Troubleshooting

Mobile-Phase Buffers, Part II — Buffer Selection and Capacity

Guest Author
G. William Tindall

When is a buffer a buffer?

Last month's "LC Troubleshooting" reviewed the basic concepts of pH in aqueous and partially aqueous solutions, including the changes that occur when an organic solvent is added to an aqueous buffer (1). Rules predict these changes for concentrations of methanol or acetonitrile less than approximately 50%; neutral acids such as acetic acid and anionic acids such as dihydrogen phosphate get weaker following the addition of organic solvent, and cationic acids such as ammonium and protonated amines get stronger.

Surprises can occur in partially aqueous mobile phases when analysts fail to consider these changes, as illustrated in a publication about silica-column stability (2). Claessens and co-workers (2) prepared aqueous pH 10 phosphate and glycine buffers. They prepared the mobile phases by mixing equal amounts of these buffers with methanol and then they measured the stability of a silica column in those mobile phases. The authors were surprised to find that silica dissolved 10 times faster in the phosphate-buffered mobile phase than it did in the glycine-buffered mobile phase. Ever since this study, analysts have accepted that some property of the phosphate ion resulted in the aggressive attack on the silica.

However, the pK_a rules summarized in last month's column would have predicted that these results were caused by a pH shift. I prepared identical mobile phases and measured the pH of the water–methanol solution after mixing. After adding methanol, the aqueous pH 10 phosphate buffer became more *basic* by 0.7 pH units, and the glycine-buffered mobile phase became more *acidic* by 0.1 pH units. The 0.8 pH unit difference in these mobile phases is consistent with the differences in the observed dissolution rate.

Unexpected reversals in retention order also can occur when aqueous pH data for mobile phases or samples are extrapolated to partially aqueous mobile phases (3). Reversals could occur when the samples or buffers contain amines as well as neutral or anionic acids, in which case the pK_a rules will predict opposite retention time response to changes in organic modifier.

This month's "LC Troubleshooting" installment will cover the selection of buffers for use in partially aqueous mobile phases.

What is a Buffer?

A buffer is a solution that resists changes in pH when small increments of acid or base are added to it. Buffered mobile phases are used commonly in liquid chromatography (LC) when the sample contains acidic or basic components or when the column contains acidic or basic sites, as do ion-exchange columns, for example. The pH of the mobile phase will determine the degree of ionization of the sample or column, which in turn affects retention. For example, neutral compounds are more hydrophobic in reversed-phase LC separations and thus more retained than polar, ionized components. Peak shapes also can be affected by the degree of ionization of a column or sample. Therefore, often it is essential to buffer the mobile phase to control selectivity and to achieve reproducible separations with acceptable peak shape.

Buffer pH Selection

All that might be known about the pK_a of the sample and the buffer — and about the pH stability of the column and sample — at the beginning of method development is based upon pH and pK_a data in water. Commonly, this knowledge can be applied to the separation without considering the changes in pK_a and stability that can occur after adding an organic modifier.

The pK_a values of many potential buffer acids in methanol–water and, to a lesser extent, acetonitrile–water mixtures are available (3–5), but it is very unlikely that pK_a values for typical samples will be found. By considering the rules of how pK_a values of various acid types change after the

John W. Dolan
LC Troubleshooting Editor

addition of organic modifier, analysts can estimate relative changes for sample and buffer pK_a values and cautiously apply this information to buffer selection. Often the sample and buffer will be neutral acids, so the pK_a changes will be in the same direction and, with luck, of similar magnitude if they are similarly charged. Therefore, buffer selection can be less empirical than my discussion so far might have implied, at least for mobile phases that contain less than 50% organic modifier. Finally, pH measurements of relative acidity in similarly modified mobile phases are a reliable means to predict trends in retention and stability.

Strategy for Buffer pH Selection

In the simplest case of buffer selection, the buffer may be required only to keep acids protonated during the separation. Seldom is there a price to pay for buffering at too low a pH level, except that stationary-phase stability can be reduced at aqueous pH levels of pH 2 or lower. Most aliphatic and aromatic acids have aqueous pK_a values of 3 or more in water. At pH 2 these acids would be nearly fully protonated and therefore retained almost as much as possible. A buffer could be prepared from a mixture of phosphoric acid and dihydrogen phosphate, but as I will describe later, phosphoric acid alone will provide good buffering at pH 2 in water. If the separation is performed in a completely aqueous mobile phase, I have enough information to predict that a dilute solution of phosphoric acid probably will provide maximum retention and acceptable column life.

But suppose a methanol gradient is necessary. After methanol is added to the system, the pK_a of the sample and buffer will change by some unknown amount, as will the hydrogen ion activity of the mobile phase and possibly the column stability. In this example, both the buffer and sample pK_a values increase with increasing methanol concentration, according to the rule for neutral acids. Because the target pH is set generously low, it usually will not matter if moderate differences occur in how much the pK_a values change after adding methanol; the acids will remain protonated. Column stability will be no problem because the acidity of the modified mobile phase will decrease with increasing methanol concentration as a result of dilution and a decrease in phosphoric acid pK_a. For this simple case, the application of aqueous buffer and sample data usually leads to a successful separation. In fact, 0.002 M phosphoric acid buffer with a buffer capacity of only 0.001 commonly is used to separate a great many aliphatic (6) and aromatic acids (7) in methanol–water or acetonitrile–water mobile phases. This strategy can be extended to amines with acidic or basic buffered mobile phases when it might be desirable to separate either the protonated or free amine.

In some cases precise pH control is essential. If a pair of acids with different pK_a values will not separate when fully protonated at low pH, they could separate readily at some higher pH level at which one or both acids are partially dissociated. However, retention will be affected strongly by small changes in pH, because pH will have a large effect upon the relative amounts of dissociation. Precise pH control also is required for compounds that have both acid and basic functionality; for example, peptides. The charge on the molecule will depend upon pH, and precise charge control could be necessary for a separation. In these cases, it is very unlikely that data available for the compound in aqueous solution could be used successfully to predict an optimum buffer pH for a partially aqueous mobile phase, so an empirical optimization of the separation as a function of pH would be necessary.

Buffer Capacity

After selecting a target pH, the next step is to select a buffer that will have adequate buffering capacity to maintain the target pH. Buffer capacity — or buffer index (8) — is a quantitative measure of how well a buffer resists changes in pH when a small amount of acid or base is added. Buffer capacity is illustrated by the titration curve of a weak acid such as acetic acid with sodium hydroxide (Figure 1).

In this titration, the pH is recorded with the incremental addition of base. I think of the titration as a way to test the buffering capacity. At the beginning when acetic acid mainly is present, the pH rises quickly even as small increments of base are added, which demonstrates that acetic acid by itself is a poor buffer. In the mid-range when the ratio of acetic acid to acetate is between approximately 10:1 and 1:10, the pH changes more slowly as base is added incrementally. The buffer capacity therefore is large in this pH range, which illustrates the well-known point that mixtures of weak acids and their salts are good buffers. Near the end point of the titration when the solution mainly contains acetate, the pH rises rapidly, which indicates a small buffer capacity.

Well past the end point, as the concentration of hydroxide becomes significant, the pH again changes slowly. It often is unappreciated, but a strong base such as sodium hydroxide is an excellent buffer at pH levels higher than pH 12. Similarly, at pH levels lower than pH 2, a strong acid such as hydrochloric acid provides excellent buffer capacity.

In the following discussion, I will ignore the activity coefficients to simplify the presentation. Activity coefficients affect buffer capacity and pH, but ignoring them will not change the qualitative effects described below. A mathematical definition of buffer capacity (β) is

$$\beta = d(C_b)/d(\text{pH}) = 1/\text{titration curve slope} \quad [1]$$

where $d(C_b)$ represents a small addition of base and d(pH) is the resulting differential change in pH (9). This definition states that buffer capacity simply is the reciprocal of the slope of a titration curve.

If I write an equation for the concentration of species present during the titration of a weak acid (HA) and this equation is differentiated according to equation 1, then the resulting general equation for buffer capacity is derived as

$$\beta = 2.3[\text{H}] + 2.3[\text{OH}] + \frac{2.3K_{HA}C_{HA}[\text{H}]}{\left(K_{HA} + [\text{H}]\right)^2} \quad [2]$$

where [H] and [OH] are the concentrations of hydrogen ions and hydroxide in the solution, K_{HA} is the dissociation constant of the weak acid, and C_{HA} is the total concentration of the weak acid plus its salt (HA + A). Reference 9 and most advanced

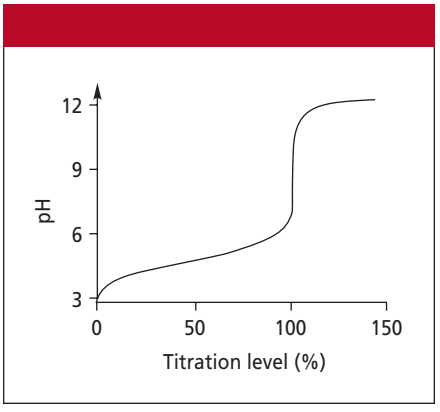

Figure 1: Titration of acetic acid with sodium hydroxide. Maximum buffering is obtained ± 1 pH unit from the pK_a; pH 4.8 ± 1 in this example, as well as pH 12 and greater.

texts dealing with buffers describe the derivation of this expression.

This equation reveals several important points about buffers. The buffer capacity of a solution is the sum of the buffer capacity of the components, which in this case are H^+, OH^-, and HA.

If a buffer is prepared from a strong acid such as 0.01 M hydrochloric acid, then [H] equals 0.01 M. The second and third terms of equation 2 are insignificantly small, so the buffer capacity is

$$\beta = 2.3[H] = 0.023 \qquad [3]$$

In the mid-pH range (approximately pH 2–12), the first two terms of equation 2 are insignificant, and the buffer capacity is determined by the weak acid.

The buffer capacity of the weak acid depends upon the ratio of H^+ to pK_a and the buffer concentration (C_{HA}). It is independent of buffer composition. Buffering capacity reaches its maximum when pH is equal to pK_a:

$$\beta_{max} = 0.58C_{HA} \qquad [4]$$

At ± one pH unit from a weak acid's pK_a, the buffer capacity is 0.19 C_{HA} or approximately one-third less than the maximum. At ± two pH units from the weak acid's pK_a, the buffer capacity is 25 times smaller than at the maximum or $0.023C_{HA}$. For comparison, the buffer capacity of water is 4.6×10^{-7}. Even when buffers are prepared well beyond their optimum pH range, they provide vastly greater buffering capacity than no buffer at all.

Let me compare the buffer capacity of a pH 2 buffer prepared from a 0.01 M solution of a strong hydrochloric acid with that of an 0.01 M acetic acid–sodium acetate buffer at its maximum buffer capacity — pH 4.6. The 0.01 M strong acid buffer capacity is 0.023 from equation 3, and the maximum 0.01 M acetate buffer capacity is 0.0058 from equation 4, or approximately fourfold less. This example illustrates how simple solutions of strong acids make excellent buffers at low pH levels. Because of potential corrosion, hydrochloric acid normally would be avoided when preparing a low-pH buffer for chromatography, but phosphoric, trifluoroacetic, formic, and sulfuric acids make excellent low-pH buffers.

Calculating a minimum buffer capacity necessary to achieve a reproducible separation would not be an easy task, but in most cases the buffer capacity would be small. The stationary phase is exposed to a relatively large volume of mobile phase, so it should take little buffering capacity to maintain the column in a constant state of protonation. After a separation is developed within a column, the concentration of components normally is very small, so a small buffer capacity would be sufficient to maintain these components at a constant protonation. Therefore, low concentrations of buffer often are sufficient, and it can be possible to use buffers one or more pH units from their pK_a values with acceptable results.

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Generally, the buffer capacity is most challenged during injection. The sample might be concentrated, and the sample pH might be very different from the target pH of the mobile phase. A high buffer capacity could be necessary to adjust the sample to the mobile-phase pH after injection but before the sample reaches the head of the column. If the buffer capacity of the mobile phase is insufficient, the sample could be less than fully protonated and therefore improperly retained as it encounters the column. A split or badly fronting peak can result. This problem can be fixed by increasing the acidity of the sample, increasing the buffer capacity of mobile phase, or injecting a smaller volume.

Summary

I presented the concept of buffer capacity and discussed the factors that affect buffer capacity. Buffers prepared from weak acids and their salts have their best buffer capacity in the mid-ranges of pH at ± 1 pH unit from the weak acid's pK_a value. However, useful buffering capacity is available beyond this range. Strong acids provide the best buffers at low pH, as do strong bases at high pH. In a chromatographic separation, the buffer capacity is most challenged during injection, whereas buffer capacity is seldom an issue during the separation.

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G. William Tindall is retired from a career in analytical chemistry research at Eastman Kodak and Eastman Chemical (Kingsport, Tennessee) and now operates a consulting service, Analytical Science Solutions, 888 Christian Bend Road, Church Hill, TN 37642, e-mail bjtindall@mindspring.com. He also volunteers for the Northeast Tennessee Section of the American Chemical Society, which promotes chemistry teaching at the high-school level.

John W. Dolan
"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Walnut Creek, California, and a member of LCGC's editorial advisory board. Direct correspondence about this column to "LC Troubleshooting," LCGC, 859 Willamette Street, Eugene, OR 97401, e-mail John.Dolan@LCResources.com.

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