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Characterization of Buffers for Electrokinetic Separations

SUBIR K. BASAK,[1] AJOY VELAYUDHAN,[2] AND MICHAEL R. LADISCH*,[3]

[1]*Department of Agricultural Engineering;* [2]*Laboratory of Renewable Resources Engineering; and* [3]*Department of Agricultural Engineering, Purdue University, 1295 Potter Center, West Lafayette, IN 47907-1295*

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ABSTRACT

Buffers used in electrophoresis and electrochromatography must have a relatively low ionic strength in order to minimize ohmic heating in the presence of an applied potential. Calculation of pH, ionic strength, and the van Slyke buffer capacity, β, is therefore important. This paper describes the *a priori* calculation of these parameters for tris buffer made up with either glycine (a zwitterion) or HCl. A quadratic expression for pH, valid over wide ranges, is obtained for both buffer systems. The calculated values of pH, ionic strength, and buffer capacity are shown to agree with experimental results as a function of tris, HCl, and glycine concentrations ranging from 1 to 50 m*M*. A new parameter, the electrokinetic buffer effectiveness factor, is introduced to characterize buffers being considered for use in electrokinetic systems such as electrochromatography, and is used to determine the appropriate composition ranges for the buffer components.

Index Entries: Zwitterions; buffer; electrochromatography; pH; buffer-capacity; ionic strength; tris; glycine; HCL.

Nomenclature: b, amount added to strong alkali/acid, moles; c_i, concentration of each type of ionic species, moles/L; $^+gly^-$, zwitterionic form of glycine; gly^-, negatively charged form of glycine; ^+gly, positively charged form of glycine; H^+, hydrogen ion; I, ionic strength,

*Author to whom all correspondence and reprint requests should be addressed.

mM; K_a, dissociation constant of amino group of glycine (Eq. 2), M; K_c, dissociation constant of carboxyl group of glycine (Eq. 3), M; K_{Na}, dissociation constant of NaOH (Eq. 16), M; K_t, dissociation constant of tris (Eq. 4), M; k_w, dissociation constant of water (Eq. 5), M; tris, 2-amino-2-hydroxymethyl-1,3-propanediol, $tris^+$, protonated form of tris; U, intermediate expression defined by Eq. (26), M; z, charge of an ion, Coulomb; [x], represents concentration of a species x, M; $[x]_t$, represents total concentration of a species x, M; *Greek Symbols:* β, buffer capacity, M; η, electrokinetic buffer effectiveness, dimensionless; *Acronyms:* HEPES, *N*-2-hydroxyethyl-piperazine-*N*'-2-ethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; MOPS, 3-(*N*-morpholino)propanesulfonic acid; TEA, triethanolamine; TES, *N*-Tris (hydroxymethyl)methyl-2-aminoethanesulfonic acid.

INTRODUCTION

Electrophoresis separations are based upon differences in surface charge density and zeta potential among target molecules. These differences can be enhanced by a suitable choice of buffer. Since electrolysis occurs at the electrodes, the buffer must negate possible changes in pH because of the reverse flow of ions, and maintain a constant charge on the molecules being separated (*1*). In addition, low ionic strengths are desirable since this increases the target molecules' zeta potentials and mobilities and, in general, has been found to give larger differences in mobility (*1*). Buffers of low ionic strength have the additional advantage of minimizing ohmic losses (*2*) (and consequently, heating effects), as well as minimizing the current through the system (*3,4*) subject to the constraint that the ionic strength be high enough to keep the solutes dissolved.

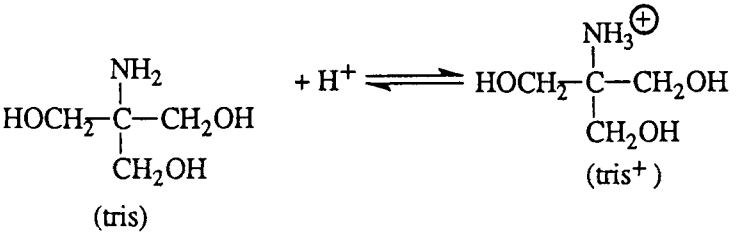
Joule heating is defined as heating caused by energy dissipation in a medium due to the passage of electric current through that medium. This effect can be minimized by reducing ionic strength since the heat generated is directly proportional to the conductivity of the solution. Haber (*5*) has demonstrated the use of extremely low conductivity buffers in obtaining protein separations in a system that "looks like standard electrophoresis" (*6*). His electromolecular propulsion technology (EMP) is based on charge transfer effects between protein molecules. The present work addresses prediction of the pH, ionic strength, buffer capacity, and effectiveness of tris-glycine buffer.

BACKGROUND

Ampholytes and zwitterions have a natural tendency to act as buffers by virtue of dissociating into both positively and negatively charged

species (*7,8*). In the physiological pH range of 6–9, several zwitterions, such as MOPS, TES, and HEPES have been widely tested (*9*). Bushey and Jorgenson (*10*) used zwitterionic buffers in the free zone electrophoresis of proteins in untreated fused silica capillaries to enhance separation efficiency and circumvent problems encountered with buffers of high ionic strength. The zwitterions associate with the proteins, thereby both reducing their adsorption to the capillary wall and disrupting protein–protein interactions. Since zwitterions do not contribute to the conductivity of the operating buffer, higher voltages can be used and migration times can be shortened.

Tris is a monoacidic buffer that has been widely used in biochemical studies after having been recommended in 1946 for pH control in the range of 6.5–9.7 (*11,12*). Tris, or tris-(hydroxymethyl)aminomethane, represented by the IUPAC name of 2-amino-2-hydroxymethyl-1,3-propanediol, is a stable, crystalline solid (*13*) with a pK of 8.08 (*14*):



(1)

Tris buffers are widely used in electrophoresis and chromatography (*8*). Poulik used a dilute tris-citrate buffer of pH 8.65 (76 m*M* Tris, 5 m*M* citric acid) in 1957 for protein separations (*15*). Pilz and Johann used tris-acetic acid buffer for starch gel electrophoresis in 1966 (*16*).

Tris-glycine mixtures in the presence of chloride were used as buffers in disc electrophoresis by Ornstein (*17*). Jovin, Chrambach, and Naughton (*18*) used a buffer of 3.8 m*M* tris and 5.0 m*M* glycine to reach a pH of 8.3 in polyacrylamide gel electrophoresis. Orr, Blakely, and Panagon (*19*) used a tris-glycine buffer of pH 8.9 in the preparative gel electrophoresis of a variety of enzymes. They also carried out similar runs using TEA-TES as well as imidazole-MES buffers in order to examine the relative mobility of proteins as a function of buffer systems. Recently, a buffer of 47 m*M* glycine and 3.9 m*M* tris, with a pH of 8.2 and an ionic strength of 1.5 m*M*, has been used in our laboratory (*4*) to obtain electrochromatographic resolution between β-lactoglobulin B and α-lactalbumin as well as between α-lactalbumin and bovine hemoglobin.

Bates and Bower (*20*) reported experimental values of pH as a function of the fraction of amine neutralized for a Tris-HCl buffer. McFarland and Norris (*21*) gave hydrochloric acid titration curves for the same buffer as the concentration of the hydrochloride derivative of Tris (Trizma 8.3) was increased.

THEORETICAL

When glycine is dissolved in water, it is almost completely found in the charged forms $^{+}$gly-, $^{+}$gly, and gly^{-} as shown below:

$$^{+}H_3N\text{-}CH_2\text{—}C(=O)\text{—}O^{-} \underset{}{\overset{K_a}{\rightleftharpoons}} H_2N\text{-}CH_2\text{—}C(=O)\text{—}O^{-} + H^{+} \qquad (2)$$

($^{+}$gly^{-}) (gly^{-})

$$^{+}H_3N\text{-}CH_2\text{—}C(=O)\text{—}OH \underset{}{\overset{K_c}{\rightleftharpoons}} {}^{+}H_3N\text{-}CH_2\text{—}C(=O)\text{—}O^{-} + H^{+} \qquad (3)$$

($^{+}$gly) ($^{+}$gly^{-})

where $K_c = 4.458 \times 10^{-3}M$ and $K_a = 1.66 \times 10^{-10}M$. At 25°C, glycine's carboxyl group has a pK of 2.35, its amino group a pK of 9.78 (*22–24*), and its isoelectric point is 6.06 (*25*). Since $^{+}$gly^{-} acts both as a proton acceptor and a proton donor, it has two equilibrium constants given by (*22*). The corresponding dissociation constant for tris is (*14*):

$$K_t = 8.31 \times 10^{-9}\,M = \frac{[\text{tris}][H^+]}{[\text{tris}^+]} \qquad (4)$$

whereas the dissociation constant for water is:

$$K_w = 1.0 \times 10^{-14}\,M = [H^+][OH^-] \qquad (5)$$

In a dilute solution, the mass balances are:

$$[^{+}\text{gly}] + [\text{gly}^-] + [^{+}\text{gly}^-] = [\text{gly}]_t \qquad (6)$$

$$[\text{tris}] + [\text{tris}^+] = [\text{tris}]_t \qquad (7)$$

The electroneutrality condition takes the form

$$[^{+}\text{gly}] + [\text{tris}^+] + [H^+] = [OH^-] + [\text{gly}^-] \qquad (8)$$

pH Equation

An implicit expression for the hydrogen ion concentration is reached by solving Eqs. 2–8:

$$\left[H^+\right]^2 = \frac{K_w + \dfrac{[gly]_t K_a}{\left(\dfrac{\left[H^+\right]}{K_c} + \dfrac{K_a}{\left[H^+\right]} + 1\right)}}{1 + \dfrac{[tris]_t}{\left(K_t + \left[H^+\right]\right)} + \dfrac{[gly]_t}{K_c\left[\dfrac{\left[H^+\right]}{K_c} + \dfrac{K_a}{\left[H^+\right]} + 1\right]}} \tag{9}$$

On the basis of previous literature, and from experience in our laboratory, a useful range of tris-glycine concentrations, resulting in a pH of 6–9, is typically below 50 m*M* for both glycine and tris. In this restricted range, the following approximation is valid:

$$\frac{\left[H^+\right]}{K_c} + \frac{K_a}{\left[H^+\right]} + 1 \approx 1 \tag{10}$$

Eq. 9 then reduces to the following expression:

$$\left[H^+\right]^2 = \frac{[gly]_t K_a + K_w}{\dfrac{[tris]_t}{\left(K_t + \left[H^+\right]\right)} + \dfrac{[gly]_t}{K_c} + 1} \tag{11}$$

In the physiologically useful buffer range of pH 6–9, the first term in the denominator of Eq. 11 dominates the other two terms and the first term in the numerator dominates the second, giving rise to the simple quadratic expression:

$$\left[H^+\right]^2 = \frac{K_a\left(\left[H^+\right] + K_t\right)[gly]_t}{[tris]_t} \tag{12}$$

or

$$\left[H^+\right]^2[tris]_t - \left[H^+\right]\left(K_a[gly]_t\right) - K_a K_t[gly]_t = 0 \tag{13}$$

The solution of Eq. 13 gives

$$pH = -\log\left[H^+\right] = -\log\left\{\frac{K_a[gly]_t + \sqrt{\left\{K_a[gly]_t\right\}^2 + 4K_a K_t[tris]_t[gly]_t}}{2[tris]_t}\right\} \tag{14}$$

Eq. 14 is only valid for tris concentrations below 50 m*M*, and glycine concentrations below 50 m*M*. When either of these conditions is not met, the general result given in Eq. 9 must be used.

Ionic Strength Equation

The ionic strength of a buffer is defined as

$$I = \Sigma \frac{1}{2}\left(c_i z^2\right)$$

where c_i in the concentration of each type of ionic species (in moles/L) and z is the charge of the ion (*26,27*). The ionic strength of the tris-glycine buffer is given by:

$$I = \frac{1}{2}\left(\left[\text{tris}^+\right] + \left[\text{gly}^-\right] + \left[{}^+\text{gly}\right] + \left[\text{H}^+\right] + \left[\text{OH}^-\right]\right) \tag{15}$$

The concentrations of tris and tris$^+$ can be determined from Eqs. 4 and 7, using the H^+ concentration, which can be obtained through Eq. 14. The hydroxyl (OH^-) concentration is determined from Eq. 5. The concentrations of gly^-, ^+gly, and $^+gly^-$ are similarly determined from Eqs. 2, 3, 6, and 8.

Buffer Capacity Equation

The Van Slyke buffer capacity β, is defined as $db/d(\text{pH})$, where b represents the number of moles of strong alkali (or -db/d(pH) where b represents the number of moles of strong acid) added to 1 L of buffer solution. The dissociation of NaOH is described by:

$$K_{Na} = \frac{\left[\text{Na}^+\right]\left[\text{OH}^-\right]}{\left[\text{NaOH}\right]} \tag{16}$$

where $pK_{Na} = -0.77$ (*28,29*). A mass balance on NaOH results in:

$$\left[\text{Na}^+\right] + \left[\text{NaOH}\right] = \left[\text{NaOH}\right]_t \tag{17}$$

Combining Eqs. 5, 16, and 17 gives:

$$\left[\text{NaOH}\right] = \frac{\left[\text{NaOH}\right]_t}{\left[1 + \dfrac{\left[\text{H}^+\right]K_{Na}}{K_w}\right]} \tag{18}$$

In the presence of NaOH the electroneutrality equation becomes:

$$\left[{}^+\text{gly}\right] + \left[\text{Na}^+\right] + \left[\text{tris}^+\right] + \left[\text{H}^+\right] = \left[\text{gly}^-\right] + \left[\text{OH}^-\right] \tag{19}$$

The expression for the hydrogen ion concentration in the tris-glycine buffer on addition of NaOH is given by

$$[H^+]^2 = \frac{K_a[gly]_t + K_w}{\left[\frac{[gly]_t}{K_c} + \frac{[NaOH]_t}{[H^+]} + \frac{[tris]_t}{K_t + [H^+]} + 1\right]} \tag{20}$$

In may be noted that Eq. 20 has the same form as Eq. 11, except for the additional NaOH term. Substitution of Eqs. 2–7 and 16–18 into Eq. 19 gives:

$$[NaOH]_t = \left[\frac{K_a[gly]_t + K_w}{[H^+]} - \frac{[H^+][gly]_t}{K_c} - \frac{[H^+][tris]_t}{(K_t + [H^+])} - [H^+]\right] \tag{21}$$

Differentiation of Eq. 21 with respect to pH gives

$$\beta \equiv \frac{d[NaOH]_t}{d(pH)} = -2.303H\frac{d[NaOH]_t}{dH} =$$
$$2.303\left[\frac{[tris]_t K_t[H^+]}{(K_t + [H^+])^2} + \frac{K_a[gly]_t}{[H^+]} + \frac{[H^+][gly]_t}{K_c} + [H^+] + \frac{K_w}{[H^+]}\right] \tag{22}$$

When the concentration of glycine and tris are such that the pH is between 6–9, the buffer capacity is determined almost wholly by the first two terms on the right-hand side of Eq. 22. Over this range, a maximum in buffer capacity does not exist since β increases with both pH and tris/glycine concentration.

MATERIALS AND METHODS

Reagent grade Trizma base (mol wt 121.1) was obtained from Sigma Chemical Co. (St. Louis, MO) and glycine (mol wt 75.07) was furnished by Fisher Scientific (Fair Lawn, NJ). Distilled deionized water was used to prepare the buffers. One set of buffer solutions was made in 47 m*M* glycine and varying concentrations of Tris (0–4 m*M*). Another set of buffer solutions was made in 3.9 m*M* Tris and varying concentrations of glycine (0–50 m*M*). The pH of the tris-glycine buffer was determined using a Corning pH/ion meter 150 (Curtin Matheson Scientific, Houston, TX) and a Corning combination X-EL calomel electrode. The electrode was rinsed with deionized water after each measurement to remove any ions adsorbed to its surface before the next measurement. All runs were carried out at ambient temperatures.

The buffer capacity of the tris-glycine buffer was determined by adding very small amounts (5–10 mL) of 0.1*N* NaOH to 5 mL of buffer solution and measuring the change of pH. The ratio of the amount of NaOH added to the change in pH was assigned as the buffer capacity corresponding to the arithmetic means of the pHs before and after the addition of NaOH. Inaccuracies in the measurement of capacity were minimized by using small changes in pH.

RESULTS AND DISCUSSION

Characterization of Tris-Glycine Buffer

The quadratic approximation for pH given in Eq. 14 is tested against experiment in Figs. 1 and 2. In Fig. 1, the glycine concentration is held constant at 47 m*M*, whereas the tris concentration varied between 0–5 m*M*. The solid line represents Eq. 14, while the triangles are experimental data. It can be seen that the agreement is excellent. For comparison, the pH of tris alone, in the absence of glycine, is shown as the dashed line. It should be noted that this result can be obtained directly from Eq. 14 by setting the glycine concentration to zero. The dashed line may be viewed as an upper limit that would be reached as the initial fixed value of glycine decreases to zero. The other limit, that of zero tris concentration, is simply the y-intercept of the solid line, i.e., a point. The ionic strength of the tris-gly buffer, as calculated from Eq. 15, is shown as a dotted line. The ionic strength remains low, not exceeding 2 m*M*, making this buffer attractive for electrokinetic applications. By contrast, conventional ionic buffers such as phosphate have ionic strengths on the order of 100 m*M*.

Figure 2 shows the analogous situation when the tris is kept constant (at 3.9 m*M*) and the glycine concentration varies. Again the calculated values agree with the measured pH. The dashed line, representing the limit of zero tris concentration, is again obtained directly from Eq. 14. The ionic strength remains low.

Another important property of a buffer is its Van Slyke buffer value, or buffer capacity, β. This is calculated from Eq. 22 and depicted as a function of pH (calculated from Eq. 14) in Fig. 3. The increasing curve represents varying tris concentrations; the glycine concentration is constant at 47 m*M*. The decreasing curve represents changing glycine concentrations when the tris level is fixed at 3.9 m*M*. Reasonable agreement with experiment is seen.

The buffer properties discussed above—pH, buffer capacity, ionic strength—are functions of both tris and glycine concentrations, are best depicted as three-dimensional plots. Fig. 4 illustrates pH as a function of the two independent variables, the initial gly and tris concentrations. The desired pH range then determines a range of possible tris and glycine concentrations. An interesting feature of Fig. 4 can be deduced from Eq.

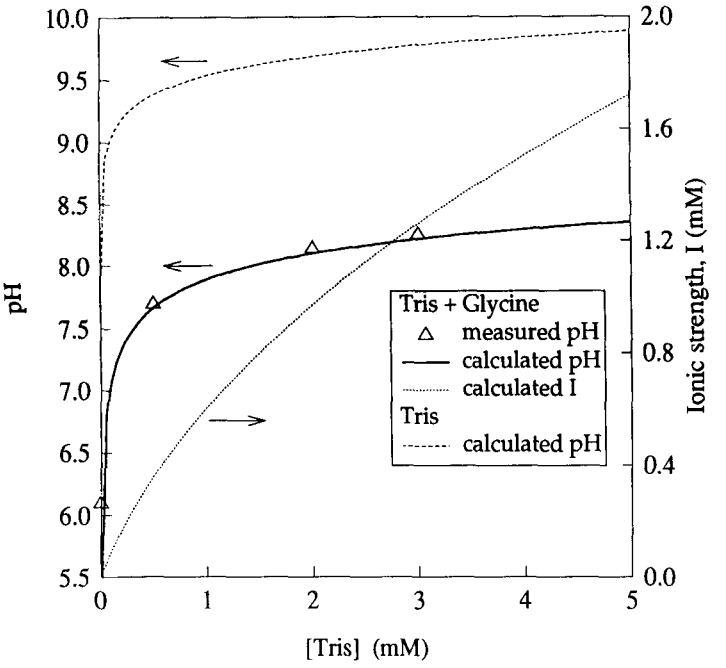


Fig. 1. pH and ionic strength of tris-glycine buffer and pH of tris buffer as a function of tris concentration for a constant glycine concentration of 47 m*M*. Experimental pH values of this buffer system were found at ambient temperature. pH was calculated using Eq. 14 and ionic strength was calculated using Eq. 15.

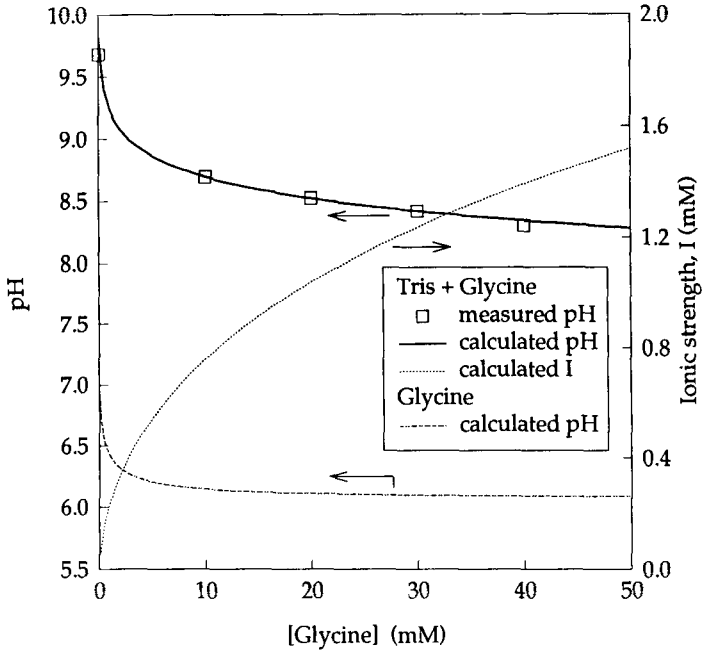


Fig. 2. pH and ionic strength of tris-glycine buffer and pH of glycine buffer as a function of glycine concentration for a constant tris concentration of 3.9 m*M*. Experimental pH values of this buffer system were found at ambient temperature. pH was calculated using Eq. 14 and ionic strength was calculated using Eq. 15.

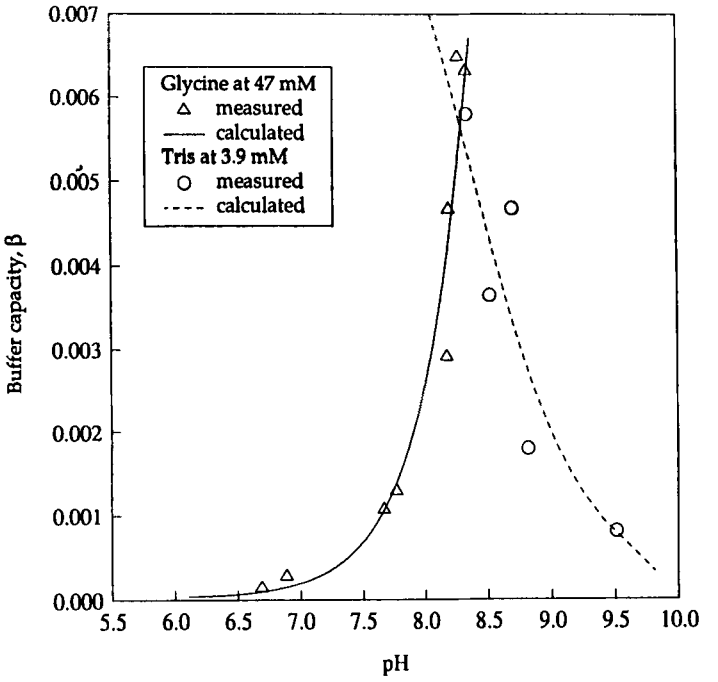


Fig. 3. Buffer capacity, β, for tris-glycine buffer for changing tris concentrations at constant glycine concentration of 47 m*M* (increasing curve) and for changing glycine concentrations at constant tris concentration of 3.9 m*M* (decreasing curve).

12: at constant pH, the initial glycine and tris concentrations are linearly related. This implies that every horizontal section plane in Fig. 4 intersects the pH surface in a straight line. In particular, when the initial concentrations of tris and glycine are equal, the pH is constant at 8.9. This can be deduced from either Eqs. 12 or 13:

$$\mathrm{pH} = -\log\left\{\frac{K_a + \sqrt{(K_a)^2 + 4K_aK_t}}{2}\right\} \tag{23}$$

Using the values previously given for the dissociation constants gives the desired result.

In an analogous fashion, three-dimensional plots for the buffer capacity and the ionic strengths are given in Figs. 5A and B, respectively. This figure depicts a monotonic increase in both β and I with increase in either tris or glycine level. However, the constraints of electrochromatography, and within certain limitations, electrophoresis, and other allied electrokinetic separation techniques require that β be as large as possible and I as small as possible (with the added requirement that I be large enough to keep all the adsorbates in solution). Mikkers et al. (*30*),

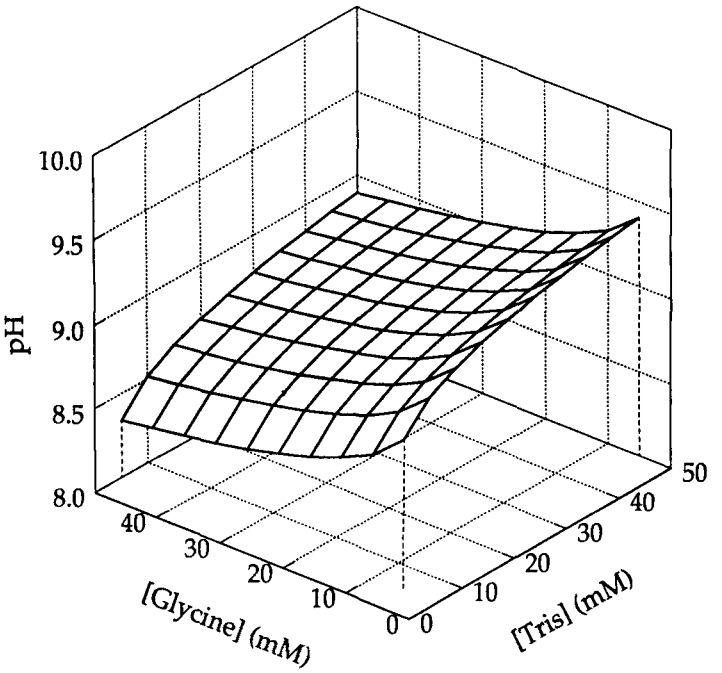


Fig. 4. pH of tris-glycine buffer as a function of tris and glycine concentrations. The range of tris concentration is 5–50 m*M* and that of glycine concentration is 5–50 m*M*.

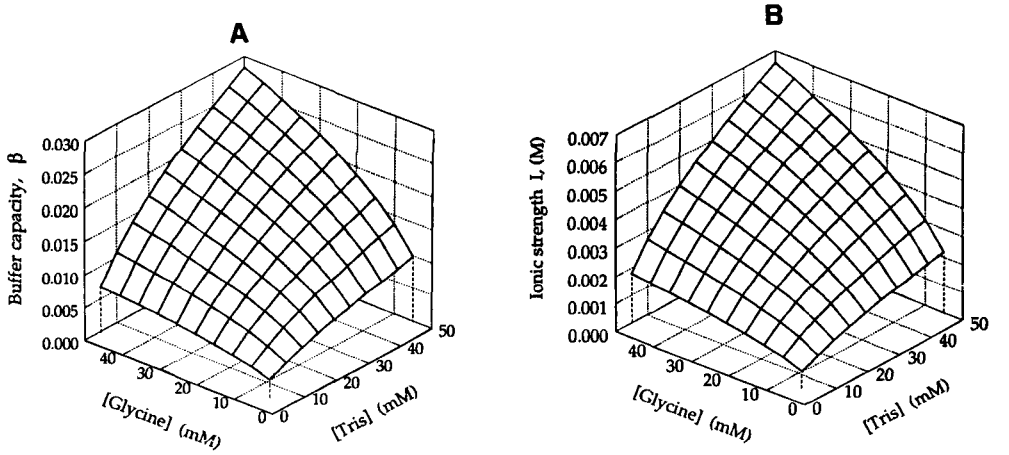


Fig. 5. Buffer capacity (Fig. 5A) and ionic strengths (Fig. 5B) of tris-glycine buffer as a function of tris and glycine concentrations.

reported the dominance of electromigration dispersion over Joule heating effects when the ratio of the sample zone concentration to the carrier zone concentration is greater than 0.01. However, this does not affect use of zwitterionic buffers, which at even high concentrations maintain low ionic strengths when compared to other conventional buffers. Hjerten (*31*) had suggested that the lower the conductivity of the buffer, the

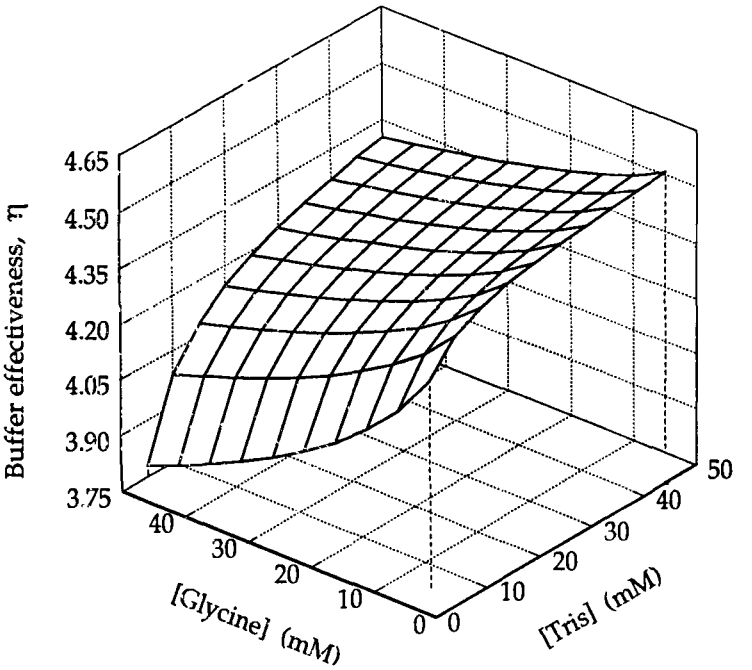


Fig. 6. Buffer effectiveness (η) as a function of tris and glycine concentrations for tris-glycine buffer.

greater would be the risk of peak asymmetry. However, for macromolecules such as proteins, low conductivity values would be preferred (*31*). Thus we define a dimensionless parameter, the electrokinetic buffer effectiveness factor, η, as the ratio of β to I. For the tris-glycine buffer, η is readily expressed as

$$\eta = 2.303 \frac{\dfrac{K_a[gly]_t}{[H^+]} + \dfrac{K_t[tris]_t[H^+]}{(K_t + [H^+])^2} + \dfrac{[H^+][gly]_t}{K_c}}{\left[\dfrac{K_a[gly]_t}{[H^+]} + \dfrac{[tris]_t[H^+]}{K_t + [H^+]}\right]} \tag{24}$$

This result is shown as a three-dimensional plot in Fig. 6. The surface is qualitatively very similar to that of the pH (cf. Fig. 4); again, η is constant (=4.3) when the initial concentrations of tris and glycine are equal. It can be seen that, in general, decreasing the glycine level and increasing the tris level improves buffer effectiveness. A more detailed and intuitive picture can be drawn by using specific constraints. Let us say we wish our buffer to operate at a pH below 9, with a buffer capacity of at least 0.02, but with an ionic strength of less than 15 m*M*. These constraints and the

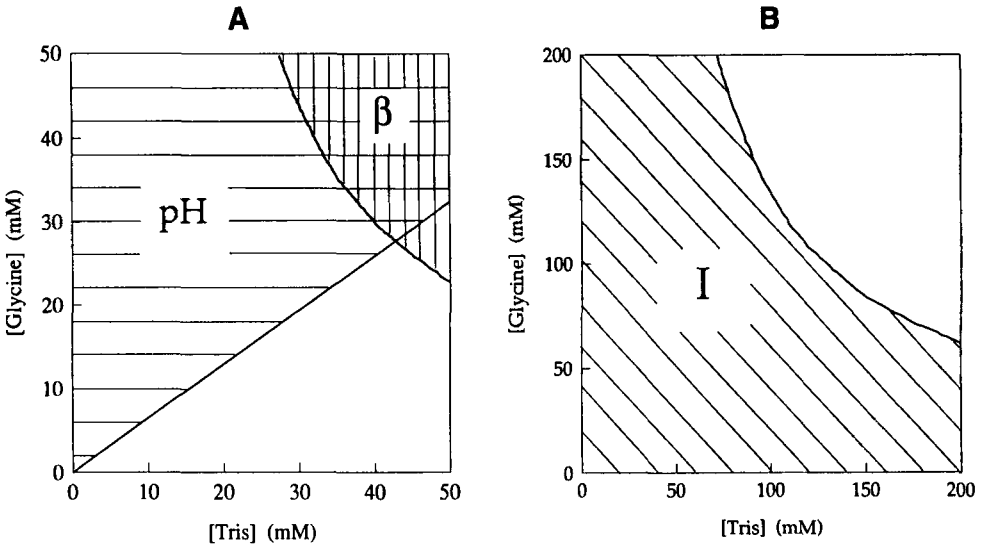


Fig. 7. Constrained optimization of buffer composition. The pH and buffer capacity constraints (9 and 0.02, respectively) for a suitable electrokinetic buffer are represented in Fig. 7A by the shaded regions for tris and glycine concentrations varying from 0–50 m*M*. The intersection of these two regions represents a region where both constraints are satisfied simultaneously. The corresponding ionic strength constraint (upper limit of 0.015*M*) is shown in Fig. 7B. The shaded region represents tris and glycine concentrations that correspond to an ionic strength of 0.015*M* or less.

consequent regions of admissible tris and glycine concentrations are shown in two-dimensional form in Fig. 7. Figure 7A graphs the pH and β constraints: The horizontal hatching indicates the region over which the pH condition is met, and the vertical hatching similarly describes the β constraint. Thus the cross-hatched region is where β and pH constraints are satisfied simultaneously. The ionic strength constraint is shown in Fig. 7B: The tris-glycine buffer has such a low ionic strength that the constraint is not met only at concentrations of tris and glycine on the order of 100 m*M*. Thus this constraint does not provide any serious restriction in practice, compared to the other two constraints.

The ionic strength constraint was relatively unimportant in the example discussed above. However, it is likely to be extremely significant for other buffer systems. For example, the common ionic buffers such as phosphate and citrate, which have ionic strengths on the order of 100 m*M* would be excluded on the basis of the I constraint.

The preferred ranges of the concentrations of the individual components of a multicomponent buffer to be used in electrophoretic applications can thus be made by specifying the pH, placing an upper bound on ionic strength and a lower bound for buffer capacity.

Characterization of Tris-HCl Buffer

This approach of theoretically calculating equilibrium values of pH, buffer capacity, ionic strength, and buffer effectiveness was applied to another common buffer, Tris-HCl. The derivation of the expression for pH is analogous to that for tris-glycine, and gave the result:

$$\left[H^+\right]^2 + U\left[H^+\right] - [HCL]_t K_t = 0 \tag{25}$$

where

$$U = K_t + [tris]_t - [HCl]_t \tag{26}$$

The solution of Eq. 25 is:

$$pH = -\log\left[H^+\right] = -\log\left\{\frac{-U + \sqrt{U^2 - 4\left[H^+\right]\left[HCl\right]_t K_t}}{2}\right\} \tag{27}$$

The pK_a of HCl was taken as -6.1 (*28*). Measured pH values for this buffer (*17*) were consistent with values calculated from Eq. 27 (Fig. 8). The buffer capacity of the tris-HCl buffer was found to be

$$\beta = -\frac{db}{d(pH)} = -2.303\left[H^+\right]\frac{db}{dH} = 2.303\left[\frac{[tris]_t K_t\left[H^+\right]}{(K_t + \left[H^+\right])^2} + \left[H^+\right] + \frac{k_w}{\left[H^+\right]}\right] \tag{28}$$

This expression was found to be consistent with an expression derived for a buffer system composed of amine hydrochloride and the free amine (*32*). The ionic strength is given by

$$I = \frac{1}{2}\left[tris^+ + Cl^- + H^+ + OH^-\right] = \frac{1}{2}\left[\frac{\left[H^+\right][tris]_t}{\left(K_t + \left[H^+\right]\right)} + [HCl]_t + \left[H^+\right] + \left[OH^-\right]\right] \tag{29}$$

The ionic strength is dominated by the first two terms on the right-hand side of Eq. 29. These terms are in fact equal, to a very good approximation, as can be seen from the electroneutrality equation for this system:

$$\left[tris^+\right] + \left[H^+\right] = \left[Cl^-\right] + \left[OH^-\right] \tag{30}$$

Again, the first term on both sides of Eq. 30 dominates (in the pH range of 6–9), giving

$$\left[tris^+\right] \cong \left[Cl^-\right] \cong [HCl]_t \tag{31}$$

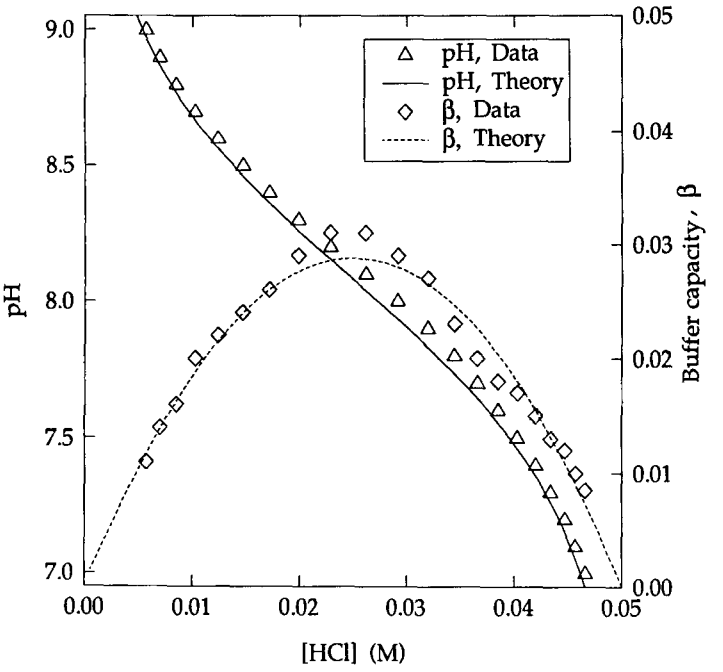


Fig. 8. pH and buffer capacity of tris-HCl buffer as a function of HCl concentration. Tris concentration maintained at 50 m*M*. Experimental values of pH and buffer capacity were taken from Bates and Bower (*20*).

However, $tris^+$ is determined from Eqs. 4, 7, and 30 as

$$\left[tris^+\right] = \frac{\left[H^+\right][tris]_t}{K_t + \left[H^+\right]} \tag{32}$$

Consequently, Eq. 29 for the ionic strength can be rewritten as

$$I \cong \frac{1}{2}\left\{\left[tris^+\right] + [HCl]_t\right\} \cong \left[tris^+\right] \cong [HCl]_t \tag{33}$$

Bates (*20*) has used the last form of Eq. 33, $I = [HCl]_t$, to calculate ionic strength when the initial tris concentration is held constant and the HCl concentration varies. Our more general result, Eq. 33, shows that $I = [tris^+]$ when the HCl concentration is held constant and the initial tris concentration varies, and, in the general case, is the sum of these quantities. The electrokinetic buffer effectiveness factor, η, is then obtained as the ratio of β to I from Eqs. 28 and 29.

In the context of electrochromatographic buffers, although tris-HCl has a low ionic strength in the pH range 7–9, its buffering capacity is small at moderately acid pH. It therefore has a relatively low effectiveness

factor, with a maximum of $\eta = 2$ at pH = 8.9. By contrast, the η of tris-gly buffer is well above 3 throughout and is above 4 at pH = 8.9. Hjerten (*31*) had suggested the use of Orstein's (*17*) discontinuous buffer systems in CZE and one of them is Tris/glycine of comparable composition. Thus zwitterionic buffers, such as the tris-gly buffer described above, are preferable, particularly for biopolymeric solutes.

CONCLUSIONS

The simple quadratic expression for pH given in Eq. 14 is valid for many classes of practical buffers. It will apply whenever the buffer system consists of a monoprotic zwitterionic component (e.g., glycine) added to a monoprotic ionic component (e.g., acetate, HCl), and the pK values are sufficiently far apart, allowing the approximation in Eq. 10 to be made. Thus simple explicit expressions for pH are available for systems that have been found useful, such as CHES-K_2SO_4 (*10*). As outlined in the Appendix, similar results can be obtained for a buffer consisting of two monoprotic zwitterionic species.

Buffer capacity relative to ionic strength is a key specification for tris-glycine and tris-HCl buffers in electrophoretic separations. An electrokinetic buffer effectiveness has been defined which facilitates *a priori* computation of those buffer compositions best suited to electrophoretic and electrochromatographic separations. These calculations reflect the benefits of low ionic strength and the advantages of zwitterionic species. A method to select the composition of the buffer (tris) and the buffer additive (glycine) was demonstrated with calculated and experimental results giving satisfactory agreement.

APPENDIX

The properties of a buffer consisting of two zwitterions can be calculated in a manner entirely analogous to that carried in the text for a buffer consisting of a zwitterionic and an ionic species. Consider two monoprotic zwitterionic species, $^+A^-$ and $^+B^-$. The acid dissociation constant for species $^+A^-$ is described by

$$K_{Aa} = \frac{\left[{}^+A^-\right]\left[H^+\right]}{\left[{}^+A\right]} \qquad \text{(A-1)}$$

and its basic dissociation constant by

$$K_{Ab} = \frac{[A^-][H^+]}{[{}^+A^-]} \tag{A-2}$$

Analogous definitions obtain for K_{Ba} and K_{Bb}. The mass balances take the form

$$[{}^+A^-] + [{}^+A] + [A^-] = [A]_t \tag{A-3}$$

$$[{}^+B^-] + [{}^+B] + [B^-] = [B]_t \tag{A-4}$$

and the electroneutrality condition is

$$[H^+] + [{}^+A] + [{}^+B] = [OH^-] + [A^-] + [B^-] \tag{A-5}$$

When pK_{Aa} and pK_{Ab} are far from the operational pH range (e.g., for a pH buffering range of 6–8, $pK_{Aa} \cong 3$, $pK_{Ab} \cong 11$), the species A is found almost wholly as the zwitterion $^+A^-$. Thus

$$[{}^+A^-] = \frac{[A]_t}{1 + \dfrac{[K_{Ab}]}{[H^+]} + \dfrac{[H^+]}{K_{Aa}}} \cong [A]_t \tag{A-6}$$

A similar result is obtained for species B. Substituting these results into the electroneutrality equation, Eq. A-5, the pH can be calculated as

$$[H^+]^2 = \frac{K_w + K_{Ab}[{}^+A^-] + K_{Bb}[{}^+B^-]}{1 + \dfrac{[{}^+A^-]}{K_{Aa}} + \dfrac{[{}^+B^-]}{K_{Ba}}} \tag{A-7}$$

As before, the first terms in both the numerator and denominator of Eq. A-7 are relatively negligible, leading to

$$[H^+]^2 \cong \frac{K_{Ab}[A]_t + K_{Bb}[B]_t}{\dfrac{[A]_t}{K_{Aa}} + \dfrac{[B]_t}{K_{Ba}}} \tag{A-8}$$

This result has the same linear homogeneous dependence on the two variables, $[A]_t$ and $[B]_t$, as the earlier result, Eq. 11, derived in the text, and will exhibit many similar properties. For example, when the initial concentrations of A and B are equal (or lie in a constant ratio), the pH becomes constant.

Similar results may be deduced for the buffer capacity, ionic strength, and effectiveness factor.

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