Liquid Chromatography Problem Solving and Troubleshooting

Question:

I am having a difficult time accurately adjusting the pH of my buffer. With the addition of a small amount of acid, the pH jumps down significantly, and when I attempt to bring the pH up, a small amount of base results in a similar reverse jump. If I dilute the acid, I can adjust the pH, but will the extra volume that I add have a significant effect on the concentration of the total solution? I require an accurate pH for ensuring retention time reproducibility.

Answer:

When making the aqueous portion of the mobile phase, it is important to know the final buffer concentration, and it is therefore important that you compensate for the dilution that might occur when adjusting pH. If you use a large volume of dilute acid to adjust the pH, this may require you to adjust the pH in a beaker and then transfer the solution to a volumetric flask for the final addition of water. However, the situation that you describe may be indicative of the fact that you are not using a true buffer. A buffer will establish a pH that will not respond to small changes in the addition of an acid or a base. You may be using a buffer salt but using it outside of the buffering capacity or range.

The capacity of a buffer is its ability to maintain the pH; it depends on the concentration of the buffer and the pK_a of the buffering agent. Remember that the maximum buffering capacity occurs at the pK_a value. For HPLC, I like to use the rule of thumb that the adjustable range of the pH is ± 1 pH unit of the pK_a of the buffering ion; however, a range of ± 1.5 pH units may be possible when using higher concentrations (≥ 10mM). Table I describes the use of some commonly used buffers for HPLC and their buffering range. As I have pointed out in this column before, pH is measured in water and is not defined in a nonaqueous or partially aqueous solution. Therefore, adjust the pH of the aqueous portion of your mobile phase before combining it with the organic portion.

I have observed many papers in which the authors used a salt solution and not a true buffer. For example, using potassium phosphate (monobasic) at a pH of 4.8 is not a buffer. It may be suitable for some chromatography, but it is not a buffer; if one were having reproducibility issues with the "buffer" preparation or the chromatography, I would use a pH within the buffering range of the salt or switch to another salt that would buffer in the desired pH range. In this case, with the desired pH of 4.8, I would use disodium citrate. From Table I, it can be seen that the commonly used buffer, phosphate, does not cover much of the operating pH range for reversed-phase silica-based packing material (2–8). Citrate, on the other hand, is a very good buffer in that it can be used from approximately pH 2 up to pH 6.5, which covers quite a broad range for optimization of the chromatographic separation. Therefore, when developing future methods, you may wish to use citrate rather than phosphate if pH is a key parameter to optimize.

Table I. Common Buffers Used in HPLC

Buffer	pK_a	pH range
Potassium phosphate (monobasic)	2.1	1.1–3.1
Dipotassium phosphate (dibasic)	7.2	6.2–8.2
Tripotassium phosphate (tribasic)	12.3	11.3–13.3
Sodium citrate	3.1	2.1–4.1
Disodium citrate	4.7	3.7–5.7
Trisodium citrate	5.4	4.4–6.4

During method development, I prefer to use a concentration of 10mM buffer to start with and, if necessary, raise the concentration as high as 50mM if required, to improve chromatography. In addition to controlling the ionization of the analyte, the high buffer concentration will also "mask" silanol contributions to retention. If one uses a high concentration of buffer salt, this will mask small variations in the silanol concentration from column to column, which is also beneficial for a rugged and reproducible method. Of course, the concentration of the buffer should not exceed the recommended concentration of salt that the hardware will tolerate. Most HPLCs will accommodate 5–100mM solutions. When in doubt, ask the manufacturer.

The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC–MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward to JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting,* P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

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