

Basic principles of electrolyte chemistry for microfluidic electrokinetics.

Part I:† Acid–base equilibria and pH buffers‡§

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We review fundamental and applied acid–base equilibrium chemistry useful to microfluidic electrokinetics. We present elements of acid–base equilibrium reactions and derive rules for pH calculation for simple buffers. We also present a general formulation to calculate pH of more complex, arbitrary mixtures of electrolytes, and discuss the effects of ionic strength and temperature on pH calculation. More practically, we offer advice on buffer preparation and on buffer reporting. We also discuss “real world” buffers and likely contamination sources. In particular, we discuss the effects of atmospheric carbon dioxide on buffer systems, namely, the increase in ionic strength and acidification of typical electrokinetic device buffers. In Part II of this two-paper series, we discuss the coupling of acid–base equilibria with electrolyte dynamics and electrochemistry in typical microfluidic electrokinetic systems.

Introduction

Microfluidics is a rapidly expanding group of enabling technologies in the fields of chemistry, biology, and biomedical engineering.^{1–3} System designs are growing increasingly complex and more specialized, and a wide array of electrolyte chemistry is being employed. Electrolyte chemistry is a key consideration in nearly all microfluidic devices and techniques, and this is particularly true of electrokinetic systems. On-chip mixing, reaction, separation, purification, electrophoretic separation, and electroosmotic flow are each inherently physicochemical processes and consequently coupled to ionic concentrations, reactions, and equilibria. Further, Faraday current and

associated electrode reactions are coupled to and can significantly affect electrolyte chemistry and primary functions of a microfluidic device. Well-controlled and well-specified chemistry in electrokinetics is crucial to systematic experimental design, robust operation, and reproducibility.

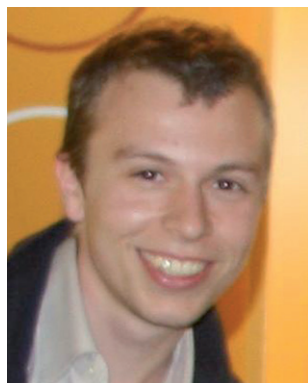
Despite its importance, the rationale behind the choice of electrolyte chemistries and pH buffers used for specific applications are rarely directly addressed in the microfluidics literature. In particular, the various principles associated with these phenomena have not, to our knowledge, been summarized in a paper easily addressable to the microfluidics community. We here present a general introduction to the fundamental principles of electrolytes and acid–base chemistry. We also summarize some misconceptions and inconsistencies we have observed in the microfluidics literature. These include errors in ionic concentration estimates; erroneous or incomplete reports of buffer chemistry; and inconsistent or incomplete reports of electrophoretic mobilities. Our intent is not to merely point these out, but to offer a basic description of the problem, suggest formulations, and provide a practical summary of how to choose electrolytes and buffers for microfluidics.

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We begin with an introduction to some essential relations and concepts in the aqueous chemistry of microfluidic electrokinesis. We then offer formulations and concepts extendable to the analysis, modeling, and practical design and control of fairly complex species transport and electrokinetic systems. We then apply these concepts to, and discuss elements of, pH buffer chemistry in the scope of electrokinetic experiments. We include practical tips associated with pH buffer selection, measurement, and reporting. In Part II of this two-paper series,⁴ we discuss the effects of acid–base equilibria on electrophoretic mobilities and conductivities, and discuss the coupling between electrolyte chemistry and electrode reactions.

Nomenclature

HA	acid form of species A
A [−]	basic form of species A
BH ⁺	acid form of species B
B	basic form of species B
H ₃ O ⁺	hydronium ion
H ⁺	proton and short notation for hydronium ion
OH [−]	hydroxyl ion
a _X	activity of X
c _{X,z}	concentration of X in valence state z (M)
I	ionic strength (M)
K _a	acid dissociation constant
K _{X,z}	acid dissociation constant of X in valence z
K _w	water autoprotolysis constant
n _X	minimum valence of species X
p _X	maximum valence of species X



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cells. He has received a Frederick Emmons Terman Faculty Fellowship ('98–'01); won the National Inventor's Hall of Fame Collegiate Inventors Competition ('01); was awarded the Outstanding Achievement in Academia Award by the GEM Foundation ('06); and was awarded a National Science Foundation Presidential Early Career Award for Scientists and Engineers (PECASE) ('03–'08). Santiago has given 13 keynote and named lectures and more than 100 additional invited lectures. He and his students have been awarded nine best paper and best poster awards. Since 1998, he has graduated 14 PhD students and advised 10 postdoctoral researchers. He has authored and co-authored 95 archival publications, authored and co-authored 190 conference papers, and been awarded 25 patents.

R	gas constant = 8.314 J K ^{−1} mol ^{−1}
T	temperature (K)
γ _X	activity coefficient of species X
γ _±	mean activity coefficient of a cation/anion pair

Equilibrium chemistry and pH

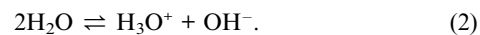
Until the 18th century, acid and basic substances were classified only on rudimentary observable or sensed properties such as taste (the word “acid” comes from the Latin “*acetum*”, sour),⁵ tactile sensation (*e.g.*, the soapy feeling of bases), or chromatic properties.⁶ After the pioneering work of Lavoisier (*ca.* 1777) on the chemistry of acidity and in the light of the first experiments on electric conduction in water, Arrhenius (*ca.* 1880) hypothesized that charged molecules were responsible for current flow through liquids, and proposed that hydrogen ions carry current in acids and hydronium ions do in bases.⁵ Arrhenius' seminal work is at the basis of the modern definition of acids and bases given by Brønsted and Lowry:^{7,8} an acid (*resp.* a base) is a proton donor (*resp.* acceptor).

We consider the general dissociation reaction of an acid HA into its conjugate base A[−]:

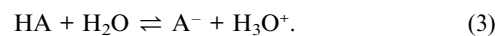


In this reaction, H⁺ is merely a designation for the donated proton, which is most generally accepted by a solvent molecule. In this paper, we will only consider aqueous solutions.

Water is both an acid and a base, as it is both a proton donor and a proton acceptor. The exchange of a proton between two water molecules is called autoprotolysis:



The reaction of dissociation of an acid HA in water is:



We can see from equation (3) that qualitatively, the amount of hydronium ions H₃O⁺ in solution is a measure of the affinity of A[−] for protons (in the rest of the paper, we will often use the shorthand H⁺ to designate the hydronium ion). From Le Chatelier's principle, reaction (3) shows that increasing the concentration of H₃O⁺ reduces the degree of dissociation of HA. This observation encouraged Sorensen⁹ to develop a scale to measure hydronium concentration in his study on enzymatic activity: this is the pH scale.

As of today, the official definition of pH is:¹⁰

$$\text{pH} \equiv -\log_{10} a_{\text{H}^+}, \quad (4)$$

where *a*_{H⁺} is the activity of the hydronium ions on the molal scale. This definition holds for pH ranging between 2 and 12, and ionic strength below 100 mM.¹⁰ In this paper, we will assume this definition is equivalent to the measurement given by a (calibrated) Harned cell pH-meter.^{10,11} The activity *a*_X can be viewed as an effective concentration of the species X. It is defined from the chemical potential *G*_X as:

$$a_X \equiv \exp\left(\frac{G_X - G_X^\circ}{RT}\right), \quad (5)$$

where G_X° is the chemical potential of X in some specified standard state,^{12,13} for example a pure state. In this paper, we will consider the case of sufficiently diluted solutes so that density of water is approximately 1 kg L⁻¹. In this conditions, the molal and molar scale activities are equal.¹²⁻¹⁴ We define the molar scale activity coefficient γ_X as $a_X = \gamma_X c_X / c^\circ$, where $c^\circ \equiv 1$ M.¹³ As is common practice, we will omit c° in such activity relations and replace it by its value so the quantity c_X / c° is written simply as c_X . γ_X is a dimensionless quantity which measures the departure of the concentration of component X from its ideal behavior. That is, it quantifies the departure from a hypothetical state where it is infinitely diluted.¹³ The activity coefficient depends on the component's environment in solution, including ionic strength, solvent permittivity, and temperature. The law of mass action applied to equation (3) defines the acid dissociation constant K_a of the pair HA and A⁻:

$$K_a = \frac{a_{\text{H}} a_{\text{A}^-}}{a_{\text{HA}} a_{\text{H}_2\text{O}}} = \frac{c_{\text{H}} c_{\text{A}^-}}{c_{\text{HA}}} \frac{\gamma_{\text{H}} \gamma_{\text{A}^-}}{\gamma_{\text{HA}}}, \quad (6)$$

the activity of the solvent being unity.¹³ Taking the logarithm of equation (6) relates pH and species activities:

$$\text{pH} = \text{p}K_a + \log_{10} \frac{\gamma_{\text{A}^-} c_{\text{A}^-}}{\gamma_{\text{HA}} c_{\text{HA}}}, \quad (7)$$

where $\text{p}K_a \equiv -\log_{10} K_a$.

The interpretation of equation (7) is difficult because pH is expressed in terms of activities. There are no generally applicable models which can be used to relate concentrations to activity coefficients. For example, key parameters determining this functional dependence include valence, ionic strength, temperature.^{12,15} Therefore, the relation of these equations to the concentrations in solution created using known amounts of electrolytes remains complex.¹⁴ For example, direct measurements of pH yields a quantification of the relation between activities, while direct measurement of an amount of weak electrolyte and its titrant (*e.g.*, by weighing) typically yields estimates of their total concentrations. In the Section on non-ideal solutions later, we provide one relatively simple theory by Davies¹⁶ which can be used to estimate activity coefficients.

For now, we can obtain more insight from equation (7) in the limit of an infinitely diluted solute. In this limit, activity coefficients are near unity and $\gamma_X \approx 1$, so that equation (7) becomes the Henderson Hasselbalch equation:^{17,18}

$$\text{pH} = \text{p}K_a + \log_{10} \frac{c_{\text{A}^-}}{c_{\text{HA}}}. \quad (8)$$

Williams *et al.*¹⁹ compiled a large database of $\text{p}K_a$ (mostly at infinite dilution) which is available online. Knowledge of the solution pH then yields the relative concentration of base A⁻ to its conjugate acid HA. In particular, for a monovalent acid

- At $\text{pH} > \text{p}K_a + 1$, $c_{\text{A}^-} > 10 c_{\text{HA}}$, so that A is mostly in its basic form A⁻ (nearly fully ionized)

- At $\text{pH} < \text{p}K_a - 1$, $c_{\text{HA}} > 10 c_{\text{A}^-}$, so that A is mostly in its acid form HA

- At $\text{pH} = \text{p}K_a$, $c_{\text{A}^-} = c_{\text{HA}}$, so that A is half dissociated.

These simple rules show that the relative concentrations of acid and base are sensitive to pH changes. At equilibrium (and

kinetic rates much faster than the timescales of interest), pH therefore sets the time fraction during which the acid is ionized. In this example, the time fraction is equal to $c_{\text{A}^-} / (c_{\text{HA}} + c_{\text{A}^-})$. Endogenous or exogenous pH changes therefore modify charge distributions and alter electrolyte chemistry.

The ideal dilute solution assumption yields the strictly imprecise but common approximation that $\text{pH} = -\log_{10} c_{\text{H}^+}$.[¶] Despite the fact that it is approximate,¹¹ we will use this definition in the rest of this paper to discuss practical problems relevant to chemistry in electrokinetic experiments. Unless otherwise stated, we will assume ideally dilute solutions and substitute all solute activities with their corresponding concentrations. However, we will also discuss the relaxation of the dilute approximation and summarize the effects of ionic strength on activity coefficients. These corrections will take the form of effective $\text{p}K_a$ values which allow us to relate actual concentrations in solution to measured pH.¹⁶

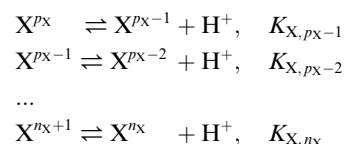
Electrolyte solutions and acid–base equilibrium

We first discuss general formulations applicable to fairly general electrolyte equilibrium chemistry problems. We then discuss key issues in preparing and analyzing buffers.

Acid–base equilibrium: conventions

Here we present conventions allowing general treatment of acid base equilibrium reactions. These conventions are useful in implementing codes solving for equilibria of a large number of species. We will later leverage this convention to present a general formulation of acid base equilibria involving an arbitrary number of electrolytes.

For each simple reaction involving a proton donated by a molecule X, we can write the general dissociation reaction $X^z \rightleftharpoons X^{z-1} + \text{H}^+$. We here write the set of acid–base dissociation reactions involving X, whose minimum valence is $z = n_X$, and maximum valence is $z = p_X$. As a useful convention, we write all equilibrium reactions as acid dissociations:



where we purposely omitted the hydrogen ion in the notation of each acid X (*e.g.*, we do not show a prefix “H”). As such, we use only acid dissociation constants K_a and never the related base equilibrium constants K_b (so we may often drop the “a” with no ambiguity). We can substitute a tabulated value of K_b by K_a using the relation $K_a = K_w / K_b$. We use X to represent all ionization states of the species. For example, in the case of the ionization states of aqueous phosphoric acid, X is a set (or “family”)

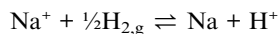
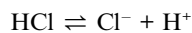
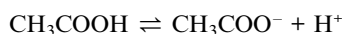
¶ We note that it is mathematically improper to take the logarithm of a quantity with dimensions (here c_{H^+} in M). More properly we should first divide c_{H^+} by a reference concentration c° as in $\text{pH} = -\log_{10} c_{\text{H}^+} / c^\circ$, where the value of c° is commonly set to 1 M.¹³ However, this notation is ingrained in the literature²⁹ and we will use it here, with the understanding that the division by c° in the argument of the logarithm is implied.

including PO_4^{-3} (i.e., X^{-3}), HPO_4^{-2} (X^{-2}), $\text{H}_2\text{PO}_4^{-}$ (X^{-1}), and H_3PO_4 (X^0). For each reaction from valence ($z + 1$) to z , we can write generally the acid dissociation constant $K_{\text{X},z}$ as:

$$K_{\text{X},z} = c_{\text{X},z}c_{\text{H}}/c_{\text{X},z+1}, \quad (9)$$

where c_{H} is the hydronium ion concentration and $c_{\text{X},z}$ and $c_{\text{X},z+1}$ are respectively the concentrations of X^z and X^{z+1} . We account for strong bases and acids using similar equilibrium reactions but where the values of K are respectively small and large. So, correspondingly, $\text{p}K_{\text{X},z}$ is (typically) below 2 for strong acids and above 12 for strong bases.

For clarity, we here give example reactions which can be, by the current convention, written as an acid dissociation (i.e., with H^+ on the right hand side and c_{H} in the numerator of the expression of K). Respectively, the dissociation of a weak acid (acetic acid CH_3COOH), a weak base (ammonia NH_3), strong acid (hydrochloric acid HCl), and a strong base|| (sodium hydroxide NaOH) can be written as



We note that salts are strong electrolytes, but their dissolution does not involve the acceptance or donation of a proton, and may be written as $\text{Y}_{\nu_+}\text{X}_{\nu_-}(\text{s}) \rightleftharpoons \nu_+\text{Y}^+ + \nu_-\text{X}^-$ (e.g., as in potassium chloride $\text{KCl}(\text{s}) \rightleftharpoons \text{K}^+ + \text{Cl}^-$, or disodium phosphate $\text{Na}_2\text{HPO}_4(\text{s}) \rightleftharpoons 2\text{Na}^+ + \text{HPO}_4^{2-}$). We can account for the addition of a salt by accounting for an equivalent addition of equal amounts of corresponding acid and base. For example, dissolution of sodium acetate $\text{NaCH}_3\text{COO}(\text{s}) \rightleftharpoons \text{Na}^+ + \text{CH}_3\text{COO}^-$ mathematically introduces two associated $\text{p}K_a$ values. Therefore, the addition of 10 mM sodium acetate can be treated as adding both 10 mM acetic acid and 10 mM sodium hydroxide. This formulation wherein all dissociation constants are written in terms of K_a is very powerful in developing general electrolyte formulations, as we shall show below. In the next sections, we will discuss the formulation and mathematical solutions of mixtures of an arbitrary number and type of electrolytes.

Simple weak electrolyte (non-buffering case)

Before proceeding to general formulations, we briefly analyze the simple case of a single weak electrolyte solution. We use the

|| The reaction $\text{Na}^+ + \frac{1}{2}\text{H}_{2,\text{g}} \rightleftharpoons \text{Na} + \text{H}^+$ is written here simply for convenience as a way of storing in a database a proton donating species (Na^+) which account for additions of NaOH to the solution. Physically, the species sodium hydroxide dissolves completely and sodium does not participate in a proton exchange. Here, for systematic and convenient treatment, useful in computations, we arbitrarily assign a large $\text{p}K_a$ to the sodium ($\text{p}K_a = 14$). We can then set the total amount of sodium in solution to the initial amount of sodium hydroxide. This approach has been used successfully by, for example, Hirokawa *et al.*⁸⁰ and Gas *et al.*⁸¹ Similar formulations can be used to account for other strong bases such as potassium hydroxide.

general equations from law of mass action, mass balance, and electroneutrality (a.k.a. charge balance). Consider a simple weak electrolyte A whose acidic form dissociates as $\text{HA} \rightleftharpoons \text{A}^- + \text{H}^+$. The relevant equilibrium reactions have dissociation constants $K_w = c_{\text{H}}c_{\text{OH}}$ and $K_{\text{A},-1} = c_{\text{A},-1}c_{\text{H}}/c_{\text{A},0}$ (recall that, in our notation, $c_{\text{A},-1} = c_{\text{A}^-}$ and $c_{\text{A},0} = c_{\text{HA}}$ for this case). The total concentration of A (a.k.a. the analytical concentration) is $c_{\text{A}} = c_{\text{A},-1} + c_{\text{A},0}$, where c_{A} is the known amount of total weak acid dissolved in solution (e.g., weighed solid crystal or a measured volume of specified stock solution). Lastly, we have the electroneutrality relation $c_{\text{A},-1} + c_{\text{OH}} = c_{\text{H}}$ (see below). These four equations can be combined to yield an implicit third order polynomial in c_{H} :

$$c_{\text{H}}^3 + K_{\text{A},-1}c_{\text{H}}^2 - c_{\text{H}}(K_{\text{A},-1}c_{\text{A}} + K_w) - K_{\text{A},-1}K_w = 0. \quad (10)$$

This polynomial has a unique real positive root (from Descartes' rule of signs). We can numerically solve eqn (10) to find that root, which yields a pH below 14 for weak electrolytes.

The process above can be repeated for a weak acid of the form $\text{BH}^+ \rightleftharpoons \text{B} + \text{H}^+$. This also yields four equations which can be combined into a third order polynomial in c_{H} . We can plot the solution to both of these polynomials in a single plot known as Flood's diagram.¹⁴ Fig. 1 shows Flood's diagram of $\log_{10} c_{\text{X}}$ versus solution pH. Here, species X can refer to a monovalent acid or a base, and X is the only species in solution. The curves below $\text{pH} = 7$ are for acids X of various $\text{p}K_a$ values; while those above $\text{pH} = 7$ are for bases X and their associated $\text{p}K_a$ values. We see that at sufficiently high analytical concentrations of weak acid, there is a linear relation between c_{H} and c_{A} . However, the associated pH increase is slower than for a strong acid. The rate of pH change for bases is also governed by $\text{p}K_a$ as shown.

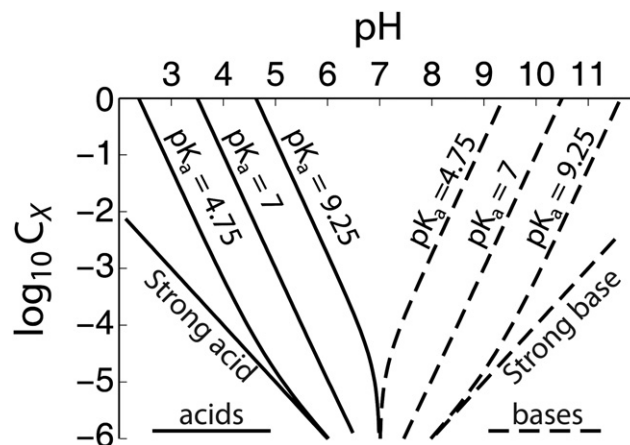


Fig. 1 Flood's diagram for strong and weak acids (solid lines), and strong and weak bases (dashed lines) in solution. The left half of the diagram corresponds to the logarithm of total concentration of a single acid as a function of pH. The pH of weak acids deviates from the strong acid relation $\text{pH} = -\log_{10}c_{\text{A}}$. pH always decreases with addition of weak acid, but more and more weakly as their $\text{p}K_a$ increases. Similarly, weak bases deviate from the effect of strong base on pH ($\text{pH} = 14 + \log_{10}c_{\text{B}}$). For the same base concentration, the pH of a stronger base (higher $\text{p}K_a$) is higher than for a weak base.

Arbitrary dilute solutions involving proton transfers and salts: A well posed problem

Before proceeding, we note that the general problem of an electrolyte composed of an arbitrary number of (strong and weak) electrolytes is well posed. We can define the number of reactions (and therefore number of $K_{X,z}$ values) per species X as $r_X \equiv p_X - n_X$. For a species X considered, we have $2 + r_X$ unknowns (including c_H), and each new family adds $1 + r_X$ unknowns. c_{OH} is an additional unknown, so we have a total of $2 + \sum(1 + r_X)$ unknowns where \sum is a summation over all families.

In comparison, our formulation yields r_X independent equilibrium relations (of the form $K_{X,z} = c_{X,z}c_H/c_{X,z+1}$) for each species set (family) X; yielding a total of $\sum r_X$ equations. We add to this one conservation-of-mass relation for each family, the electroneutrality approximation, and the water autoprotolysis relation $c_{OH}c_H = K_w$. This yields $2 + \sum(1 + r_X)$ mathematically independent equations.

Formulation and solutions for general mixtures involving acid–base reactions and salts

In this section, we provide a general formulation for solving species concentrations (including c_H which yields pH) of a solution composed of an arbitrary number of electrolytes. We formulate this general problem following the conventions summarized above. We then derive a compact polynomial formulation of the problem which can be solved systematically using common root-finding routines. The formulation is useful for general multi-species, multi-valent chemical equilibrium problems, and was verified and applied by Bercovici *et al.*²⁰ to generalized electrokinetics transport problems. This formulation allows for direct (rather than the typical iterative) solutions of pH and other ion densities.

As we mentioned earlier, the total concentration (or so-called analytical concentration) of a species set X can be expressed as the sum of all ionic states of that species family:

$$c_X = \sum_{z=n_X}^{p_X} c_{X,z}. \quad (11)$$

We note that c_X is typically a known quantity (*e.g.*, the total amount of a solute added to the solution). Again, n_X and p_X are respectively the minimum and maximum valences of the species X (*e.g.*, $n_X = -1$, $p_X = +2$ for histidine). Using this notation, the electroneutrality approximation** is given by:²¹

$$\sum_X \sum_{z=n_X}^{p_X} z c_{X,z} + c_H - c_{OH} = 0. \quad (12)$$

Note we find it most useful to treat water and its ions as a separate “family” (outside of the summation over N species sets).

** Electroneutrality is an excellent approximation for electrolyte transport analyses in devices whose length scales are much larger than the typical Debye length of a solution (*i.e.*, this is valid for volume averages in length scales much larger than characteristic charge shielding length scales). The approximation is accurate for typical microfluidic electrokinetic problems for regions outside the electric double layers.

Also, we will later use the equilibrium reaction of water to eliminate the variable c_{OH} from the problem.

As shown by Stedry *et al.*,²² the concentration of each ionic state can be related to the concentration of the neutral state, $c_{X,0}$, and the hydronium concentration, c_H . For example, multiplication of all K values from $z' = 0$ to $z' = z - 1$ yields:

$$\begin{aligned} K_{X,0}K_{X,1}K_{X,2}\dots K_{X,z-1} &= \frac{c_{X,0}}{c_{X,1}} \frac{c_{X,1}}{c_{X,2}} \dots \frac{c_{X,z-1}}{c_{X,z}} c_H^z \\ &= \frac{c_{X,0}}{c_{X,z}} c_H^z. \end{aligned} \quad (13)$$

Generalizing this result for both positive and negative ionic states yields

$$c_{X,z} = c_{X,0} L_{X,z} c_H^z, \quad (14)$$

where

$$L_{X,z} = \begin{cases} \prod_{z'=z}^{-1} K_{X,z'} & z < 0 \\ 1 & z = 0 \\ \prod_{z'=0}^{z-1} K_{X,z'}^{-1} & z > 0 \end{cases} \quad (15)$$

Substituting (14) into (11) yields the concentration of each ionic state to the total concentration of that species

$$c_{X,z} = c_X \frac{L_{X,z} c_H^z}{\sum_{z=n_X}^{p_X} L_{X,z} c_H^z}. \quad (16)$$

In Part II of this two-paper series, we will use a form similar to equation (16) to express the effective electrophoretic mobility of a weak electrolyte in solution. Substituting (16) into the electroneutrality equation (12) and using the autoprotolysis constant K_w , we obtain an algebraic equation for c_H :

$$\sum_{X=1}^N \sum_{z=n_X}^{p_X} z c_X \frac{L_{X,z} c_H^z}{\sum_{z=n_X}^{p_X} L_{X,z} c_H^z} + c_H - \frac{K_w}{c_H} = 0. \quad (17)$$

Here the values $L_{X,z}$, n_X , p_X and K_X are all known, and iterative numerical solutions can be used to converge to a positive and real value of c_H . However, more conveniently, since only integer powers appear in the expression, additional algebraic manipulation of this equation results in a polynomial form:

$$\sum_X c_X P_X + Q = 0, \quad (18)$$

where

$$Q = \prod_Y Q_Y, \quad (19)$$

$$P_X = c_H \prod_Y \left(\sum_{z'=n_Y}^{p_Y} L_{Y,z'} (1 + (z' - 1) \delta_{XY}) c_H^{z' - n_X} \right), \quad (20)$$

$$Q_0 = (c_H^2 - K_w), \quad (21)$$

$$Q_Y = \prod_Y \left(\sum_{z'=n_Y}^{p_Y} L_{Y,z'} c_H^{z'-n_Y} \right), \quad (22)$$

δ_{XY} is the Kronecker delta function, which is unity when $X = Y$ and 0 otherwise. The expressions for P and Q represent the coefficients of a polynomial in c_H , and can be computed automatically from the known values $L_{Y,z'}$. The roots of the polynomial can be computed directly by solving for the eigenvalues of the companion matrix (a matrix whose characteristic polynomial corresponds to eqn (18)). The ‘roots’ command in Matlab (The Mathworks Inc., Natick, MA, USA), for example, uses this method to find the roots of a polynomial. We give more details on the construction of this polynomial in Appendix A. A free open-source Matlab implementation of this equilibrium problem for N general species is available on the internet at <http://microfluidics.stanford.edu/download/>.

The generalized non-iterative algorithm presented above is a flexible and robust method for solving complex acid–base equilibria. In particular, it is easily incorporated into custom simulation and optimization codes. However, we also note the software packages CurTiPot, Peakmaster,²³ Simul,²⁴ and Spresso,²⁰ all of which are available online,^{††} can calculate the pH of solutions of multiple general ampholytes, including the effects of ionic strength on activity coefficients, and each includes a user-expandable chemical database. CurTiPot, a Microsoft Excel (Redmond, WA, USA) spreadsheet, presents particularly detailed information on ionization states and activity coefficients. It also simulates virtual acid–base titrations, and performs multiparametric nonlinear regression to recover concentrations and/or pK_a 's of multiple species from experimental titration data. PeakMaster, while unable to automatically simulate or analyze acid–base titrations, can calculate ion mobilities and buffer conductivities in addition to buffer pH. Finally, both Spresso and Simul simulate non-linear electrophoresis processes, calculating ion mobilities, buffer pH fields, conductivity fields, spatio-temporal ion distributions, *etc.* associated with electrokinetic transport processes. Of these packages, we recommend CurTiPot for most acid–base problems, PeakMaster for simple electrophoresis problems where ion mobility and buffer conductivity are relevant; and Simul or Spresso for acid–base equilibria in full-scale electrophoresis simulations.^{‡‡}

On assuming “moderate pH”

In the pH range 4–10, the concentrations of hydronium and hydroxyl ions remain below 0.1 mM. Typical electrolytes in microfluidics have at least millimolar concentrations, so that the solute concentrations remain larger than those of hydronium and

^{††} CurTiPot: Available http://www2.iq.usp.br/docente/gutz/Curtipot_.html; Peakmaster and Simul: Available <http://www.natur.cuni.cz/~gas/>; Spresso: Available <http://microfluidics.stanford.edu/download/>

^{‡‡} We have validated this code using comparisons to analytical solutions in the moderate pH regime, including equations (25) and (28). We have also validated the code for problems outside the moderate pH range, including problems similar to those plotted in Figs. 3 and 4. In all cases, our code agrees with these analytical solutions to within at least three significant figures on concentration variables (*e.g.*, $c_{A,-}$ and c_H). Our solution method is used in Spresso, and also agrees to three significant figures or better with solutions of weak electrolyte problems solved using Simul 5.

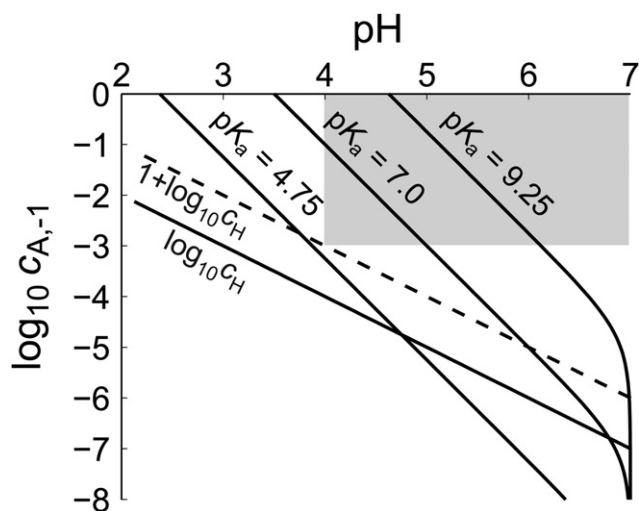


Fig. 2 The concentration of the conjugate base of three weak acids as a function of pH illustrates the moderate pH approximation in acidic conditions. The lines labelled $\log_{10}c_H$ and $1 + \log_{10}c_H$ represent the $1\times$ and $10\times$ concentration of hydronium ions, respectively. The moderate pH zone is the width of the grey area. In this zone, buffer ion concentrations of about 1 mM and higher (height of grey zone) ensure $c_{A,-1} \geq 10 c_H$. Note that in the case of a strong acid (resp. base) alone in solution, the moderate pH approximation cannot hold as $c_{A,-1} = c_H$ (resp. $c_{B,+1} = c_{OH}$ for a strong base).

hydroxyl ions. In this regime, it is useful to assume a “moderate pH”;²⁵ *i.e.*, a pH ranging between about 4 and 10. This approximation is useful in determining solution pH and species concentrations in simple buffer systems.

For moderate pH and solute concentrations of 1 mM and above we can simplify the electroneutrality relation (12) as

$$\sum_X \sum_{z=n_i}^{p_i} z c_{X,z} \cong 0 \quad (\text{moderate pH}). \quad (23)$$

In other words, we here ignore the contributions of H^+ and OH^- to the net charge density summation. In example formulations below, we will use the approximation of moderate pH range. We will see that this simplification reduces the complexity of most buffer calculations and can be used to gain insight on buffer design and preparation. This often yields simple, yet fairly accurate pH and concentration estimates. For example, in the case of a weak acid buffer titrated with a strong base, the fourth order polynomial in c_H derived from (18) becomes a second order polynomial under this assumption. Fig. 2 summarizes the principle of the moderate pH approximation in the low pH regime.

Buffer with single weak electrolyte and a strong electrolyte titrant

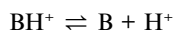
We first review the fairly “classic” buffer case of a single weak electrolyte and a (relatively) strong titrant. The purpose of the titrant is to shift the equilibrium of the weak electrolyte to reach the desired pH. We summarize various common cases in Table 1. The table shows example buffers constructed of a weak acid titrated with a strong base; a weak base titrated with a strong acid; a weak acid and a salt containing its conjugate base; and a weak base and a salt containing its conjugate acid. The titrant

Table 1 Common buffer classifications and a few examples of titrant

Buffer type	Weak electrolyte example	Titrant examples		
		Conjugate salt	Weak acid/base	Strong acid/base
Weak acid	Phosphoric Acid HEPES MES MOPS Acetic acid	(Mono/bi/tri) sodium phosphate	Tris, Bis-Tris	NaOH, KOH
Weak base	Tris Bis-Tris	Sodium acetate Tris-HCl	Boric acid, acetic acid	HCl

of a weak acid can be a strong base, a weak base with $pK_a \sim 2$ units above the solution pH, or a salt containing the buffer's conjugate base. For example, to make an acetate buffer, we can titrate an acetic acid solution with sodium hydroxide, Tris, or sodium acetate. We will focus on two examples, but note that the principles and formulations are all very similar; and numerous examples can be found in textbooks.^{26,27}

Consider a weak acid and strong base titrant. The strong base dissociates completely, while the weak acid is in equilibrium with its conjugate base. Following our convention, we write these as:



The relevant acid dissociation constants are $K_{A,0} = c_{A,-1}c_{\text{H}}/c_{A,0}$, $K_{\text{w}} = c_{\text{H}}c_{\text{OH}}$, and $K_{B,0} = c_{B,0}c_{\text{H}}/c_{B,1}$. With our convention, the value of the latter is very low (smaller than 10^{-12}) so that $c_{B,0} \approx 0$. The conservation equations are $c_{\text{A}} = c_{A,-1} + c_{A,0}$ and $c_{\text{B}} = c_{B,1}$ (*i.e.*, full dissociation), while electroneutrality (assuming moderate pH) can be written as $c_{A,-1} \approx c_{B,1}$. We see that the titrant (here the strong base) determines directly the degree to which the weak base is dissociated. Combining these equations and solving for c_{H} ,

$$c_{\text{H}} = K_{A,-1}(c_{\text{A}}/c_{\text{B}} - 1). \quad (24)$$

Taking the negative logarithm:

$$\text{pH} = \text{p}K_{A,-1} + \log_{10} \frac{c_{\text{B}}}{c_{\text{A}} - c_{\text{B}}}. \quad (25)$$

We use this relation to show the titration of a weak acid by a strong base about its pK_a in Fig. 3. In the same plot, we show an analogous titration of a weak base by a strong acid (equations not shown). At the beginning of the titration, pH varies strongly with addition of titrant. For example, pH quickly drops when adding a strong acid to a weak base below $c_{\text{A}} = 0.2 c_{\text{B}}$. When the titrant concentration approaches half of the weak electrolyte concentration, pH is more robust to addition of titrant; and, at $c_{\text{A}} = 0.5 c_{\text{B}}$, $\text{pH} = \text{p}K_{\text{weak}}$, which is called the half titration point. Adding further titrant increases the pH until the weak electrolyte and titrant reach equal concentration. This is known as the equivalence point. In the plot, we show extensions of the curves (as dashed lines) beyond the moderate pH zone. These dashed curves are accurate and calculated using a full formulation of the problem (*i.e.*, not with eqn (25)). The low slopes of the pH curve near $c_{\text{titrant}}/c_{\text{weak}} = 1.5$ are due to further addition of titrant.

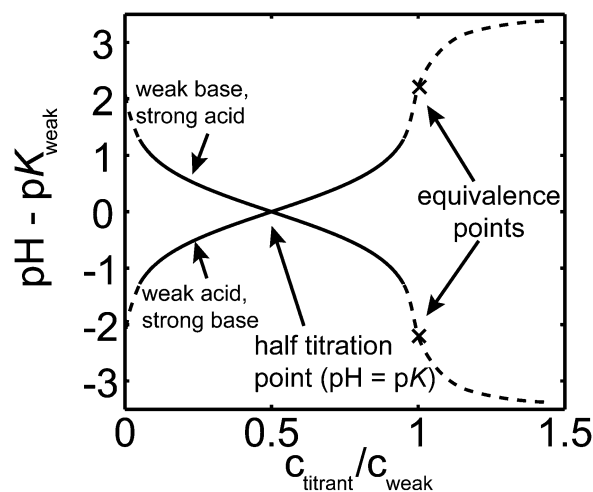
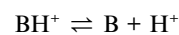
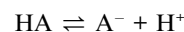


Fig. 3 Titration of a weak acid (or base) by strong base (or acid). The difference between the buffer's pH and the weak electrolyte's pK_a ($\text{p}K_{\text{weak}}$) is plotted as a function of the concentration ratio of strong electrolyte (titrant) to weak electrolyte $c_{\text{titrant}}/c_{\text{weak}}$. The solid portion of the curves assume moderate pH. The buffer's sensitivity to titrant is lowest when $c_{\text{titrant}}/c_{\text{weak}} = 0.5$, at which point $\text{pH} = \text{p}K_{\text{weak}}$, also called the half titration point. Buffer pH is most sensitive to addition of titrant when $c_{\text{titrant}} \approx c_{\text{weak}}$ or when $c_{\text{titrant}} \approx 0$. In these regions (marked by dashed lines), the moderate pH assumption breaks down, and the exact shape of the curve will depend strongly on the species' pK_a and the weak acid's concentration. The inflection point which occurs at $c_{\text{titrant}} = c_{\text{weak}}$ (marked by 'x') is known as the equivalence point.

As we shall see below, the buffering strength of a simple buffer (one weak electrolyte and one relatively strong electrolyte) is highest for the case where solution pH is near the pK_a of the weak electrolyte. For $\text{pH} = \text{p}K_a$, equation (25) reduces to $c_{\text{B}} = c_{\text{A}}/2$. This result is general for simple buffers. Namely, for optimal buffering conditions, the total strong electrolyte concentration is half the total weak electrolyte concentration. This implies that half the total amount of weak electrolyte is dissociated. For the case above, $c_{A,-1} = c_{A,0} = c_{\text{A}}/2$.

Two weak electrolyte buffer

Consider a solution composed of two weak electrolytes with equilibrium equations of the form:



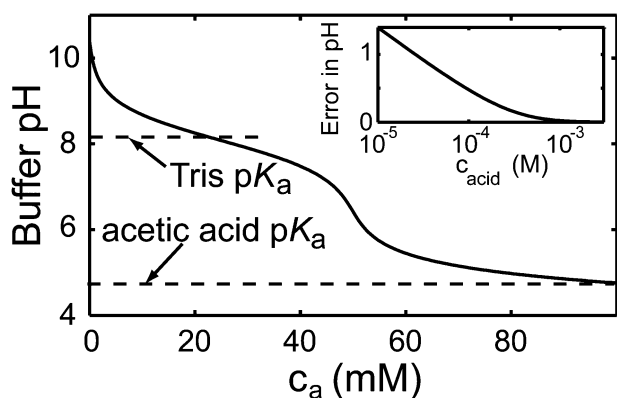


Fig. 4 Titration of 50 mM Tris with up to 100 mM acetic acid (acetic acid denoted as “a”). We here use concentration values which describe the total (analytical) concentration of acetic acid in solution. This is unlike the typical approach of plotting titration curves with respect to added volume (or number of moles) of titrant, but has the advantage of making the curve universal for all added volumes. At $c_a = 0$, the pH is elevated so that the concentration of Tris^+ ions is approximately equal to that of OH^- (and “moderate pH” assumption is violated). The buffer pH is dominated by the $\text{p}K_a$ of Tris (~ 8.1) at low (~ 25 mM) acetic acid concentrations, and by the $\text{p}K_a$ of acetic acid (~ 4.8) at high (~ 75 to 100 mM) acetic acid concentrations. The optimal buffer regions (*i.e.*, the lowest slopes in the curve) occur, as expected, near $\text{pH} = 4.8$ and 8.1 . The inset shows the overprediction of eqn (28) due to its moderate pH assumption. Note that the error, while substantial at low titrant concentrations (over 1 pH unit for 10 μM acetic acid), is negligible even for 1 mM acetic acid.

Examples of such buffers include Tris-borate, Tris-acetate, Tris-glycine, and BisTris-MES. The three relevant equilibrium constants are $K_{A,-1} = c_{A,-1}c_{\text{H}}/c_{A,0}$, $K_{B,0} = c_{B,0}c_{\text{H}}/c_{B,1}$, and $K_w = c_{\text{H}}c_{\text{OH}}$. The two conservation equations are $c_A = c_{A,-1} + c_{A,0}$ and $c_B = c_{B,0} + c_{B,1}$, and the electroneutrality at moderate pH, $c_{A,-1} \cong c_{B,1}$. Combining these equations,

$$c_B \frac{c_{\text{H}}/K_{B,0}}{1 + c_{\text{H}}/K_{B,0}} = c_A \frac{K_{A,-1}/c_{\text{H}}}{1 + K_{A,-1}/c_{\text{H}}} \quad (26)$$

Again, we can arrange this into polynomial form as

$$c_{\text{H}}^2 + K_{A,-1}(1 - c_A/c_B)c_{\text{H}} - K_{A,-1}K_{B,0}c_A/c_B = 0, \quad (27)$$

so that c_{H} is simply

$$c_{\text{H}} = \frac{K_{A,-1}}{2} \left((c_A/c_B - 1) + \sqrt{(1 - c_A/c_B)^2 + 4 \frac{K_{B,0}c_A}{K_{A,-1}c_B}} \right) \quad (28)$$

In Fig. 4 we show the titration of 50 mM Tris (a weak base with $\text{p}K_a = 8.1$) by acetic acid (a weak acid with $\text{p}K_a = 4.8$). The inset shows the deviation of this relation from the exact solution (obtained without assuming moderate pH). Note the local minima in the slope of the pH line very near the respective $\text{p}K_a$ values of these weak electrolytes. We will discuss the relation between this slope and buffering capacity in the next section.

Buffer capacity

In a pH buffer solution, a weak electrolyte (acid or base) maintains the solution’s pH. For example, addition of an acid to the

buffer solution induces a displacement of the weak acid equilibrium towards consumption of the proton, as per Le Chatelier’s principle. The ability of a buffer to resist changes in pH is called the buffer capacity (or buffer index). It can be expressed as the amount of strong acid or base required to induce a small change in pH:

$$\beta \equiv \frac{\partial c_B}{\partial \text{pH}} \quad (\text{addition of strong base}), \quad (29)$$

$$\beta \equiv -\frac{\partial c_A}{\partial \text{pH}} \quad (\text{addition of strong acid}), \quad (30)$$

where c_B (resp. c_A) is the amount of strong base (resp. strong acid) added to the buffer.

We now calculate the buffer capacity for a specific case of a weak acid buffer titrated with a strong base discussed above. In the moderate pH range, differentiation of equation (24) yields

$$\frac{\partial c_{\text{H}}}{\partial c_B} = -K_{A,-1}c_A/c_B^2. \quad (31)$$

We derive the buffering capacity (here, taking into account the self-ionization of water so that the expression applies even outside of the moderate pH range):^{28,29}

$$\beta = \frac{\partial c_B}{\partial \text{pH}} = 2.30 \left(c_A c_{\text{H}} K_{A,-1} / (c_{\text{H}} + K_{A,-1})^2 + c_{\text{H}} + K_w / c_{\text{H}} \right). \quad (32)$$

For the case of a weak base and strong acid buffer, the equivalent expression is

$$\beta = -\frac{\partial c_A}{\partial \text{pH}} = 2.30 \left(c_B c_{\text{H}} K_{B,0} / (c_{\text{H}} + K_{B,0})^2 + c_{\text{H}} + K_w / c_{\text{H}} \right). \quad (33)$$

As a rule of thumb, buffers are most effective when c_{H} is roughly equal to the K_a of one of the buffering species, *i.e.* $\text{pH} \approx \text{p}K_a$. Further, for fixed pH, higher concentrations of buffering species yield approximately proportionally higher buffering capacities. As seen in Fig. 3 the solution pH is least sensitive to addition of strong acid or base around $\text{pH} = \text{p}K_a$. Conversely, a small amount of titrant dramatically changes pH when pH is far from the buffer $\text{p}K_a$. The least titrant is needed to effect pH changes when the titrant concentration is much smaller than, or similar to, the weak electrolyte concentration. The most titrant is needed to effect the same pH change when $c_B = c_A/2$ (that is, when $c_{\text{H}} = K_{A,-1}$).

Lastly, we note that buffering strength is lost rapidly as we deviate away from the optimum conditions. For example, when the pH of a buffer differs from the $\text{p}K_a$ of a weak acid by 1 unit, the amount of titrant needed for a given pH change is over 3-fold lower than the amount needed when $\text{pH} \approx \text{p}K_a$. For a 2-pH-unit difference, the discrepancy is ~ 25 -fold. The buffering capacity as defined in equations (29) to (33) is plotted in Fig. 5 for several cases. We note that buffering capacity in polyprotic weak electrolytes (weak acids and bases with more than one $\text{p}K_a$), or in buffers with multiple weak electrolytes, is more complex. For instance, if two $\text{p}K_a$ ’s are within about 2 pH units of each other, then the pH (or pH’s) at which buffer

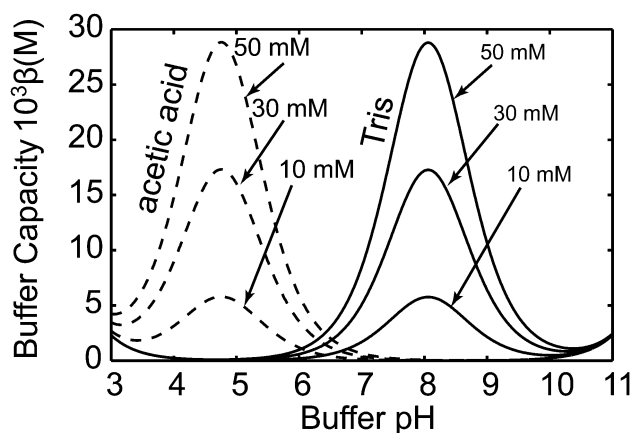


Fig. 5 Buffering capacity for a weak acid buffer (acetic acid, $pK_a = 4.75$, dashed lines) and a weak base buffer (Tris, $pK_a = 8.1$, solid lines), at several concentrations. As evident from equations (32) and (33), buffers are most effective at high concentrations and near their pK_a 's. At both high pH (pH > 10) and low pH (pH < 4) the self-ionization of water provides an additional buffering effect.

capacity is maximized tend to be in between the weak electrolytes' pK_a 's.³⁰

Introduction to non-ideal solutions and activity coefficients

We here summarize the dependence of activity coefficients on ionic strength. This section is intended as a short review of the relevant physics. In the next section, we will present practical equations useful for design and optimization of buffers for electrokinetic processes.

The effect of ionic strength on acid–base equilibria is often ignored. General modeling of the phenomena is complex and numerous models exist,¹² many of which require species-specific empirical parameters. Further, in modeling of electrokinetic systems, ionic strength couples with solution composition. This typically requires iterative solution schemes and always makes analytical models more difficult. Ionic strength is defined as:^{§§}

$$I = \frac{1}{2} \sum_X \sum_z z^2 c_{X,z}. \quad (34)$$

In the current context, I can be interpreted as a measure of the deviation from the infinite dilution approximation. We will briefly describe the physics and mechanisms involved in the correction for ionic strength in Part II of this two-paper series, along with a discussion on correction of electrophoretic mobilities.

Ions in solutions are always associated with counterions, so that each species' activity coefficient cannot be measured individually. Because we cannot perform measurements of activity of a single ionic species, we typically define the mean activity

^{§§} We note that ionic strength can be alternatively defined as a dimensionless quantity, by simply dividing the right hand side of (34) with $c^\circ \equiv 1 \text{ M}$.¹³ This does not have a significant effect on calculations (only the units of empirical constants change).

coefficient γ_{\pm} of an ion pair $\nu_+ Y^{z_+}, \nu_- X^{z_-}$ by $(\gamma_{\pm})^{\nu_+ + \nu_-} \equiv (\gamma_+)^{\nu_+} (\gamma_-)^{\nu_-}$ (from electroneutrality, valences z_{\pm} , and the number of ions ν_{\pm} , we see that $z_+ \nu_+ + z_- \nu_- = 0$). For example the mean activity coefficient of a magnesium chloride salt MgCl_2 in solution is $\gamma_{\pm}^3 = \gamma_+^1 \gamma_-^2$ (i.e., $\gamma_{\pm} = (\gamma_+ \gamma_-^2)^{1/3}$). Or for a weak acid HA which dissociates into H^+ and A^- , $\gamma_{\pm}^2 = \gamma_{\text{H}} \gamma_{\text{A}^-}$. The acid dissociation constant (6) therefore becomes:

$$K_a = \frac{c_{\text{H}} c_{\text{A}^-}}{c_{\text{HA}}} \frac{\gamma_{\pm}^2}{\gamma_{\text{H}}}. \quad (35)$$

While the treatment of the activity coefficient of the neutral species is rather simple,^{12,13} the physics involved in the mean activity coefficient for charged species are complex.¹² Debye and Hückel³¹ first recognized the effects of ionic atmosphere on activity coefficients, and derived the Debye–Hückel law for a binary electrolyte in a solution of ionic strength I :

$$\log_{10} \gamma_{\pm} = -\frac{A z_+ |z_-| \sqrt{I}}{1 + aB \sqrt{I}}, \quad (36)$$

where the constants A and B depend on temperature and dielectric constant of the solvent (in aqueous solutions at 25 °C, $A = 0.51 \text{ M}^{-1/2}$ and $B = 0.328 \text{ \AA}^{-1} \text{ M}^{-1/2}$), a is the ionic diameter of the ion in \AA . A good approximation for small ions is to assume $a = 3 \text{ \AA}$, which yields a product aB of approximately unity.¹³

The Debye–Hückel law captures qualitatively the effect of ionic strength up to 100 mM, however it cannot be used for accurate quantitative prediction of activity coefficients.¹³ There is abundant literature reporting more advanced and more successful models and semi-empirical relations for correction of activity coefficients; many of these are discussed and reviewed by Robinson and Stokes.¹² Davies¹⁶ proposed an improvement over the Debye–Hückel equation which shows better agreement with experimental values of mean activity coefficients:^{¶¶}¹⁴

$$\log_{10} \gamma_{\pm} = -0.51 z_+ |z_-| \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3I \right), \quad (37)$$

which can be also written for a single ion in solution:^{14,16}

$$\log_{10} \gamma_{X,z} = -0.51 z^2 \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3I \right) \quad (38)$$

In Part II, we will summarize the usage and effect of activity coefficients in various conservation laws.

pK_a at finite dilution: Effect of ionic strength

The Davies equation is commonly used for correction of dissociation constants.¹³ We can apply the Davies equation to an acid–base dissociation (e.g., reaction (3) with an acid dissociation constant given by (35)). We note that the activity coefficient of the neutral species γ_{HA} is approximately unity,^{13,14} so that starting with equations of the form of (7) and (38) we can derive:

$$pK_a' = pK_a + 2 \log_{10} \gamma_{\pm}, \quad (39)$$

^{¶¶} The coefficient of the linear term is here taken equal to 0.3 versus 0.2 as initially reported by Davies.¹⁶ 0.3 shows better agreement with experimental data.¹⁴

where $2\log_{10}\gamma_{\pm}$ (*e.g.*, from eqn (37)) represents a correction for pK_a . Using the Davies equation for single ions (38), we can derive a more general correction of pK_a for the dissociation of an acid HA^- of valence z as

$$\Delta pK_a = -1.02(1-z) \left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.3I \right) \quad (40)$$

Then, the pK_a at the ionic strength of interest, pK_a' , is

$$pK_a' = pK_{a, I=0} + \Delta pK_a. \quad (41)$$

The Davies equation is a rather popular relation,^{13,14} and also strikes a balance between accuracy and complexity that is appropriate for typical, practical evaluations of non-dilute effects in electrolyte solutions. In Part II of this two-paper series, we will explore the effects of ionic strength on the (fully ionized) electrophoretic mobility of an ionic species. We will present a model correcting mobilities at finite dilution and use it along with the Davies equation to show the dependence of the electrophoretic mobility of fluorescein fluorescent dye with pH and ionic strength.

Temperature dependence of pK_a

We here briefly summarize temperature effects on buffering solutions, and give examples as to where such effects can be important. The acid dissociation constant is a thermodynamic quantity which depends on the enthalpy of dissociation of the buffering species. For example, in polymerase chain reactions (PCR), the typical buffer Tris experiences important changes as temperature is varied from 25 °C to 95 °C. Over this range, the pK_a of Tris varies between 8.2 at 25 °C to 6.3 at 95 °C, and so pH of the solution is reduced by about two units below its room temperature value. In microchip electrophoresis applications, Joule heating can cause non-negligible temperature changes which can impact the separation buffer pH and therefore the (observed) effective mobility of species.³²

The acid dissociation constant K is related to the enthalpy of acid dissociation through the Van't Hoff equation:¹⁴

$$\frac{d \log_{10} K}{dT} = \frac{\Delta H^0}{2.3RT^2}, \quad (42)$$

where ΔH^0 is the standard enthalpy of reaction. ΔH^0 is a function of temperature, usually expressed in a polynomial form. The general expression of the acid dissociation constant as a function of temperature becomes:³³

$$pK_a = \frac{\lambda_1}{T} + \lambda_2 + \lambda_3 \log_{10} T + \lambda_4 T + \dots, \quad (43)$$

where the coefficients λ_i are typically determined from fitting experimental data. For example, Harned and Ehlers³³ fitted the measurement of the temperature dependence of acetic acid pK_a as follows:

$$pK_{CH_3COOH} \approx \frac{1501}{T} - 18.67 + 6.5 \log_{10} T + 0.007T.$$

Over small temperature differences, pK_a dependence on temperature is approximately linear,²⁹ and so it is common to report the temperature coefficient dpK_a/dT . We report this coefficient in our list of common buffers in Table 2. More

comprehensive treatment of pK_a is warranted to analyze significant temperature differences (as in the case of PCR experiments).

Common pH buffers and recommendations

Experimentalists use a relatively narrow range of buffering chemicals compared to the wide variety of compounds listed in pK_a databases. For instance, by far the most commonly used strong titrants are hydrochloric acid HCl for weak base buffers and sodium hydroxide NaOH for weak acid buffers. Many of the most popular weak electrolytes were described by Good and colleagues,^{34,35} and are widely known as “Good’s buffers”.^{||||} These buffers were designed for the biologically relevant range of pH [5.5, 8.6], and to have minimal experimental side effects. Many of Good’s buffers have pK_a values between 6 and 8, have low absorbance between 240 nm and 700 nm, are highly soluble, inexpensive, non-toxic, and stable. Further, Good’s buffers and several other popular buffers do not have primary amine groups, which are highly reactive.²⁹ As we mention later, both primary and secondary amine groups can react with dissolved CO_2 (*e.g.*, atmospheric CO_2) to form carbamates. Table 2 presents a list of common buffers, including Good’s buffers, with their molecular formulas, pK_a ’s, temperature dependences, valences, electrophoretic mobilities, and other relevant properties.

Notes on preparing pH buffers

In microchip electrokinetic experiments, pH and ionic strength affect most major aspects of system performance including separation efficiency and migration times,³⁶ binding constants,³⁷ Joule heating,³² biomolecular adsorption characteristics,³⁸ power requirements, and overall reproducibility of the assay. For example, a pH change of 2 units and 100 fold factor in ionic strength can each change zeta potential (and electroosmotic mobility) by approximately two folds (*e.g.* for the case of silica between pH 6 and 8, and 1 mM to 100 mM ionic strength).³⁹ The main functions of a buffer are (i) to set the solution pH, and (ii) to stabilize the solution chemistry by maintaining pH and conductivity during and between experiments. The first step is the selection of pH, which is typically entirely driven by the application. For example, Tris hydrochloride buffer at pH = 8.0 is excellent for yield and specificity in polymerase chain reaction; however, an electroosmotic pump device may benefit from the 9.2 pH of a borate buffer, despite the typical problems with borate in biochemical studies (*cf.* Table 2).⁴⁰ Other concerns include temperature dependence, solubility, availability, cost, cross-reactions, *etc.* For example, the organic propionic acid has low solubility but its pK_a is less sensitive to temperature changes than, say, Tris. Many of these issues are summarized in Table 2.

The second step is determining ionic strength of the electrolyte which at least in part determines Debye length, zeta potential, surface charge density, and conductivity among other key parameters.²¹ Designing for both specified pH and ionic strength typically requires computational tools. The most straightforward design approach is to fix weak electrolyte identity (given pH

|||| The original buffers described by Good *et al.* were MES, ADA, PIPES, ACES, BES, MOPS, TES, HEPES, EPPS, Tricine, Bicine, CHES, and CAPS.

Table 2 Common weak electrolytes used as buffering ions. Buffer species are presented in order of increasing pK_a for the respective monovalent reaction

Name	Formula	pK_a^a	dpK_a/dT^b	(Valence, mobility) ^c	Amine group ^d	Notes
Citric acid	C ₆ H ₈ O ₇	3.13 (0, -1)		(-1, 28.7)		
		4.76 (-1, -2)		(-2, 54.7)		
		6.40 (-2, -3) ⁴³		(-3, 74.4) ⁴³		
Succinic acid	C ₄ H ₆ O ₄	4.21 (0, -1)		(-1, 33.0)		
		5.64 (-1, -2) ⁴³		(-2, 60.9) ⁴³		
Acetic acid	CH ₃ COOH	4.76 (0, -1)	-0.0002	(-1, 42.4) ⁴³	Primary	Corrosive, fumes are an irritant. ²⁹
Creatinine	C ₄ H ₇ N ₃ O	4.83 (+1, 0) ⁴³		(+1, 37.2) ⁴³		
MES	C ₆ H ₁₃ NO ₄ S	6.21 (0, -1)	-0.011	(-1, 26.8) ⁴⁵	Tertiary	May complex with protonated amines. ^{46e,f}
Bis-tris Carbonate	C ₈ H ₁₉ NO ₅ H ₂ CO ₃	6.35 (+1, 0)	-0.02	(+1, 26) ²⁴	Tertiary	pK_a has high temperature dependence. ²⁹
		6.35 (0, -1)	-0.0055	(-1, 46.1) ⁴⁷		Usually used in tissue culture and other specialist applications. ²⁹ Can precipitate metal ions, and evaporation due to temperature changes can cause differences in pH. ⁴⁸
		10.3 (-1, -2)	-0.009	(-2, 71.8) ⁴⁷		Contributes strongly to ionic strength due to its valence. Can bind metal ions. ^{29e,f}
ADA	C ₆ H ₁₀ N ₂ O ₅	6.96 (-1, -2)	-0.011		Primary	Low UV absorbance at 260–280 nm. ^{29e,f}
ACES	C ₄ H ₁₀ N ₂ O ₄ S	6.99 (0, -1)	-0.02	(-1, 31.1) ⁴⁷	Secondary	
MOPSO	C ₇ H ₁₅ NO ₅ S	7.0 (0, -1)	-0.011	(-1, 26.6) ⁴⁷	Tertiary	
PIPES	C ₈ H ₁₈ N ₂ O ₆ S ₂	7.14 (0, -1)	-0.0085		Tertiary	
Imidazole	C ₃ H ₄ N ₂	7.15 (+1, 0) ⁴⁷		(+1, 52.0) ⁴⁷	Secondary	Reactive and unstable. ⁴⁹
Phosphate	H ₃ PO ₄	2.15 (0, -1)	+0.0044			Can interfere with biological reactions, and precipitate divalent metal ions. ^{29,48} Can complex with some organic compounds and lead to poor reproducibility. ^{46,50}
		7.21 (-1, -2)	-0.0028			
		12.33 (-2, -3)	-0.026			
BES	C ₆ H ₁₅ NO ₅ S	7.26 (0, -1)	-0.016	(-1, 26.7) ⁴⁷	Tertiary	Low UV absorbance at 260–280 nm. ²⁹ Binds metal ions (e.g. Cu ²⁺). ^{51e,f}
MOPS	C ₇ H ₁₅ NO ₄ S	7.31 (0, -1)	-0.011	(-1, 24.4) ⁴⁵	Tertiary	
TES	C ₆ H ₁₅ NO ₆ S	7.61 (0, -1)	-0.02		Tertiary	May bind metal ions such as Cu ²⁺ . ^{51e,f}
HEPES	C ₈ H ₁₈ N ₂ O ₄ S	7.66 (0, -1)	-0.014	(-1, 21.8) ⁴⁵	Tertiary	Biocompatible. ²⁹ May complex with protonated amines. ⁴⁶ May bind metal ions such as Cu ²⁺ . ^{51e,f}
TAPSO	C ₇ H ₁₇ NO ₇ S	7.71 (0, -1)	-0.02	(-1, 24.0) ⁴⁷	Secondary	
HEP(SO) (HEPPSO)	C ₉ H ₂₀ N ₂ O ₅ S	7.91 (0, -1)	-0.014		Tertiary	Similar to HEPES. May bind metal ions such as Cu ²⁺ . ^{51,29f}
Tris	C ₄ H ₁₁ NO ₃	8.06 (+1, 0)	-0.028	(-1, 29.5) ⁴³	Primary	Has a reactive primary amine, ³⁴ and can damage electrodes. ²⁹ Reacts with some metal cations. ⁴⁸
Tricine	C ₆ H ₁₃ NO ₅	8.26 (0, -1)	-0.021	(-1, 26.6) ⁴⁷	Secondary	
Bicine	C ₆ H ₁₃ NO ₄	8.46 (0, -1)	-0.018		Tertiary	
TAPS	C ₇ H ₁₇ NO ₆ S	8.51 (0, -1)	-0.02	(-1, 22.9) ⁴⁷	Secondary	
Ammediol	C ₄ H ₁₁ NO ₂	8.78 (+1, 0) ⁴³		(+1, 29.5) ⁴³	Primary	
Bis-tris propane	C ₁₁ H ₂₆ N ₂ O ₆	6.8 (+2, +1)		(+1, 21.6) ⁴⁷	Secondary	
		9.0 (+1, 0) ⁵²				
Borate	H ₃ BO ₃	9.23 (0, -1)	-0.008			Can interact with carbohydrates. ²⁹ Often used with Tris and EDTA as the electrophoresis buffer. Weakly toxic. ⁴⁸ Complexes with many organic compounds, including deprotonated amines and vicinal diols. ^{34,46,50,53,54} Use with caution.
Serine	C ₃ H ₇ NO ₃	9.33 (0, -1) ⁴⁴		(-1, 34.3) ⁴⁴	Primary	
CHES	C ₈ H ₁₇ NO ₃ S	9.41 (0, -1)	-0.018	(-1, 25.1) ⁴⁷	Secondary	Low UV absorbance at 260–280 nm. ^{29f}
Ethanolamine	C ₂ H ₇ NO	9.50 (+1, 0) ⁴³		(+1, 44.3) ⁴³	Primary	
CAPSO	C ₉ H ₁₉ NO ₄ S	9.71 (0, -1)	-0.018		Secondary	Low UV absorbance at 260–280 nm. ^{29f}
Glycine	C ₂ H ₅ NO ₂	2.35 (+1, 0)	-0.002	(+1, 39.5) ⁴⁴	Primary	Can promote microbial growth, and has reactive primary amine. ²⁹ Not recommended unless necessary (e.g., for its high mobility). ^f
		9.78 (0, -1)	-0.025	(-1, 37.4) ⁴⁴		
CAPS	C ₉ H ₁₉ NO ₃ S	10.5 (0, -1)	-0.018		Secondary	Low UV absorbance at 260–280 nm. ^{29f}

^a pK_a at infinite dilution at 25 °C, taken from Beynon and Easterby²⁹ unless otherwise noted. Column also lists valences corresponding to the pK_a values reported here. ^b From Beynon and Easterby,²⁹ in K⁻¹. ^c Fully ionized electrophoretic mobilities, in 10⁻⁹ m² V⁻¹ s⁻¹. ^d As noted in the text, both primary and secondary amine groups can react with dissolved CO₂ for form carbamate. ^e One of the original buffers designed by Good *et al.*³⁴ ^f Zwitterionic buffer. As noted by Good *et al.*,³⁴ these buffers are often highly soluble and biocompatible.

needs), then simultaneously and proportionally vary both weak electrolyte total concentration and titrant concentration (although this is just approximate). For example, the online tool by Beynon and Easterby****,²⁹ allows for specification of both ionic strength and pH given a weak electrolyte. The results can then be checked for buffer capacity, temperature dependence, *etc.* In contrast, calculations using the software tools we summarize above all would require iteration for this particular problem. A second way of setting ionic strength is to add a so-called “neutral salt” such as KCl or NaCl. This allows for quick hand calculations, but such are again only approximate since ionic strength affects pK_a (unless the program used for calculation takes these effects into account). Also, the resulting buffer has less buffering capacity than, say, a weak electrolyte buffer and titrated with a strong electrolyte with equal ionic strength. Specifying both conductivity and pH is even more complex and typically always requires iteration since ionic strength affects both pK_a (Part I of this two-paper series) and electrophoretic mobility (Part II).

Titrant choice can also be important. For example, most strong electrolyte titrants typically have higher electrophoretic mobilities than typical weak electrolyte titrants. So, for a fixed pH value, titrating Tris with hydrochloric acid results in a different conductivity than titrating it with acetic acid. This means these two titrant choices result in difference conductivities, mobilities (and consequently electropherograms) and propensities to Joule heating; all of these drastically affect performance and the likelihood that other laboratories will be able to reproduce reported results.³²

A first and important advice in buffer preparation is the following: *Prepare your own buffers*. Many chemical suppliers offer premade buffer stock solutions such as Tris acetate EDTA (TAE) for agarose gel electrophoresis, Tris borate EDTA for polyacrylamide and agarose gel electrophoresis, phosphate buffered saline for cell culture, Tris hydrochloride for polymerase chain reaction, Tris glycine for SDS page, *etc.* We do not advise using premade buffers for quantitative microchip electrokinetics work for reasons including: (i) premade buffers often contain additives and preservatives (*e.g.*, EDTA, SDS, salts); (ii) the experimentalist does not have full control of pH and ionic strength; (iii) the manufacturer does not always provide exact content; (iv) the name of the buffer can be misleading (*e.g.*, typical Tris EDTA buffer (TE) contains chloride ions); and (v) these buffers are not always cheap. We advise preparation of buffers by hand starting from solid crystals or pure liquid electrolytes of (at least) reagent grade.

We advise preparation of a large amount of buffer stock solution for better reproducibility. Overly frequent preparation of fresh buffer may yield variations in pH. For example, causes include the temperature dependence of pH (despite temperature correction of some pH meters), and inaccuracy of weighing scales. However, this should be weighed against other practical considerations such as the possibility of culturing bacteria in the buffer, time kept in refrigeration, *etc.* For example, a Tris HCl buffer at pH = 8.2 can likely be kept out of a refrigerator for months without bacterial growth; but a pH = 7.3 phosphate

buffer requires refrigeration and ultimately replacement.⁴¹ To ensure that quoted buffer concentrations are correct, aliquots of stock solutions can be titrated with a strong acid or base to confirm that the buffer behaves as predicted. Accuracy in buffer pH also requires proper use of the pH meter. A pH meter is not trivial to use, or to keep calibrated over many measurements.^{11,42} Before each set of titrations (or final verification of predicted pH), the pH meter must be calibrated with fresh standard solutions. We also advise titration of a large volume of weak electrolyte with a high concentration titrant. The initial volume of weak electrolyte should be on the same order of magnitude as the final volume (*e.g.*, start with 50 mL of Tris for a final volume of 100 mL); this for convenience of measurement and accuracy. Once the pH reaches the correct value, addition of solvent to reach the final volume may slightly modify the pH as ionic strength decreases. The pH of the stock solution is, of course, the pH measured at the end of this process, and not the targeted pH value. Avoid the practice of “backpedaling” the titration pH after an overshoot, to keep tight control of ionic strength.

If buffer chemistry is solely determined based on calculations (and the buffer is not actually titrated), then the final measured pH should be reported (and ideally the predicted pH). Roughly speaking, if you care about pH, you should titrate your buffer. If you want to fix pH and ionic strength independently (*e.g.*, and care about individual mobility values) you can validate your buffer calculations (with experiments), use calculations to specify buffer contents; and then verify and report the resulting measured pH. Detailed description of buffer preparation and titration procedures are available in several textbooks.^{29,42} Also, see the next section for suggestions on how to report buffers.

Lastly, we recommend appropriate safety practices always be followed. In Appendix B, we offer some brief safety tips.

On reporting buffers

Surprisingly, despite their importance, buffers and electrolytes are often reported incompletely or incorrectly, and incorrect assumptions are made about electrolyte solutions. For instance, we searched the ISI Web of Science for articles containing “microfluidics” or “electrokinetics” in the title, keywords, or abstract. Of these, we selected papers focused on experimental microchip electrophoresis or closely related work—where buffer and electrolyte chemistry is particularly relevant and crucial. We started in 2008 and worked toward previous years. Among the 40 articles we surveyed (see ESI†), we estimate that 30% incorrectly report electrolyte and pH buffer characteristics and ~12% seem to make incorrect assumptions regarding electrolytes. Common incorrect reporting includes not reporting pH, not reporting buffer concentration, not reporting titrant, and reporting a “standard” chemistry which leaves significant ambiguity. Common incorrect assumptions include assuming that “pure” deionized water has a pH = 7 and/or a Debye length of 1 μm ,^{55,56} buffers used well beyond their buffering range (*e.g.*, pH 1 + units from pK_a),^{57–59} ideal, “pure” water as an electrolyte or buffer,^{60,61} and (low) buffer concentrations which are clearly on the order of expected bicarbonate and/or carbonate ion concentrations (see below).^{62–64} We summarize this brief, limited survey in the ESI.†

We suggest buffers be reported as follows:

**** Available free at <http://www.liv.ac.uk/buffers/buffercalc.html>

- For a titrated buffer: Report weak electrolyte concentration, titrant, and pH. An example would be “100 mM Tris titrated with hydrochloric acid to pH = 8.5.”

- For a buffer prepared by quantifying components: Report weak electrolyte concentration, titrant concentration, and the final measured pH. An example: “Buffer was 100 mM Tris and 100 mM HEPES with a measured pH = 7.8.”

We also suggest researchers consider reporting several useful items including temperature at which pH was measured, conductivity measurement, specific manufacturer and model of pH meter, and predicted ionic strength along with associated assumptions. If the stock solution is to be diluted significantly (*i.e.*, 10-fold), it is useful to report pH after dilution.

Lastly, we note that some typical buffers have a standard composition. Examples include Tris acetate EDTA (TAE) or Tris borate EDTA (TBE), phosphate buffered saline (PBS). The concentrations of these is by convention described by a dilution factor from a standard concentration (*e.g.*, 1× TAE or 0.5× TBE). We believe this is an acceptable report of a buffer, provided the buffer is well known. However, if in doubt, they should be reported fully. For example, so-called “TES” (Tris EDTA sodium) buffer is not “obvious,” as it is easily confused with the weak base TES (*i.e.*, 3- $\{$ tris(hydroxymethyl)methylamino $\}$ -ethanesulfonic acid).

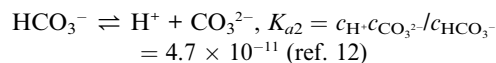
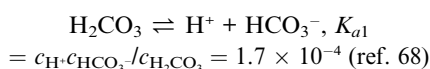
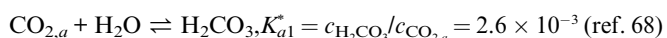
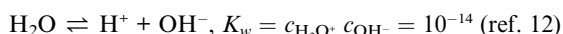
Water and impurities

“Pure” water as an electrolyte

Water ionizes with the dissociation $2\text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{OH}^-$. The chemical kinetics associated with this equilibrium are relatively fast ($k_{rev} = 1.4 \times 10^{11} \text{ M}^{-1}\text{s}^{-1}$, $k_{fwd} = k_{rev}/K_w = 1.4 \times 10^{25} \text{ M}^{-1} \text{ s}^{-1}$).¹³ A general equilibrium constant can be defined as $K_w \approx c_{\text{H}_3\text{O}^+} c_{\text{OH}^-} = 10^{-14}$ at 25 °C (see Robinson and Stokes¹² for data on the temperature dependence of K_w). This yields theoretically minimum ion densities of $c_{\text{H}_3\text{O}^+} = c_{\text{OH}^-} = 0.1 \mu\text{M}$, yielding pH = 7.0, ionic strength of 0.1 μM , and Debye length of approximately 1 μm .⁶⁵ However, neither these ion densities nor the aforementioned pH describe water in practical on-chip electrokinetic experiments. In most experiments, impurities contribute to and even dominate ion density.

Atmospheric carbon dioxide

A major source of impurity ions arises from the effect of atmospheric carbon dioxide. Water uptakes $\text{CO}_{2,g}(\text{gas})$ from the atmosphere to form carbonic acid H_2CO_3 , which dissociates to successively form bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions.⁶⁶ Typical, practical laboratory water chemistry at 25 °C therefore includes at least the following equilibrium reactions:



where the equilibrium constants are expressed in the dilute approximation. H_{CO_2} is simply the Henry’s law coefficient for carbon dioxide, and p_{CO_2} is the partial pressure of carbon dioxide in the atmosphere. The latter is currently $\sim 39 \times 10^{-5} \text{ atm}$ (390 ppm⁶⁹) (note CO_2 is respectively 30 and 50 times more soluble in water than oxygen and nitrogen¹³). The kinetics and thermodynamics of these forward and reverse reactions have been studied extensively.⁷⁰ The limiting step is often the relatively slow hydrolysis of carbon dioxide (time scale order 10 s) and its subsequent convective diffusive transport into the volume of the water.

In the absence of other impurities or buffer ions, we can solve the equations governing the carbon dioxide reaction mechanism to yield the pH of otherwise pure water (*i.e.*, water that has been exposed to atmosphere).⁶⁶ This value is pH ~ 5.6 , which is consistent with our own typical measurements for deionized (DI) water. Typical sources for DI water include clean room deionized water plants or a supplier such as Fischer Scientific (Pittsburg, PA) or Invitrogen (Carlsbad, CA). Therefore, assuming that the pH of deionized water is pH neutral is not realistic, as is often the case in electrokinetics literature.^{65,71} Another common mistake is reporting sub 1 μM concentrations and a 1 μm Debye length.^{55,56} The Debye length of deionized water reduces to about 0.2 μm , a practical lower limit for typical experiments. This suggest that on-chip electrokinetic experiments with “pure” water are likely difficult to control and reproduce.

Buffered systems can stabilize pH and ionic strength (*versus* leaving it to the vagaries of electrolysis and local CO_2 concentration). For pH < 5.5, the dissociation of $\text{CO}_{2,a}$ is negligible and

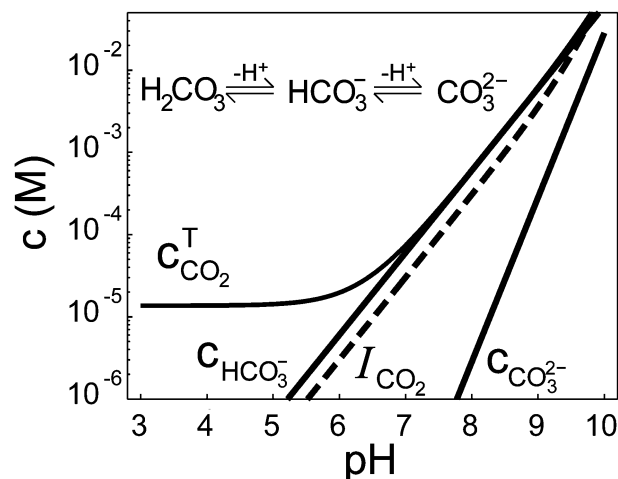


Fig. 6 Carbonic acid concentration and its coupling with pH. At pH < 5, most of the dissolved carbon dioxide remains protonated, in neutral form and asymptotes to the value determined by its Henry’s constant. In this regime, the contribution of carbon dioxide and related species to ionic strength, I_{CO_2} , is less than 1 μM . For pH > 5.5, $c_{\text{HCO}_3^-}$ is non-negligible and the equilibrium total concentration of carbonate species in solution $c_{\text{CO}_2}^T$ dramatically increases. $c_{\text{HCO}_3^-}$ is the primary contribution to I_{CO_2} , which reaches 3.6 mM at pH = 9.0. For pH > 9.5, $c_{\text{CO}_3^{2-}}$ is significant. At high pH, $c_{\text{CO}_3^{2-}}$ has a dramatic effect on ionic strength. Theoretically, the ionic strength contribution from CO_2 reaches 87 mM at pH = 10.

so $c_{\text{HCO}_3^-}$ and especially $c_{\text{CO}_3^{2-}}$ are negligible compared to the total carbonic acid concentration $c_{\text{CO}_2^T}$. For $\text{pH} > 5.5$, $c_{\text{HCO}_3^-}$ and $c_{\text{CO}_3^{2-}}$ can increase to significant quantities. In Fig. 6, we plot $c_{\text{HCO}_3^-}$ and $c_{\text{CO}_3^{2-}}$ on a log scale *versus* solution pH. The contribution of carbonate to ionic strength can grow to 0.4 mM at $\text{pH} = 8.1$ (e.g., in a typical Tris buffer), and to 8.3 mM at $\text{pH} = 9.3$ (e.g., in a typical borate buffer). We conclude that in a system buffered to moderately high pH, one must take into account the contribution of carbonate to ionic strength, conductivity, transference numbers, *etc.*

Carbon dioxide and buffer pH

We here discuss the effects of carbon dioxide on the pH of a typical basic buffer; in particular when its concentration is on the same order as carbonate ions. We showed above that the concentration of carbonate species can be on the order of millimolar at basic pH. At $\text{pH} = 9$, the concentration of carbonate species reaches 3.6 mM (*cf.* Fig. 6). Intuitively, a 1 mM buffer around $\text{pH} = 9$ will be more acidic than expected. Fig. 7 shows such an example, where we present the pH of three Tris buffers in atmospheric (with CO_2) and ideal conditions (without CO_2). We obtained the non-ideal case curves from resolution of the full acid–base problem, using the general formulation we presented

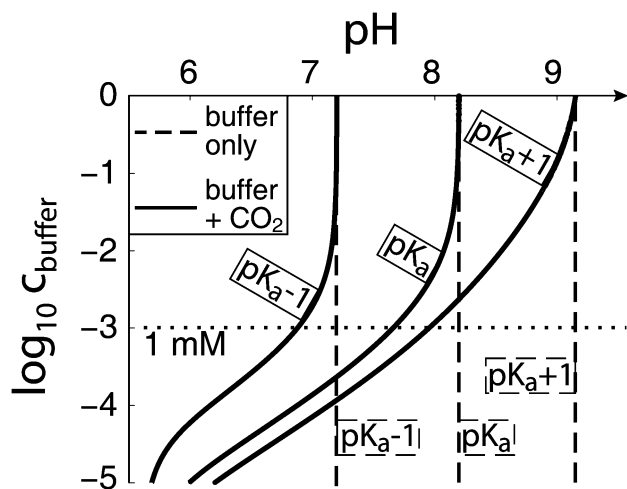
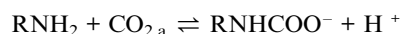


Fig. 7 Flood's diagram for three different Tris hydrochloride ($\text{p}K_a = 8.2$ at 20°C) buffers under the influence of dissolved CO_2 and carbonate species. The dashed vertical lines correspond to three idealized cases where the atmospheric carbon dioxide concentration is neglected, and moderate pH. The solid curves correspond to a realistic buffer subject to atmospheric effects. The curve labels indicate the pH value in idealized conditions (free of CO_2). The middle curve (labelled $\text{p}K_a$) corresponds to a buffer prepared with c_{buffer} of Tris and $0.5 c_{\text{buffer}}$ of HCl, so that, ideally, $\text{pH} = \text{p}K_a = 8.2$ (from Henderson–Hasselbalch equation (21)). For $c_{\text{buffer}} > 100$ mM, the buffer pH has values reasonably close to those predicted by neglecting CO_2 . However, we see strong deviation from ideal pH for buffer concentrations smaller than 10 mM. For example, a 1 mM Tris, 0.5 mM HCl solution has an actual pH about 7.7; while a 0.1 mM, Tris 0.05 mM HCl solution has an actual pH of about 6.9 (*vs.* 8.2). The left (labelled $\text{p}K_a - 1$) and right-hand (labelled $\text{p}K_a + 1$) curves correspond to buffers prepared with c_{buffer} of Tris and respectively 0.91 c_{buffer} and 11 c_{buffer} of HCl; and so, in the absence of CO_2 , would yield respectively $\text{pH} = \text{p}K_a - 1 = 7.2$ and $\text{pH} = \text{p}K_a + 1 = 9.2$.

earlier with carbonate and buffer equilibrium reactions. Each solid curve corresponds to the calculated pH of a buffer with the relative amount of Tris and HCl is constant, but where their absolute concentrations varies, and pH changes accordingly with the contribution from atmospheric CO_2 . From left to right, the curves respectively correspond to $c_{\text{titrant}} = 11 c_{\text{buffer}}$, $0.5 c_{\text{buffer}}$ and $0.9 c_{\text{buffer}}$ (so that the pH values in the absence of CO_2 effects are $\text{p}K_a - 1$, $\text{p}K_a$, and $\text{p}K_a + 1$, respectively). The expected pH calculated from just the Henderson Hasselbalch equation (25) can significantly overestimate the actual pH value. There is a clear deviation between ideal, carbon-dioxide-free pH and (practical) pH exposed to carbon dioxide when buffer concentration is below about 10 mM. For example, a solution of 100 mM Tris and 50 mM HCl has pH about 8.2 (near the ideal value), while a solution of 0.1 mM Tris, and 0.05 mM HCl has a pH of about 6.9, more than one unit below the carbon-dioxide-free pH. For the buffer prepared at $\text{p}K_a + 1$, the pH at 1 mM can be more than 1 unit below the carbon-dioxide-free value of 9.2. The effect of carbonate species is strongest for the most basic buffers. We conclude that buffers prepared according to a “buffer recipe” (e.g., by weighing weak electrolyte and titrant and not by titration) based solely on dissociation of Tris (*i.e.*, neglecting CO_2), and/or buffers diluted one or more order of magnitudes may not reach the targeted pH.

In summary, this effect can significantly impact buffer preparation when: (i) diluting a stock solution more than $10\times$, or (ii) when preparing buffers from “recipes”, *i.e.* from amounts of reagents calculated according to an ideal case. Carbonate effects highlight the necessity of buffer titration and careful pH measurement.

Buffer ions may also interact and react with atmospheric gases. For example, as discussed and observed experimentally by Khurana and Santiago,⁷² $\text{CO}_{2,a}$ can react with uncharged primary and secondary amine groups of buffer components (e.g., glycine or Tris) or analyte ions (e.g., amino acids) and strongly affect electrokinetic experiments. The reaction forms carbamate ions as summarized below:^{73,74}



where “R” denotes an arbitrary functional group. Typically, the forward reaction has a characteristic time scale of ~ 0.1 s and the equilibrium constant a typical value of 10^{-5} .⁷⁹ Commonly used weak electrolyte buffer species with primary or secondary amine groups include glycine, epsilon amino-caproic acid, Tris, bis-Tris propane, alanine, and benzylamine among others (see Table 2). Presumably, carbamate ions may represent a source of extra peaks in electropherograms; and are certainly an important issue in displacement assays such as isotachopheresis.⁷²

On the use of barium hydroxide to mitigate CO_2 effects

We note that one method for reducing dissolved CO_2 (and therefore carbonate and in some cases bicarbonate and carbamate ions and their effects on pH) is to add barium hydroxide ($\text{Ba}(\text{OH})_2$) to a buffer. Barium hydroxide is a moderately soluble strong base;⁷⁶ it can also serve as a titrant or one of multiple titrants (e.g., along with NaOH) in a buffer. Barium hydroxide

reacts with carbon dioxide to form barium carbonate, BaCO_3 . BaCO_3 is practically insoluble in water⁷⁶ and rapidly precipitates as a solid.⁷⁷

The effect of bicarbonate, carbonate, and carbamates as background ions can be especially important in displacement type assays such as isotachopheresis.⁷⁸ An example visualization of carbonate and carbamate zones, their effect on isotachopherograms, and the mitigation of these effects with barium hydroxide is discussed by Khurana and Santiago.⁷² Khurana and Santiago introduced $\text{Ba}(\text{OH})_2$ at 5 mM concentrations into a 250 mM glycine buffer. This addition was performed both during the buffer preparation step, and also introduced real-time into a microchip reservoir as a crystalline solid. In that particular application, electroosmotic flow helped to prevent entrainment of precipitant particles into the channel entrance.

Other impurities

On the subject of impurities, it is worth mentioning that improperly cleaned glassware and even “pristine” plastic-ware can contaminate water. One notable example is the recent study by McDonald *et al.*⁷⁹ showing that commonly used disposable plastic ware such as pipette tips and plastic tubes can leach chemicals and contaminate buffers with order μM concentrations of biologically active DiHEMDA and *cis*-Oleamide. These contaminants can significantly modify experimental results of biological assays.

Conclusion

We have presented a review of the theory, formulations, design, and practical implementations of aqueous electrolyte chemistry. We first presented a brief summary of acid–base equilibria, electrolyte activity, and discussed the relation between activity and ion concentrations. We then presented a general formulation for the well-posed problem of a solution with an arbitrary number of weak and strong acids; weak and strong bases; and salts. This formulation leverages a systematic notation and yields a compact polynomial equation which allows for direct (*vs.* typical iterative) solutions. We described available (free, online) software which can be used to solve arbitrary electrolyte problems, including transport problems in on-chip electrokinetic devices.

We then presented a description and consequences of the moderate pH approximation for electrolyte solutions in the common pH range of 4 to 10. We leveraged this approximation in presenting intuitive formulations, solutions, and graphical visualization of typical pH buffer equilibria. This included simple, yet accurate estimates of pH and ion densities in typical pH buffer systems involving weak bases and/or weak acids. We provided a simple classification of types of buffers and listed example choices of weak electrolyte and associated titrants. We ended the latter discussion with a quantitative description of buffering capacity and the very strong dependence of buffering capacity on proper titration.

Ionic strength plays a major role in determining electrolyte conductivity (obviously) and pH (less obviously). Ionic strength can be viewed as a quantification of the deviation from the ideal

dilute approximation. At high ionic strength, ions in solutions shield each other more efficiently, therefore reducing activity. We presented an overview of corrections of activity coefficients for ionic strength. For most on-chip electrokinetic applications, we recommended the use of the Davies equation which shows a good fit with experimental measurements of activity coefficients. We provided a formulation which can be used in practical applications to estimate the effect of ionic strength on $\text{p}K_a$.

To help experimentalists developing chemistry for electrokinetic devices, we presented discussions around practical issues in the selection, design, preparation, and reporting of pH buffer solutions. We first presented a summary of the effects of (and how to estimate) temperature on buffer chemistry and pH. We then presented a summary of commonly used buffers (Table 2). This table summarizes key information including $\text{p}K_a$ and its associated valence, chemical formulas, and temperature dependence of $\text{p}K_a$. The notes column of the table summarizes practical information including known reactions with analytes and other buffer ions, optical properties, biocompatibility, and reported propensity to culture microbial growth.

Lastly, we presented a summary of the effects of impurities in electrolytes. Most importantly, we highlighted the known and expected effects of carbon dioxide on solution ionic strength and buffer pH. Atmospheric carbon dioxide dissolves in water and dissociates into bicarbonate and carbonate ions. Contribution to ionic strength theoretically varies from approximately 1 μM at pH 5.6 (typical pH of deionized water) to approximately 90 mM at pH = 10. We showed that carbon dioxide can have a significant effect on buffer equilibria and titration. For example, 100 \times dilution of a 100 mM Tris hydrochloride buffer titrated to pH = 8.2 reduces the pH to 7.2, solely from carbon dioxide acidification. We concluded our presentation of common impurities by discussing reaction between amine buffers and carbon dioxide (to form carbamate), as well as impurities from common disposable plastic containers.

In Part II of this two-paper series we continue our review of electrolyte chemistry and its practical implementation to electrokinetic systems. We will discuss the effects of acid–base equilibrium on electrophoretic mobilities. We also use acid–base reactions to describe the chemistry of electrode reactions and its effect on electrokinetic experiments. In parallel, we present general electrochemical theory and discuss the relation between electrode and electrolyte potentials.

Appendix A

Example construction of polynomials for generalized electrolyte problems

As an example for the construction of the polynomials for the generalized electrolyte formulation, consider the case of a mixture consisting of a bivalent ampholyte (*e.g.*, glycine), a divalent weak acid (*e.g.*, carbonic acid), and monovalent weak base (*e.g.*, Tris). We summarize the properties in Table 3: The matrix L can then be constructed according to eqn (15) as

$$\mathbf{L} = \begin{bmatrix} 10^{-9.8} & 1 & 10^{2.3} \\ 10^{-10.3} 10^{-6.4} & 10^{-6.4} & 1 \\ 1 & 10^{8.1} & 0 \end{bmatrix}$$

Table 3 Summary of properties for a three electrolyte example formulation

Number	Component	Valences	pK_a	n_x	p_x
1	Glycine	[-1 0 + 1]	[9.8 2.3]	-1	1
2	Carbonic acid	[-2 -1 0]	[10.3 6.4]	-2	0
3	Tris	[0 + 1]	[8.08]	0	1

The construction of the polynomial Q is equivalent to the multiplication of the polynomials

$$Q = (-10^{-14} + c_H^2)(10^{-9.8} + c_H + 10^{2.3}c_H^2) \dots (10^{-16.7} + 10^{-6.4}c_H + c_H^2)(1 + 10^{8.1}c_H)$$

However, to reduce this calculation to a purely numerical algorithm, the coefficients of the resulting polynomial can be computed by convolving the four vectors of coefficients:

$$Q = [-10^{-14}, 0, 1] * [10^{-9.8}, 1, 10^{2.3}] * [10^{-16.7}, 10^{-6.4}, 1] * [1, 10^{8.1}, 0]$$

The result is a vector of size 1×8 , representing the coefficients of a 7th order polynomial.

The polynomials P_i are computed in a similar manner with some modifications to the matrix L due to a multiplication with the valence. For example, P_1 , P_2 , P_3 and P_4 would be computed by convolving the row of

$$\hat{M}_1 = \begin{bmatrix} -10^{-9.8} & 0 & 10^{2.3} \\ 10^{-16.7} & 10^{-6.4} & 1 \\ 1 & 10^{8.1} & 0 \\ 0 & 1 & 0 \end{bmatrix}$$

$$\hat{M}_2 = \begin{bmatrix} 10^{-9.8} & 1 & 10^{2.3} \\ -2 \cdot 10^{-16.7} & -10^{-6.4} & 0 \\ 1 & 10^{8.1} & 0 \\ 0 & 1 & 0 \end{bmatrix}$$

$$\hat{M}_3 = \begin{bmatrix} 10^{-9.8} & 1 & 10^{2.3} \\ 10^{-16.7} & 10^{-6.4} & 1 \\ 0 & 10^{8.1} & 0 \\ 0 & 1 & 0 \end{bmatrix}$$

respectively, where the last row in each matrix represents the multiplication by c_H appearing in eqn (20). As an example, we solved for pH of a solution composed of 10 mM Tris, 10 mM carbonic acid and 100 mM glycine. The resulting pH is about 7.2.

Appendix B

A note on safety practices

We advise to wear appropriate protective equipment when manipulating chemicals (e.g. gloves, safety glasses). In particular, use extreme caution when manipulating strong acids or bases; we recommend manipulation of those chemicals in a laboratory fume hood.

We also advise that researchers consult the material safety data sheet (MSDS) before use of any chemicals. Note that health effects of many chemicals are unknown. For example, according to the National Institute for Occupational Safety and Health (NIOSH), only 2% of chemicals have been tested for carcinogenicity. More detailed safety practices can be found on the NIOSH website (<http://www.cdc.gov/niosh>).

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