Mobile-Phase Buffers, Part III — Preparation of Buffers

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What is the best way to prepare a buffer?

In Part I of this series, I discussed the interpretation of pH in aqueous and partially aqueous mobile phases.[1] This topic is a source of great confusion because discussions often treat pH as if it were equivalent to $-\log a_H$ or even $-\log[H]$, where [H] is hydrogen ion concentration and a_H is hydrogen ion activity. Only in special instances is the number displayed on a pH meter equal to $-\log a_H$, and it could be said that the meter never measures hydrogen ion concentration.

Hydrogen ion activity in the mobile phase is the value that is important for predicting retention, as well as both column and sample stability. The pH measurement made in the aqueous buffer cannot be used easily to calculate an absolute hydrogen ion activity in the organic-modified mobile phase. The change in hydrogen ion activity following the addition of the organic modifier could be one to two orders of magnitude. Therefore, pH measurements in aqueous buffers are less reliable for predicting partially aqueous mobile-phase retention and column and sample stability than many chromatographers realize. However, properly made measurements of relative pH in partially aqueous mobile phases can provide more-accurate predictions of retention and stability than can pH measured in the aqueous buffer and I showed some examples in Part II of this series.[2]

In Part II, I discussed the concept of buffer capacity. Buffer capacity is a measure of a buffer's ability to resist changes in pH. Modest buffer capacity is often sufficient to achieve a reproducible separation, but a large buffer capacity is necessary when the sample is buffered at a pH that is much different from that of the mobile phase. I showed that strong acids have excellent buffer capacity at low pH and that buffers can often be used successfully well outside of their optimum ranges of buffer capacity.

In this final instalment of the mobile-phase buffer series, I will use the concepts and limitations discussed in Parts I and II to propose strategies for buffer preparation. I will also suggest guidelines for reporting buffer procedures.

Buffer Preparation

Target pH known: Chromatographers encounter two general situations in aqueous buffer preparation: either the exact target pH is known or an approximate range is known and the optimum target pH must be found. In the first instance, analysts can consult compilations of buffers or tables of pK_a to find an acceptable recipe. For example, references 3 and 4 contain recipes for preparing common buffers at a target pH. If the target aqueous pH is approximately pH 2 or lower, phosphoric acid or trifluoroacetic acid is a good buffer. In the mid-pH range, compilations of weak acid pK_a will aid the selection of possible buffers.

Optimum buffer capacity will be achieved at a pH that is ± 1 unit from the acid pK_a. For example, if a moderate-capacity aqueous buffer of pH 4.0 is required, then analysts could consult a table of acid pK_a, which would reveal that acetic, citric and phthalic acids have a pK_a within ± 1 unit of pH 4. Any of these acids would provide good buffer capacity at a concentration of 0.01 M. For UV detection, acetic acid would provide the lowest background absorbance. However, if detection were performed at 200 nm, acetate would be an unacceptable choice because of its high absorbance at 200 nm. In this instance, phosphate, which has little absorbance at 200 nm, might be a better choice even though pH 4 is outside the best buffer range for phosphate. Although it is best to use buffers prepared within ± 1 pH unit of the acid component pK_a, this guideline can often be broken successfully because many separations do not require a large buffer capacity, especially if the sample is unbuffered.

Suppose I was going to prepare a pH 4 acetate buffer. I could consider four options for the preparation of aqueous buffers.

Option 1: I could consult a buffer calculator program such as the one found at http://www.bi.umist.ac.uk/users/mjfrbn/buffers/Makebuf.asp. This particular program takes into consideration activity coefficient corrections so the buffers prepared from this program will be more accurate than programs or hand calculations that skip this correction (e.g., http://www.zirchrom.com/buffer.asp). I would enter the buffer acid concentration and target pH, and the program would provide a recipe. The recipe to prepare 1 L of 0.01 M acetate (pH 4) calls for 0.0084 mol of acetic acid and 0.0015 mol of sodium acetate.

Option 2: I could use the equation for

dissociation of a weak acid to calculate a buffer recipe. For acetic acid, the equation is

$$[H^+]\,[OAc^-] / [HOAc] = 10^{-pk_a} = 1.7 \times 10^{-5} \quad [1]$$

By substituting pH 4 ($H^+ = 10^{-4}$) into the equation, I would obtain a ratio of OAc^-/HOAc equal to 0.17 to achieve pH 4. To prepare 1 L of 0.01 M buffer, I would weigh 0.01 mol of acetic acid into approximately 900 mL of water. The calculation indicates that I would need to add 0.0015 mol of sodium hydroxide and after this addition the volume is diluted to exactly 1 L. (Alternatively, I could prepare the buffer from 0.0015 mol of sodium acetate and 0.0085 mol of acetic acid.)

The calculated amount of base is an approximation because I ignored the activity coefficients. Activity coefficient calculations are tedious to perform by hand and ignoring them will introduce an error of only a few per cent with monovalent 0.01 M buffers. This error will probably be unimportant in most separations. However, for physiological buffers in which the salt content, and hence ionic strength, is high, errors of more than 0.1 pH units will occur if the activity coefficients are ignored.

Option 3: I could use an empirical approach to prepare this buffer. I could add something less than the calculated amount of sodium hydroxide to the acetic acid solution and check the pH. The pH should be checked in a small sample removed from the bulk to avoid contaminating the buffer by the pH probe.[5] I would add increments of sodium hydroxide until the measured pH is 4.00. Then I would dilute the solution to 1 L. I can assume that a small dilution of a dilute buffer will not affect the pH materially. For example, a 1:1 (v/v) dilution of a 0.1 M dihydrogen phosphate solution changes the pH 0.1 unit as a result of activity coefficient changes,[6] so a 10% dilution of a 0.01 M phosphate buffer should have negligible effect. However, I should caution that large dilutions of a concentrated buffer will affect the pH because of large changes in activity coefficient. This situation might be encountered when a 1 M stock of buffer is prepared and adjusted to target pH and then portions of it are diluted to 0.01 M.

I must emphasize that the measured pH and the hydrogen ion activity of this buffer will change when this buffer is mixed with organic modifier. The hydrogen ion activity of the mixture, not the pH of the aqueous buffer, determines retention and stability. Knowing the pH of the aqueous buffer is useful only if experience has shown that an aqueous pH of some value will yield an acceptable hydrogen ion activity after mixing with the organic solvent.

After I have found a pH that results in an adequate separation and acceptable column and sample stability, it is important that I can replicate these conditions conveniently and precisely in future separations. Option 3 might seem like the preferred way to replicate pH conditions because the pH can apparently be adjusted to an exact value to match the target pH. However, this approach is the least desirable because it is the most time-consuming way to prepare buffers. It is also the least precise. Although pH meters can calculate a pH value to three decimal places, in fact, day-to-day repeatability of pH measurements are seldom better than 0.1 unit. Buffers prepared by weights and volumes — Options 1 and 2 — are much faster and more precise because measuring weights and volumes will result in concentrations vastly more precise than what can be achieved by a pH measurement.

Option 4: Alternatively, I could prepare a buffer by adjusting to a target pH in the organic-modified mobile phase. This approach is generally not recommended. Although users can make reliable pH measurements in partially organic mobile phases, these measurements are more difficult, and less precise buffers will probably result from this approach. In addition to this series of "LC Troubleshooting" columns, a recent article in *LC•GC Europe* discussed pH in aqueous–organic mixtures.[7]

Target range known: Another instance of buffer preparation occurs when only a target range of pH is known and an analyst must optimize the separation by varying the pH within this range. I'll suppose that something in the range of pH 4–5 is expected to be optimum and it remains to be determined exactly what pH will be best. I would prepare a stock solution of 0.01 M acetic acid and 0.01 M sodium acetate. These stock solutions can then be mixed in any proportion to prepare 0.01 M buffers in the target range. I could mix 1:10 and 10:1, test the separation or sample and column stability at these extremes, and then optimize with other mixtures. It is unnecessary to be concerned with the pH values of these mixtures during the optimization process because the separation — not the pH meter — will reveal the best ratio.

Reporting Buffer Preparation Procedures

Historically, buffer preparation has been described in terms of weights and volumes; for example, the classic Clark and Lubs buffers.[8] In the chromatography literature, however, buffer preparation is commonly described in terms of the target pH; for example, "the mobile phase was 30% methanol–70% 0.01 molar pH 2.2 phosphate buffer." National Institute of Standards and Technology (NIST) primary and secondary pH standards are described and prepared with weights and volumes. If a more precise and accurate way existed to describe buffer preparation, then NIST would have used it. As an example, the description for preparation of the NIST pH 6.865 phosphate buffer is to prepare a solution" by dissolving 3.38 g KH_2PO_4 and 3.53 g Na_2HPO_4 in water and diluting to 1 L at 25 °C."[9] The ease of repeating this preparation, and its accuracy, should be apparent.

When writing chromatography procedures for buffer preparation, using the weights-and-volumes format will lead to efficient, unambiguous and precise replication of the procedure by others. Weighing is the most precise and accurate operation in analytical chemistry — vastly more so than pH measurement. Stating a weight has no ambiguity, whereas a poll of chemists can reveal several non-equivalent ways to prepare a 0.01 M acetate buffer (pH 4.5) in 50:50 methanol–water. For example, blending 0.01 M solutions of acid and base to reach the target pH gives a different buffer concentration than does adjusting the pH of a 0.01 M base with concentrated acid.

In addition to being more precise, the weights-and-volumes approach is also the most convenient and least time-consuming way to prepare a buffer because it involves no calibration of a pH meter. Component weights and volumes can be scaled easily to prepare any volume from litres for control laboratory chromatography to millilitres for capillary electrophoresis.

Summary

The preparation of buffers from weights and volumes is the easiest, least ambiguous and most reproducible technique for buffer preparation. On-line buffer preparation software tools can simplify obtaining a recipe for a desired buffer. The most important point about buffer preparation is that no matter what technique is used, be sure to include a complete, unambiguous description so that others can reproduce your results.

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