments to the method to enhance the separation of a pair of peaks when a small error in buffer preparation was made or column aging causes a reduction in resolution.

References

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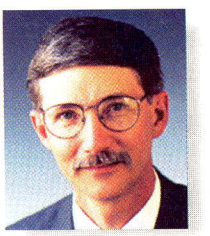

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