Buffer considerations for LC and LC-MS

Xavier Subirats*, Elisabeth Bosch and Martí Rosés

Department of Analytical Chemistry, Universitat de Barcelona

Martí i Franquès 1-11, 08028 Barcelona, Spain

*Corresponding author

Dr. Xavier Subirats

E-mail: xavier.subirats@ub.edu

Prof. Elisabeth Bosch

E-mail: e.bosch@ub.edu

Prof. Martí Rosés

E-mail: marti.roses@ub.edu

Abstract

In this article, the buffer capacity concept is revisited, particularly concerning its behavior in hydroorganic mobile phases. The buffer capacity of a polyprotic acid, or a mixture of monoprotic acids, depends upon the concentration of each weak acid-conjugate base pair, and the pH of its maximum value mainly fits to the acid-base pK_a, but it is shifted to a certain degree according to the ionic strength of the buffered solution. Consequently, when an organic solvent is added to an aqueous buffer to prepare a particular mobile phase, the buffer capacity of the hydroorganic mixture is reduced due to the dilution effect, and the maximum buffer capacity is shifted to lower or higher pH values according to the nature of the buffering acid-base pair.

In reversed-phase liquid chromatography the pH of the mobile phase is a fundamental parameter which affects significantly the retention of ionisable analytes. The buffer capacity is a very important quality of a buffer solution, since it gives information about the resistance of a solution to pH change. Between December 2002 and February 2003 a series of interesting and formative papers from Tindall and Dolan dealing with mobile-phase buffers were published in LC·GC Europe [1-3]. These papers were about pH interpretation in partially aqueous mobile phases and buffer selection and preparation. They also included a brief introduction about buffer capacity in aqueous solutions. The objective of this paper is to more fully describe buffer capacity, particularly in hydroorganic mobile phases.

Some essential foundations on buffer capacity concept

In 1922 Van Slyke [4] proposed the following differential ratio as a quantitative measure of the buffer action in aqueous solution:

$$\beta = \frac{\mathrm{d}C_{\mathrm{B}}}{\mathrm{dpH}} = -\frac{\mathrm{d}C_{\mathrm{A}}}{\mathrm{dpH}} \qquad [1]$$

expressing the relationship between the increment of the concentration of a strong base B, C_B (or strong acid A, C_A) added to a buffer solution and the resultant increment in pH. Buffer capacity (β) is always a positive numerical value: if base is added to a buffered solution the pH is increased, so both dC_b and dpH are positive and the ratio is also positive; if acid is added dC_A is positive but dpH is negative, thus the minus sign before the dC_A/dpH turns the negative ratio into a positive function. β is an additive quantity which depends on the buffer capacities of the acid-base pair which make up the particular buffer system, and the unavoidable acid-base pairs of water (H_3O^+/H_2O and H_2O/OH^-).

$$\beta = \beta_{\mathrm{H_3O^+}} + \beta_{\mathrm{acid_1-base_1}} + \beta_{\mathrm{acid_2-base_2}} + \ldots + \beta_{\mathrm{OH^-}} \qquad [2]$$

In case of a polyprotic acid buffer (e.g. phosphoric), or a mixture of monoprotic acids (e.g. ammonium carbonate or ammonium formate), the total buffer capacity of the system can be expressed in terms of [5]:

$$\beta = 2.3\left([H_3O^+] + \frac{C_1 K'_{a1} [H_3O^+]}{(K'_{a1} + [H_3O^+])^2} + \frac{C_2 K'_{a2} [H_3O^+]}{(K'_{a2} + [H_3O^+])^2} + ... + \frac{K_{ap}}{[H_3O^+]} \right) \quad [3]$$

where C_1, C_2… refers to the total concentration of each weak acid-conjugate base pair, K'_{a_i} is the concentration acidity constant at the working ionic strength, and K_{ap} is the autoprotolysis constant of water. This equation is correctly applicable to acids presenting a ratio of successive ionisation constants (K_{a2}/K_{a1}) lower than 0.05, condition fulfilled by the most of common polyprotic acids. When this condition is not satisfied (e.g. tartaric and adipic acids) more complex equations are required. For example, the exact expression for buffer capacity of a diprotic acid is [6]:

$$\beta_{H_2A} = 2.3\, C_{H_2A}\, K'_{a1} [H_3O^+] \frac{[H_3O^+]^2 + 4\, K'_{a2} [H_3O^+] + K'_{a1} K'_{a2}}{\left([H_3O^+]^2 + K'_{a1} [H_3O^+] + K'_{a1} K'_{a2} \right)^2} \quad [4]$$

Anyway, buffer capacity is proportional to the concentration of the buffering species and depends on its pK'_a. It must be noticed that activity correction has not been carried out in Eqs. [3-4]. For an acid-base equilibrium like $HA^z \rightleftarrows A^{z-1} + H^+$, the concentration acidity constant (K'_{a_i}) is defined as:

$$K'_{a_i} = a_{H^+} \frac{[A^{z\text{-}1}]}{[HA^z]} \quad [5]$$

whereas for the same equilibrium, the thermodynamic acidity constant includes the activity coefficients (γ_i):

$$K_{a_i} = \frac{a_{H^+}\, a_{A^{z-1}}}{a_{HA^z}} = a_{H^+} \frac{[A^{z\text{-}1}] \gamma_{A^{z-1}}}{[HA^z] \gamma_{HA^z}} \quad [6]$$

General interconversion between thermodynamic and concentration acidity constants is possible using the Debye-Hückel equation ($\log \gamma_i = -A z_i^2 \sqrt{I} \,/\, (1 + a_0 B \sqrt{I})$, with A=0.5 and a_0B=1.5 for water solvent) to calculate the particular activity coefficients of the acidic and

basic species of the buffer. Then, from Eqs. [5] and [6] and taking logarithms the following expression can be established:

$$\mathrm{p}K_{\mathrm{a}}^{'} = \mathrm{p}K_{\mathrm{a}} + \log\frac{\gamma_{\mathrm{A}^{z-1}}}{\gamma_{\mathrm{HA}^{z}}} \qquad [7]$$

In fact the effect of activity coefficients is not significant in diluted aqueous solution, especially in case of low ionic strength. Nevertheless activity correction can play an important role when the organic solvent content increases in the preparation of mobile phases and the ionic strength of the solution is relatively high due to charge of the buffer species. Although Eqs. [1-4] were developed for aqueous solutions, they can be generalised to any amphiprotic solvent (i.e. self-ionising solvent possessing both characteristics of Brønsted acids and bases) or mixtures of them, such as the ones used in LC mobile phases.

pH of maximum buffer capacity

According to Eq. [1], buffer capacity can be also defined as proportional to the reciprocal of the slope of a titration curve. Figure 1 shows the titration curves of tris(hydroxymethyl)aminomethane (tris) and formic acid, together with the corresponding profiles of the buffer capacity of the system. The hydrogen and hydroxide ions contributions to the buffer capacity become relevant below pH 3 and above pH 11, respectively, and they can be large as compared to the buffer capacity of the weak acid-base pair. Maximum buffer capacity of this weak acid-base pair buffer is achieved at the half titration point, where the concentration of the acid equals to the concentration of its corresponding conjugate base, or in other words, when the pH of the solution equals to the $\mathrm{p}K_{\mathrm{a}}^{'}$ of the buffer compounds. In case of the formic acid-formate pair its buffer capacity is directly overlapped by the inevitable H_2O-H_3O^+ because of its low pK_a. We should pay special attention to not confuse the $\mathrm{p}K_{\mathrm{a}}^{'}$ value of a selected buffer in a particular solution and the pK_a value often obtained in the literature corrected to zero ionic strength. In Table 1 the aqueous pK_a values (at zero ionic strength and 25ºC, from ref. [7]) of commonly used buffers are shown, together with their calculated pH values of maximum buffer capacity with activity correction (equivalent to $\mathrm{p}K_{\mathrm{a}}^{'}$) at three different concentrations covering the usual HPLC range. In case of neutral

acids (e.g. acetic acid) and neutral bases (e.g. ammonia and tris) the variation of the maximum of buffer capacity does not relevantly change with concentration, because the ionic strength is relative low (only the acid or the conjugate base has a positive charge). But for anionic acids (e.g. dihydrogencitrate, hydrogenphosphate and hydrogencitrate) and cationic bases (e.g. piperazonium) the apex of buffer capacity can be shifted in a significant way in relation to the thermodynamic pK_a, about half unit at 0.1 mol·L^{-1} for species with two charges and about 0.8 units for trivalent species. This fact is important because buffer capacity sharply decreases when the solution pH move away from the buffer pK_a'. When the pH is one unit below or above the pK_a' the buffer capacity is reduced to 1/3 of its maximum value, and it is only about 4% when pH equals to $pK_a' \pm 2$. An illustrative example is provided by hydrogencitrate-citrate buffer: according to the literature pK_a value (Table 1) one should think that the best buffer capacity for this buffer at a concentration of 0.1M is achieved when the pH of the solution is 6.40, but in fact the correct pH is 0.83 units lower, and therefore this prepared solution of pH 6.40 has only about the 40% of the presupposed buffer capacity. These phenomena are even more notable when an organic solvent is added to the aqueous buffer, because of its significant effects on activity coefficients. For example, in case of mixtures containing 50% in volume of methanol or acetonitrile and a buffer concentration of 0.05 mol·L^{-1}, the buffer capacity apex for dihydrogencitrate-hydrogencitrate or hydrogencarbonate-carbonate is shifted down about 0.5 units in relation to the thermodynamic pK_a, and about 1 unit in case of hydrogencitrate-citrate (instead of 0.34 and 0.73, respectively, for water solvent, as shown in Table 1).

What happens to an aqueous buffer when organic solvent is added?

When an organic solvent is added to an aqueous buffer to prepare a particular mobile phase two different phenomena take place: (a) the buffer capacity of the mixture is reduced due to the dilution effect, because β is proportional to the concentration of the buffer, and (b) the maximum of buffer capacity is shifted according to the pK_a' shift of the buffer species, including the contribution of the activity coefficients. There are data available about the pK_a variation of commonly used HPLC buffers in acetonitrile-[8] and methanol-aqueous[9] solution up to 60% and 80% in volume, respectively. In this studied range of solvent compositions the

pK_a of neutral and anionic acids is shifted to higher values, whereas the pK_a of cationic acids shows the reverse trend. Both phenomena are depicted in Figure 2 for phosphoric and citric acids buffering systems. It must be mentioned that in the present article the pH in the hydroorganic mixture is expressed in the ${}^{s}_{w}\mathrm{pH}$ scale, i.e. the pH quantity is referred to water as standard state. In other words, the pH is measured in the mixture, but the glass electrode is calibrated using standard aqueous buffers. After this clarification and coming back to Figure 2, the citric acid system shows a good buffer capacity in an outstanding wide range of pH, up to pH 6.5-7 in aqueous solution and up to pH 8 in 60% of acetonitrile, whereas in case of phosphoric acid system there is a poor buffered zone around pH 5 because of the large difference between the first and the second pK_a values.

MS-friendly buffer mixtures: ammonium carbonate, -acetate and -formate

Buffers composed of two buffering species are commonly used in HPLC systems. In particular, ammonium formate, ammonium acetate and ammonium carbonate are widely used when HPLC is coupled to mass spectrometry. Ammonium acetate and formate are employed in separations performed at low pH, in which the buffering species are formate or acetate, and ammonium plays only the role of a volatile MS-friendly co-ion, instead of sodium or potassium ions. Figure 3 shows buffer capacity profiles of ammonium acetate and formate in aqueous solution, and how they vary when acetonitrile is added to the aqueous buffers to prepare particular mobile phases. In these plots the individual buffer capacities corresponding to ammonium-ammonia and acetic acid-acetate or formic acid-formate species are described, together with the contribution of hydrogen ion (H_3O^+) at very acidic pH and hydroxyl ion (OH^-) at very alkaline pH. In case of ammonium acetate there is a difference of 4.6 units between the pK_a values of both buffering species (ammonium-ammonia and acetic acid-acetate) in aqueous solution, leaving a broad non-buffered zone between them. In case of ammonium formate this non-buffered zone is even wider (5.5 units) because formic is more acidic than acetic acid. These pK_a differences are progressively reduced with the addition of the organic solvent (ammonium is a cationic acid, formate and acetate are conjugate bases of neutral acids), but in spite of this fact the difference between pK_a values is still substantial at high organic solvent concentrations. Therefore when these buffers are used for low pH

separations there is no contribution from ammonium in the improvement of acetate buffer capacity, and their buffer capacity at intermediate pH values is really poor.

Ammonium hydrogencarbonate has been described as an excellent buffer for the analysis of basic drugs by HPLC-MS[10]. In fact, this mixed buffer presents a good buffer capacity in a relatively wide pH range, because the buffer capacity of ammonium-ammonia species is added up to the one corresponding to hydrogencarbonate-carbonate (Figure 4). In this case, in contrast to ammonium formate or acetate, the difference on aqueous pK_a values is only about 1 unit. Therefore, there is a wide pH range of excellent buffer capacity at least from pH 8 to 11 in aqueous solution, and if the concentration of the buffer is high enough it might be wider. When acetonitrile is added, ammonium becomes more acidic and hydrogencarbonate more basic, so differences on pK_a values are increased. But the pK_a values of the buffering species are still close enough to provide a wide range of good buffer capacity, and it widens with the addition of acetonitrile. McCalley reported[11] instability of ammonium hydrogencarbonate solutions at pH around 7, probably because of the poor buffering capacity of this buffer in the valley between the first and the second pK_a values.

Summary

Going a step forward from the “Mobile-Phase Buffers” series by Tindall and Dolan published in this journal, in the present article is discussed the dependence of maximum buffer capacity with ionic strength and organic modifier content. It is also pointed out the definition of buffer capacity as an additive quantity of buffer capacities of the acid-base pairs which constitute the particular buffer system, together with the contribution of H^+ and OH^- at very low and high pH values, respectively. Finally, the buffer capacity profile of usual MS-friendly buffers containing ammonium as co-ion is presented.

FIGURES

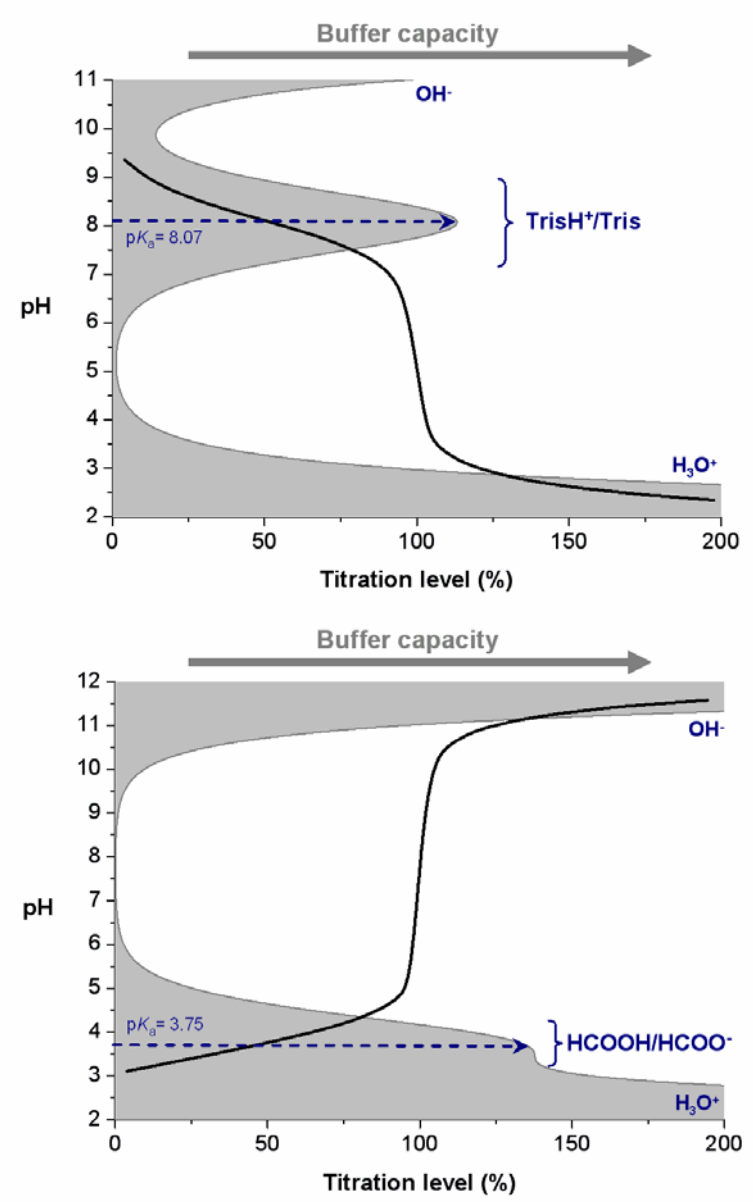

Figure 1. Titration curves of tris(hydroxymethyl)aminomethane (0.005M) with KOH (0.1M), and formic acid (0.005M) with HCl (0.1M) in aqueous solution, together with their corresponding buffer capacity.

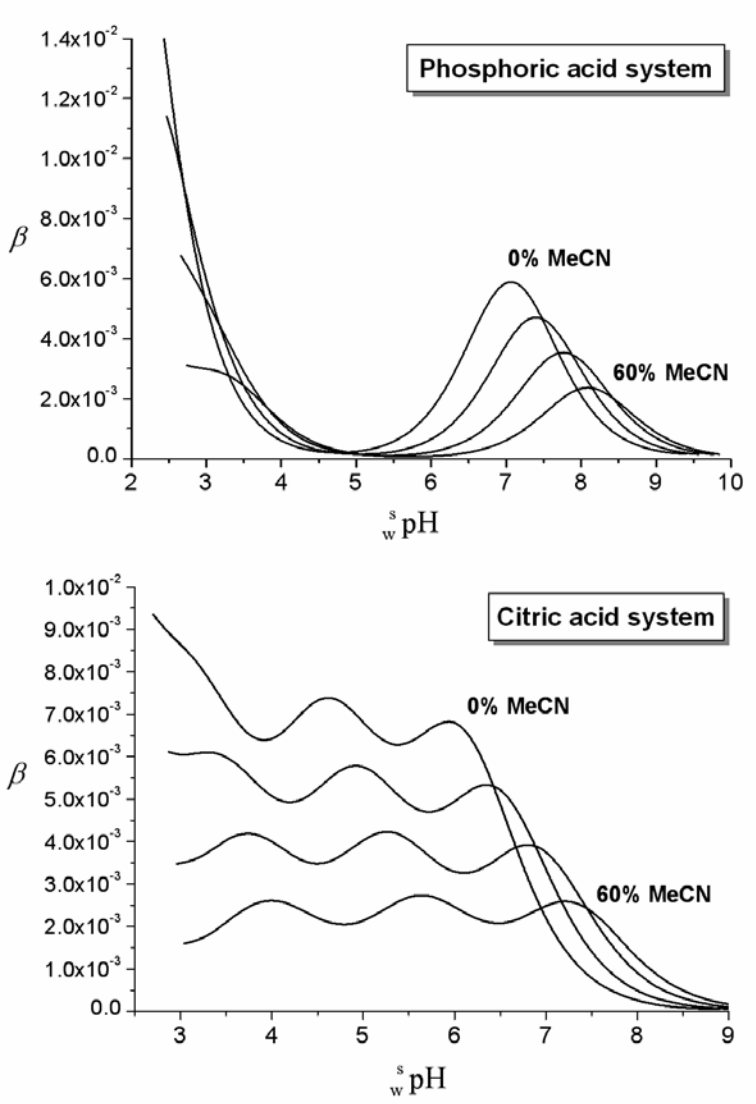

Figure 2. Buffer capacity variation of phosphoric and citric acids systems with the addition of acetonitrile. Aqueous buffer concentration: 0.01 mol·L^{-1}.

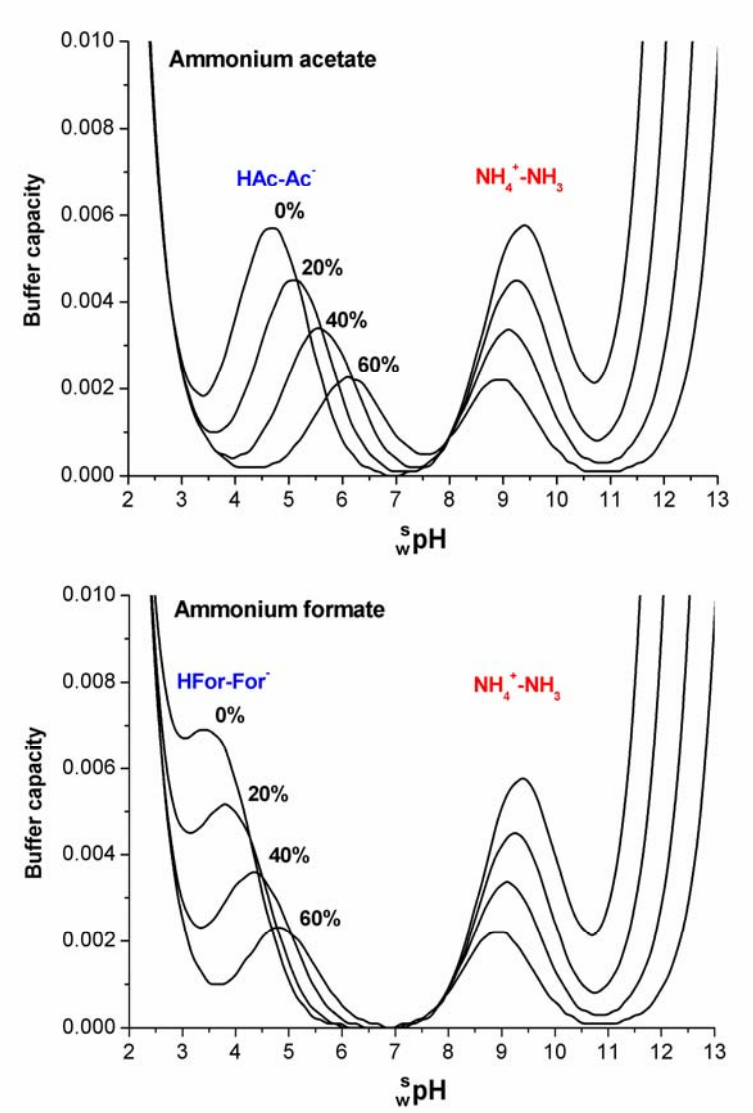

Figure 3. Variation of buffer capacity for aqueous ammonium acetate and ammonium formate solutions of concentration 0.01 mol·L^{-1} when acetonitrile is added.

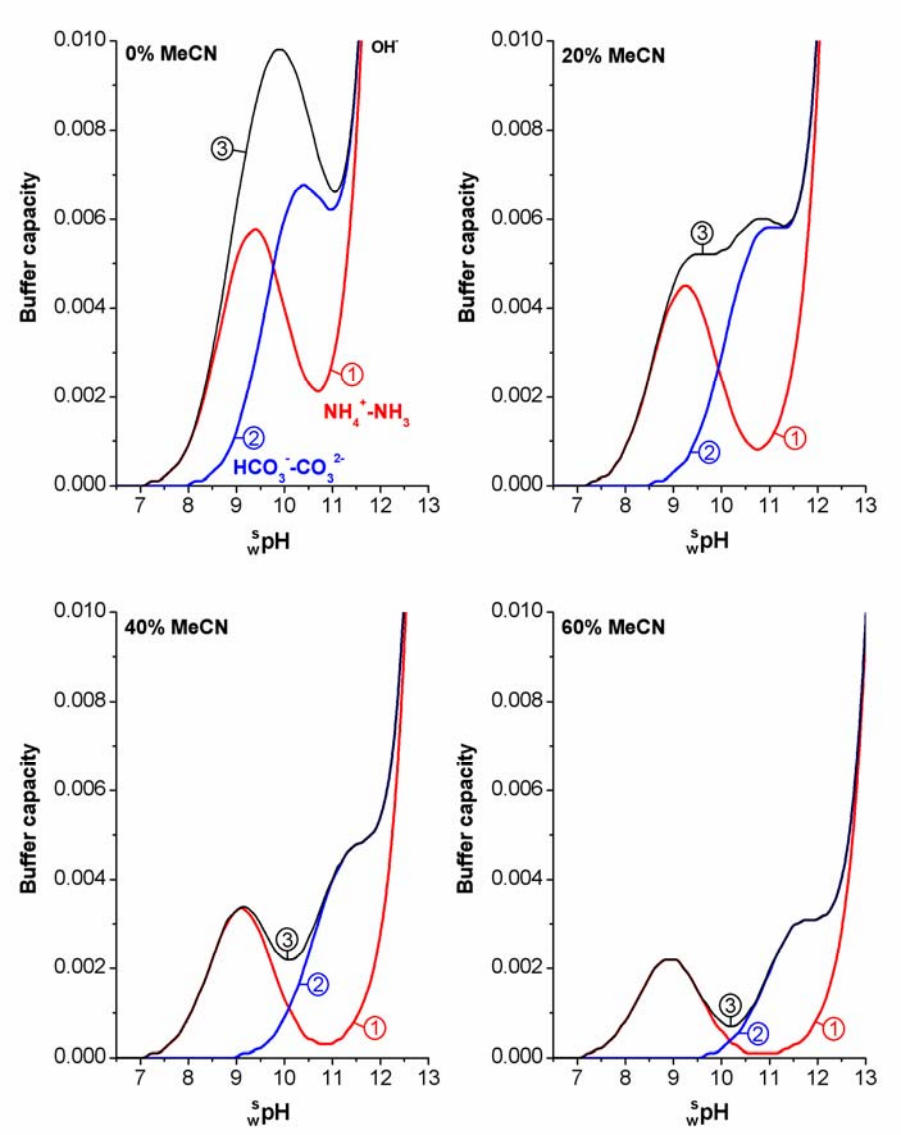

Figure 4. Variation of buffer capacity for an aqueous ammonium hydrogencarboante solution of concentration 0.01 mol·L^{-1} when acetonitrile is added. Legend: (1) ammonium-ammonia, (2) hydrogencarbonate-carbonate, (3) total buffer capacity.

TABLES

Table 1. pH values of maximum buffer capacity in aqueous solution at different concentrations.

		pH values of maximum buffer capacity		
Buffer acid-base pair	pK_a	0.01 M	0.05 M	0.1 M
Acetic acid-acetate	4.76	4.73	4.69	4.67
Dihydrogencitrate-hydrogencitrate	4.76	4.58	4.42	4.34
PiperazineH_2^{2+}-piperazineH^+	5.33	5.51	5.67	5.75
Hydrogencitrate-citrate	6.40	5.98	5.67	5.52
Dihydrogenphosphate-hydrogenphosphate	7.20	7.02	6.86	6.78
TrisH^+-Tris	8.07	8.10	8.14	8.16
Ammonium-ammonia	9.25	9.28	9.32	9.34
Hydrogencarbonate-carbonate	10.33	10.15	9.99	9.91

REFERENCES

1. G. W. Tindall and J. W. Dolan, *LC·GC Eur.*, **15**, 776 (2002).
2. G. W. Tindall and J. W. Dolan, *LC·GC Eur.*, **16**, 10 (2003).
3. G. W. Tindall and J. W. Dolan, *LC·GC Eur.*, **16**, 64 (2003).
4. D. D. Van Slyke, *J. Biol. Chem.*, **52**, 525 (1922).
5. L. Šůcha and S. Kotrlý, *Solution Equilibria in Analytical Chemistry*, Van Nostrand Reinhold, London, 1972.
6. J. N. Butler, *Ionic equilibrium. A mathematical approach*, Addison-Wesley, Reading, MA, USA, 1964.
7. R. N. Goldberg, N. Kishore, and R. M. Lennen, *J. Phys. Chem. Ref. Data*, **31**, 231 (2002).
8. S. Espinosa, E. Bosch, and M. Rosés, *Anal. Chem.*, **74**, 3809 (2002).
9. I. Canals, F. Z. Oumada, M. Rosés, and E. Bosch, *J. Chromatogr. A*, **911**, 191 (2001).
10. A. Espada and A. Rivera-Sagredo, *J. Chromatogr. A*, **987**, 211 (2003).
11. D. V. McCalley, *J. Chromatogr. A*, **987**, 17 (2003).