

# Solubility of Buffers in Aqueous–Organic Eluents for Reversed-Phase Liquid Chromatography

The authors study the solubility of five buffers commonly used in reversed-phase liquid chromatography as a function of volume fraction in binary mixtures of the aqueous buffer and methanol, acetonitrile, or tetrahydrofuran.



**W**hen preparing binary eluents containing both an organic solvent and a buffer for a reversed-phase liquid chromatography (LC), one must know the solubility of the buffer in the organic cosolvent to avoid precipitation of the buffer salt. For example, one must know the highest percent organic cosolvent (that is, %B, v/v) in which a buffer salt remains soluble. Obviously, one can test the solubility of a buffer at a desired %B by preparing a small volume of the desired eluent and waiting a sufficient time to see if a precipitate forms. Although buffer solubility is determined readily, this method is tedious and time-consuming.

One always can avoid precipitation of the buffer salt by using a very low concentration of buffer salt (<10 mM) in the aqueous portion of the eluent. However, the resulting low buffer capacity can lead to slow equilibration, irreproducible retention, and poor peak shapes (1). Furthermore, both retention and selectivity are functions of the salt concentration for ionizable solutes (2–5). Thus, knowing the relationship between the buffer's solubility limit and volume fraction of organic cosolvent is useful in reversed-phase LC.

There is a paucity of information in the literature on the solubility of common buffers in typical hydro-organic mixtures. We expect that buffer solubility should follow the same trend as the solubility of cations in water ( $\text{NH}_4^+ > \text{K}^+ > \text{Na}^+$ ). Therefore, we studied the three most common organic modifiers in combination with five of the more common buffer systems, including: ammonium acetate at pH 5.0,

ammonium phosphate at pH 3.0 and pH 7.0, and potassium phosphate at pH 3.0 and pH 7.0. We limited our studies to inorganic buffer salts, as they are less soluble in typical organic cosolvents compared with organic buffer salts. We also avoided sodium salts because they are expected to have lower solubility, which makes them less useful for reversed-phase LC.

## Experimental

**Preparation of Buffers:** High performance liquid chromatography (HPLC)-grade water was obtained from a Barnstead Nanopure deionizing system (Dubuque, Iowa). This water was boiled to remove carbon dioxide and cooled to room temperature before use. Concentrated (2.0 M) solutions of ammonium acetate, monobasic ammonium phosphate, and monobasic potassium phosphate were prepared, along with 0.75 M solutions of dibasic ammonium phosphate and dibasic potassium phosphate. Each buffer salt was used as obtained (from Fisher, Fair Lawn, New Jersey) without further purification. The ammonium acetate buffer was adjusted to pH 5.0 using formic acid, the monobasic phosphate buffers were adjusted to pH 3.0, and the dibasic phosphate buffers were adjusted to pH 7.0 using phosphoric acid. A  $\Phi$ 31 Beckman pH meter (Fullerton, California) was calibrated using pH 4.0 and pH 7.0 aqueous standard buffer solutions (Fisher). The pH of the buffers was adjusted while the buffers were constantly stirred magnetically. Each buffer was passed through a 0.45- $\mu\text{m}$  nylon filtration apparatus before use (Lida Manufacturing, Inc., Kenosha, Wisconsin).

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## Preparation of Eluents

The eluents used in this experiment were 90:10, 80:20, 75:25, 70:30, 50:50, and 25:75 (v/v) organic modifier–water. The HPLC water and each organic modifier (obtained from Pharmco, Brookfield, Connecticut) were passed through a 0.45- $\mu$ m nylon filtration apparatus before use. These eluents were prepared by weight taking the density of water, methanol, acetonitrile, and tetrahydrofuran at room temperature (25 °C) as 0.9971 g/mL, 0.78664 g/mL, 0.7761 g/mL, and 0.88415 g/mL, respectively (6). The eluents were mixed thoroughly and allowed to reach room temperature before use.

## Preparation of Eluents Using Concentrated Buffer Solutions

The solubility of each buffer was determined by dropwise addition of buffer to an aliquot of the aqueous–organic mixture until a cloudy solution or visible precipitate formed. The solution was constantly stirred magnetically during the addition. The volume of aqueous–organic mixture was varied from 15.00 to 100.00 mL to ensure that at least 0.2 mL of concentrated buffer solution was added via a 50-mL buret. Each determination was performed at least in duplicate.

The flask in which the experiment was performed was rinsed three times with HPLC-grade water after each determination to remove traces of buffer and eluent. A clean paper towel was used to remove excess water. The flask then was rinsed three times with acetone and dried using nitrogen before the next determination.

## Results and Discussion

The solubility limit and volume fraction of the organic cosolvent (that is, %B) of the final solution were calculated at the point of precipitation, knowing the volume and concentration of the buffer and the organic–aqueous mixture. We ignored the pH changes upon addition of organic solvent. Plots of salt concentration versus %B were created. To determine the relationship between buffer solubility and %B, a set of two and three term-fitting equations were used, including the Pade approximation (equation 1), the quadratic equation (equation 2), the reciprocal fit (equation 3), the reciprocal-linear fit (equation 4), and the exponential fit (equation 5). In equations 1–5, the  $\phi$  symbol represents %B to avoid confusion with the  $B$  fitting coefficient;  $y$  represents the molar solubility of the buffer salt (millimolar) in the aqueous–organic mixture at the point of precipitation.

$$y = A + \frac{B \cdot \phi}{1 + C \cdot \phi} \quad [1]$$

$$y = A + B \cdot \phi + C \cdot \phi^2 \quad [2]$$

$$y = A + \frac{B}{\phi} \quad [3]$$

$$y = A + B \cdot \phi + \frac{C}{\phi} \quad [4]$$

$$y = A + B \exp(C \cdot \phi) \quad [5]$$

The solver function in Microsoft Excel was used to obtain the fitting coefficients ( $A$ ,  $B$ , and  $C$ ) for all the equations. Overall, equation 4 provided the best fit of the experimental data for all buffer systems. For example, the average percent deviation in the measured buffer solubility and that predicted by equation 4 for the ammonium phosphate (pH 3.0) buffer system was 6.3%, 7.4%, and 5.6% for methanol, acetonitrile, and tetrahydrofuran cosolvents, respectively. Similar or lower errors using equation 4 were seen for the other buffer systems.

In fitting the experimental data to equations 1–5, we assumed that each buffer is insoluble ( $y = 0$  mM) in pure organic media (100% B). This makes sense to a first approximation; adding an aqueous solution of buffer to a pure organic solvent will change the %B of the solution. However, the fact that the solubility of ammonium acetate in pure methanol is nearly 2 M is an extreme example of the failure of this approximation. Nonetheless, we feel that this procedure provides a conservative (low) estimate of the buffer solubility.

Plots of buffer solubility versus the volume fraction of organic cosolvent are shown in Figure 1 for all the aqueous–organic mixtures investigated. Overall, the expected trends in solubility are observed. All buffers are most soluble in methanol and least soluble in tetrahydrofuran. Ammonium acetate is the most soluble buffer salt, ammonium phosphate is less soluble, and potassium phosphate is the least soluble buffer salt in each aqueous–organic mixture. Phosphate buffers at pH 3.0 are more soluble than phosphate buffers at pH 7.0. Notable exceptions to these trends exist for the potassium

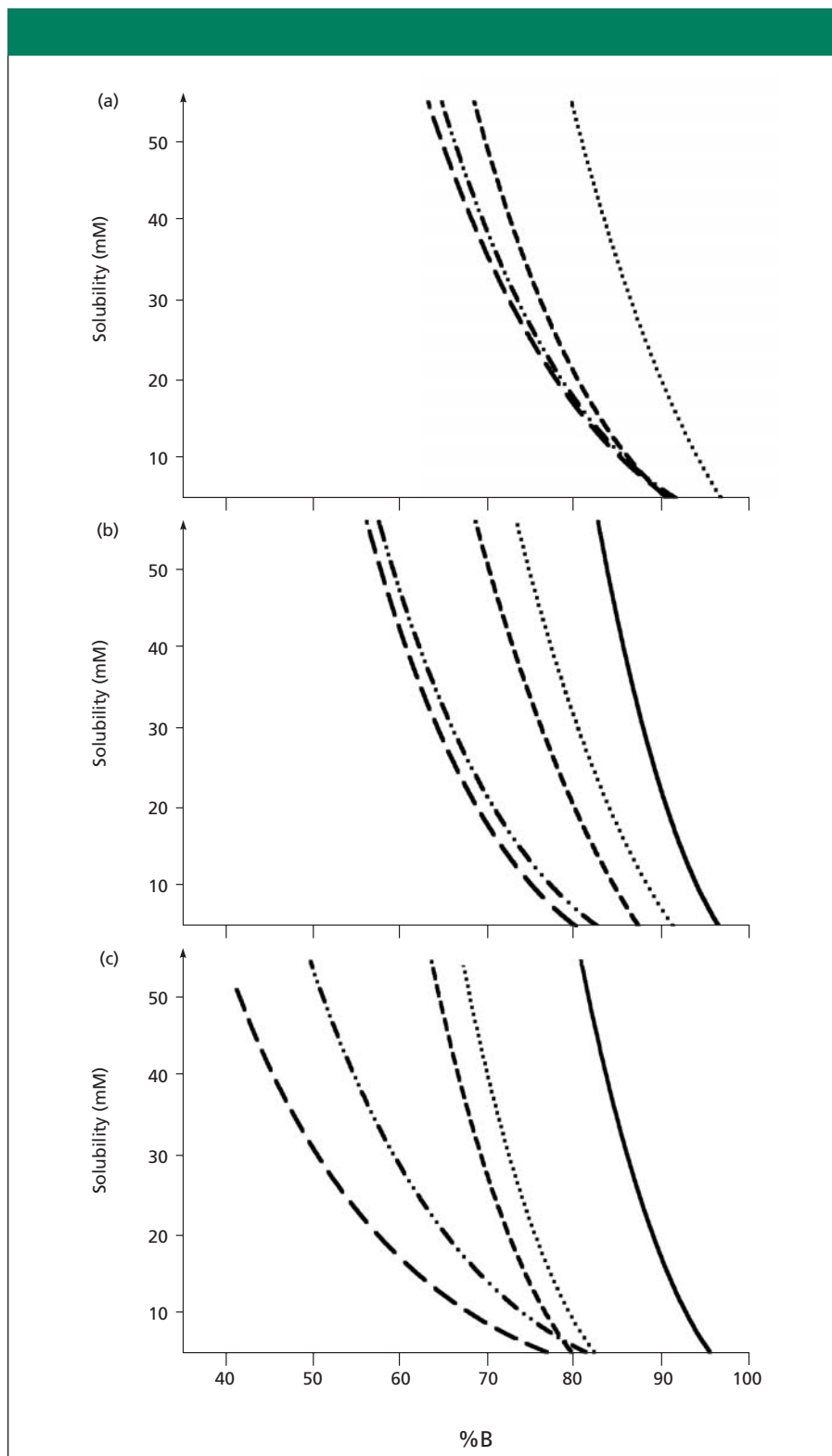
phosphate buffer at pH 3.0 and the two phosphate buffers at pH 7.0. These buffers appear to be equally soluble in both methanol and acetonitrile media.

To verify the solubility trends shown in Figure 1, we prepared 5 mM and 50 mM solutions of each buffer and then made 20 mL of eluent at the %B for which these buffer solutions should be soluble for each organic cosolvent. After sealing these solutions and allowing them to equilibrate at room temperature for one day, no precipitate or cloudiness was observed. To confirm that each solution was near or at its solubility limit, we added an amount of organic modifier that increased the organic composition by 4% (v/v). In all cases, this additional organic cosolvent was sufficient to form a cloudy solution or precipitate after allowing these solutions to equilibrate at room temperature for one day. Thus, we are confident that the buffer solubility measurements shown in Figure 1 are satisfactorily accurate as guidelines for use in buffer preparation in reversed-phase LC. Table I presents an estimate of the soluble concentration of the least soluble buffer (potassium phosphate at pH 7.0) in three organic cosolvents. In addition, the fitting coefficients used to create Figure 1 are available on our website (<http://www.chem.umn.edu/groups/carr/1carr.html>).

This study has focused on the buffer solubility in premixed aqueous–organic mixtures. In determining these values of buffer solubility, we observed that when the buffer concentration in the mixture was near the solubility limit, a single drop of concentrated buffer often formed a transient precipitate that gradually disappeared. The microenvironment of mixing might be important, especially when an LC system is used to mix the eluent (7). Thus, one LC system might rapidly mix an aqueous–organic mixture with a buffer while another

Table I: Summary of buffer solubility

%B	Solubility (mM)		
	Methanol	Acetonitrile	Tetrahydrofuran
0	>50	>50	>50
10	>50	>50	>50
20	>50	>50	>50
30	>50	>50	>50
40	>50	>50	50
50	>50	>50	25
60	>50	45	15
70	35	20	10
80	15	5	<5
90	5	0	0



**Figure 1:** Solubility of five buffers in mixtures with (a) methanol, (b) acetonitrile, and (c) tetrahydrofuran, where — represents ammonium acetate at pH 5.0, ···· represents ammonium phosphate at pH 3.0, --- represents potassium phosphate at pH 3.0, -·-·- represents ammonium phosphate at pH 7.0, and -- -- represents potassium phosphate at pH 7.0. The 95% confidence level intervals are  $\leq 3.3$  mM.

desired %B in one channel and the appropriate concentration of buffer in water in the other channel to minimize the chance of buffer precipitation. Every effort should be taken to avoid mixing a pure organic solvent with a purely aqueous buffer.

### Conclusions

In this study, we determined the maximum solubility limits for five commonly used reversed-phase LC buffers in the three most common organic cosolvents at room temperature and atmospheric pressure. Overall, these results provide useful guidelines for the preparation of strong binary eluents in which the aqueous portion contains a common reversed-phase LC buffer salt. Although there are many buffer salts used in reversed-phase LC that we did not study, we believe the general trends in buffer solubility presented here will allow chromatographers to estimate which buffer concentrations can be used. The main results of this study are as follows:

- All buffers were most soluble in methanol and least soluble in tetrahydrofuran.
- Ammonium acetate at pH 5.0 was the most soluble buffer and potassium phosphate at pH 7.0 was the least soluble buffer in each eluent.
- Buffer solubility decreases by a factor of 2 for a 10% increase in organic volume fraction in the range of 50–70% B.

Overall, these data should eliminate some of the guesswork and tedium in eluent preparation. This method has proven to be a reliable approach in estimating buffer solubility as a function of composition with minimal experimentation.

### References

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might not. Inefficient mixing will lower the apparent solubility of a machine-mixed buffer compared with the value reported in

Figure 1. Additionally, when a machine-mixed eluent is used, we recommend using an aqueous–organic mixture near the