Troubleshooting

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What happens to the pH when an organic solvent is added to a buffer?

Editor's note: This month's "LC Troubleshooting" column is the first in a three-part series about the selection and use of buffers in liquid chromatography. Of most concern is the buffer pH because it affects retention and, often, peak shape. The elementary concepts of pH in dilute aqueous solutions are inadequate to understand pH in partially aqueous or nonaqueous mobile phases. This month's column will discuss the interpretation of pH measurements in aqueous solutions and the significant changes that occur when an organic solvent is added. Next month, guest author G. William Tindall will use this information to aid in the selection and preparation of buffers for typical mobile phases.

John W. Dolan
LC Troubleshooting Editor

Mobile-Phase Buffers, Part I — The Interpretation of pH in Partially Aqueous Mobile Phases

If liquid chromatography (LC) was performed exclusively in dilute aqueous solutions, analysts would need only an elementary understanding of pH to select and prepare buffers for mobile phases. The temptation is to apply these simple concepts to partially aqueous mobile phases, which leads to questions such as, "Why does the pH of the mobile phase change when I add methanol? Which pH is correct? What pH do I need to use?" The interpretation of pH in partially aqueous solutions is different and more complicated than it is in dilute aqueous solutions. To understand buffers in partially aqueous or nonaqueous mobile phases, chromatographers first must understand the meaning of pH in these solutions.

Aqueous pH Measurement

The first definition of pH that everyone learns is "pH = −log [H$^+$]." This definition was proposed by Sorensen (1) in 1909. Researchers soon discovered that the hydrogen electrode Sorensen used, as well as the glass electrodes discovered soon after, responded to hydrogen ion *activity* (a_H), not the concentration. Therefore, a new definition was proposed: pH = −log a_H.

The glass electrode was a handy device, and it could be stuck in all kinds of things — dilute aqueous solutions, concentrated mineral acids, blood, soil, acetic acid, tissues, gels, and organic solvents. The glass electrode provided useful measures of *relative* acidity in these diverse environments, and the measurements became widely used in industry. However, solution thermodynamics was not up to the task of interpreting an absolute hydrogen ion activity from measurements in these environments, so the pH measured was not equivalent to hydrogen ion activity in many cases.

Once again, the definition of pH was at odds with how people were making and using the measurement. By the early 1950s, the world's organizations responsible for

standardization — such as the National Institute for Standards and Testing (NIST) (2,3) — began to adapt the definition of pH that still is accepted universally. This definition is based upon the difference in electrochemical response of a pH-sensitive device — for example, a hydrogen electrode or a glass electrode — between a standard buffer and the sample solution.

The modern definition of pH is

$$pH = pH_{st} + (E - E_{st})F/RT \ln 10 \quad [1]$$

where pH is the pH of the sample solution measured, E is the cell voltage measured in the sample solution, pH_{st} is the pH of the standard solution, E_{st} is the voltage measured in the standard, R is the gas constant, T is the temperature, and F is the Faraday constant. This definition says that to measure pH, you place the electrode, typically a glass pH electrode, in a standard buffer of defined pH and read the voltage. Then you place the electrode in the sample solution and read the voltage. The known and measured values are substituted into equation 1, and the pH is calculated. Modern pH meters do the substitution and calculation internally, and users read the pH of the sample solution directly from a digital display. The true pH of the solution is whatever the meter says it is. The definition of pH according to equation 1 does not necessarily imply any fundamental significance to the measurement in terms of hydrogen ion activity or concentration. I will explain below why pH and −log a_H are not necessarily equivalent.

Most definitions in chemistry are based upon some fundamental property of nature; for example, a mole is 6.02×10^{23} molecules. However, pH is an *operational definition* based upon a defined series of steps to yield a result — the pH. What makes this topic confusing is that most discussions of pH treat pH and −log a_H, or even −log [H$^+$], as though they were

equivalent, when they are not in most practical cases. Analysts should remember that pH is just a value derived from a measurement. Hydrogen ion activity is a fundamental property of the solution, and it is this value, not pH, that is important in chemical reactions. Unfortunately, often it is impossible to calculate a_H from many pH measurements.

In special cases, a measured pH can assume fundamental significance. If a NIST or NIST-equivalent buffer is used as a standard, the unknown solution is dilute and aqueous, and the pH is mid-range so that the glass electrode responds nearly ideally to a_H, then the meter will read a pH that is equivalent to $-\log a_H$. If any properties of the sample solution deviate from these criteria, as would be the case with a typical mobile phase, then the measured pH could provide only a relative indication of solution acidity.

Why is it more difficult to make a fundamental interpretation of pH in terms of hydrogen ion concentration or activity in a typical methanol–water mobile phase? Suppose a hypothetical 0.01 M pH 5.0 buffer is prepared in water from a mixture of a weak acid and its salt. This buffer solution is dilute and aqueous, and the pH is mid-range, where the glass electrode responds nearly ideally to a_H. I call these conditions the *DAM criteria* — dilute, aqueous, and mid-range. Because this solution meets the DAM criteria, the pH equals, or very nearly equals, $-\log a_H$. After the addition of some methanol, the pH now reads 7. Why did the pH change, what is the correct pH, and what does this pH tell us about the acidity of the mixed solvent? The mixed solvent can't be neutral in spite of the pH 7 reading, and, in fact, it is not.

The ability of the glass electrode to respond to hydrogen ion activity did not change in this partially aqueous buffer. A typical glass electrode responds nearly ideally to a_H in methanol, acetonitrile, water, and mixtures of these solvents (4,5). Therefore, it is entirely appropriate to make pH measurements in these solvents. If special buffers are used for calibration, it even is possible to accurately measure hydrogen ion activity in methanol–water mixtures (4).

The best way to predict buffer behavior in mobile phases is to measure the pH *after* adding organic solvent. Inappropriate use of the electrode is not the reason the pH changed in the above example. However, four important things changed when the methanol was added and caused the change in pH.

Change in Junction Potential

A combination pH electrode contains a glass pH-sensing electrode and a reference electrode. Electrical contact between them is provided by a porous material such as a ceramic frit. During measurement, a tiny current is established across this frit by the diffusion of positive ions one way and neg-

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ative ions the other. These ions come from the filling solution of the reference electrode and the ions in the sample solution. The mobility of these ions is not equal because of their different sizes and charges, so charge separation occurs and a potential is established across the frit, called the *junction potential*.

The three most important factors that affect the magnitude of the junction potential are the construction of the frit, the concentration and composition of ions in the solution, and the presence of organic solvent. In a pH measurement, the junction potentials in the calibration standard and sample ideally are identical, because they will cancel in the pH calculation (equation 1). This situation is the case when the DAM criteria are met; however, when the DAM criteria are unmet, the junction potentials between the calibration standard and the sample are unequal.

This difference in junction potential (E_{jp}) is added to the potential generated from the pH response ($E - E_{st} + E_{jp}$), which results in an error in the pH measurement. For the best-designed electrodes, the error can range from a few hundredths of a pH unit for dilute buffers in low concentrations of methanol in water to two or more pH units for high concentrations of methanol or other solvents. Colloids, zwitterions, and polyelectrolytes also create large junction potential errors even in aqueous solutions. It normally is impossible to

determine the size of the junction potential error.

Change in Autoprotolysis Constant

For water the autoprotolysis constant (K_W) is given by

$$K_W = [H^+][OH^-] = 10^{-14} \text{ at 25 °C} \quad [2]$$

Neutral is defined as the state at which H^+ equals OH^-, which occurs when H is 10^{-7} or a pH of 7. For methanol the autoprotolysis constant is

$$K_{methanol} = [H^+][CH_3O^-] = 10^{-16.6} \quad [3]$$

In methanol, neutral is when H^+ equals CH_3O^-, which occurs when H is $10^{-8.3}$ or a pH of 8.3. Methanol–water mixtures have autoprotolysis constants between 14 (water) and 16.6 (methanol), so neutral in these mixtures ranges from pH 7 to pH 8.3.

In aqueous basic solutions, the anion is OH^-, and in basic solutions that contain high concentrations of methanol, the anion will be a mixture of OH^- and CH_3O^-. Methoxide is a vastly more potent nucleophile than hydroxide, so basic methanol–water mixtures can show different chemical behavior than water alone, even when the hydrogen ion activity is the same. This fact is relevant to column stability and sample stability in basic methanol–water mixtures.

Change in pH Scale

A third change is caused by a change in the standard state between water and a water–methanol mixture. This concept runs counter to the desire for fundamental properties to remain the same in different situations. Standard state is a convention in solution thermodynamics used to establish some scale, for example, pH or electrochemical potential. Just as deciding that the atomic weight of the most abundant isotope of carbon is exactly 12 and comparing all other elements to carbon, scales for pH and electrochemical potentials are established.

Unlike the atomic weight scale in which carbon is always 12 throughout the universe, the scale in solution thermodynamics can change in value whenever the solution changes. This change leads to an awkward situation. A different pH scale exists for every temperature and every solvent composition, and these pH scales cannot be compared easily. For example, suppose a

pH electrode is calibrated in an aqueous buffer. Then, it is used to measure the pH of an aqueous solution and a methanol–water solution, each with identical hydrogen ion activity. The two measurements will be different, in spite of the fact that each has identical hydrogen ion activity. The difference will be less than 0.1 pH unit for solutions of as much as 50% methanol. In 100% methanol, the difference will be more than two pH units (5).

The standard state situation makes it difficult to compare pH-sensitive behavior between two widely different solvents even if the hydrogen ion activity of each can be measured reliably. Therefore, a separation optimized with respect to hydrogen ion activity at either a certain temperature or a particular solvent composition will be optimized at some different hydrogen ion activity under widely different conditions of temperature or solvent.

Change in Buffer pK_a
The electrode responds to hydrogen ions in the buffer from the dissociation of the weak acid. Methanol and acetonitrile have lower dielectric constants, and they are weaker bases than water; therefore, they differ in their ability to dissociate ions. Acids will dissociate differently in these solvents than in water and cause differences in pK_a between water and water–solvent mixtures. The changes generally obey the following rules upon increasing the organic solvent concentration.

- Neutral weak acids such as acetic acid and anionic acids such as $H_2PO_4^-$ typically get weaker, and the pK_a becomes larger. This behavior can be rationalized because the weaker dielectric constant hinders disassociation of the neutral acid to make ions or of the cationic acid to make more highly charged ions.
- Cationic acids such as NH_4^+ get stronger, but this trend reverses at high organic concentration and they become weaker. This behavior is more difficult to rationalize.
- Some complex acids such as boric acid exhibit a more complex response of pK_a.

Table I shows changes in pK_a with methanol concentrations of some typical acids used to prepare LC buffers (6). When methanol or acetonitrile is added to a buffer, the acidity of the buffer can increase or decrease, depending upon the details of the acid structure and how much organic modifier is added. Changes in pK_a of ±1 units can be expected between water and 50:50 (v/v) methanol–water. Larger changes are observed for acetonitrile (7). Very large changes can be encountered when the solvent contains little or no water.

Putting It to Practice
To return to my example comparing pH in aqueous and mixed solvents, what does the pH reading of 7 mean in the mixed solvent? The solution is acidic, because neutral is at a pH greater than 7 for all methanol–water mixtures at 25 °C. However, if I knew an acid sample was 50% dissociated at aqueous pH 5, I have no way to predict what the dissociation will be in methanol–water at pH 7. I don't know the pK_a of the acid in the mixed solvent, and, for the reasons described above, I can't calculate hydrogen ion activity from the pH measurement. Furthermore, if I knew the sample or column was unstable at aqueous pH 7, I could not predict the stability in the pH 7 mixed solvent because I don't know the hydrogen ion activity or the effect of the standard state change. By itself, neither the pH 5 measurement of the aqueous buffer nor the pH 7 measurement in the mixed solvent is much use in predicting retention and stability behavior in the pH 7 mobile phase.

Summary
When organic modifier is added to an aqueous buffer many factors change, and these changes cause additional changes in the pH measurement and the hydrogen ion activity of the solution. In mobile phases that contain as much as 50% methanol or acetonitrile, the most significant change will be in the pK_as of the buffer and sample components. Because of

the pK_a changes, the extrapolation of aqueous pH and pK_a data could incorrectly predict retention, column stability, or sample stability in organic solvent–modified mobile phases. By considering the rules that govern these changes, however, analysts can make better estimates of retention and stability in modified mobile phases.

Comparing pH measurements in the actual mobile phase (fixed temperature and organic composition) provides better predictions of retention and stability than do extrapolations from measurements made in aqueous components. For more information on these topics, see references 2, 3, 8, and 9.

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For an ongoing discussion of LC troubleshooting with John Dolan and other chromatographers, visit the Chromatography Forum discussion group at http://www.chromforum.com.

Table I: pK_as of typical buffer acids in water and 50:50 (v/v) water–methanol mixtures*

Acid	Water pK_a	50% Methanol pK_a
Phosphoric acid pK_1	2.11	3.21
Phosphoric acid pK_2	7.19	8.24
Acetic acid	4.77	5.54
Ammonium	9.24	8.76

*Data from reference 6.