Basic principles of electrolyte chemistry for microfluidic electrokinetics. Part I:† Acid–base equilibria and pH buffers‡§

Alexandre Persat, Robert D. Chambers and Juan G. Santiago*

Received 1st April 2009, Accepted 4th June 2009
First published as an Advance Article on the web 7th July 2009
DOI: 10.1039/b906465f

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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† For Part II see ref. 4.
‡ Part of a Lab on a Chip themed issue on fundamental principles and techniques in microfluidics, edited by Juan Santiago and Chuan-Hua Chen.

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We begin with an introduction to some essential relations and concepts in the aqueous chemistry of microfluidic electrokinetics. 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, we discuss the effects of acid–base equilibria on electrophoretic mobilities and conductivities, and discuss the coupling between electrolyte chemistry and electrode reactions.

### Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>acid form of species A</td>
</tr>
<tr>
<td>A−</td>
<td>basic form of species A</td>
</tr>
<tr>
<td>BH+</td>
<td>acid form of species B</td>
</tr>
<tr>
<td>B</td>
<td>basic form of species B</td>
</tr>
<tr>
<td>H3O+</td>
<td>hydronium ion</td>
</tr>
<tr>
<td>H+</td>
<td>proton and short notation for hydronium ion</td>
</tr>
<tr>
<td>OH−</td>
<td>hydroxyl ion</td>
</tr>
<tr>
<td>αX</td>
<td>activity of X</td>
</tr>
<tr>
<td>cX,z</td>
<td>concentration of X in valence state z (M)</td>
</tr>
<tr>
<td>I</td>
<td>ionic strength (M)</td>
</tr>
<tr>
<td>Kd</td>
<td>acid dissociation constant</td>
</tr>
<tr>
<td>Kd,X,z</td>
<td>acid dissociation constant of X in valence z</td>
</tr>
<tr>
<td>nX</td>
<td>minimum valence of species X</td>
</tr>
<tr>
<td>pX</td>
<td>maximum valence of species X</td>
</tr>
</tbody>
</table>

### 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), tactile sensation (e.g., the soapy feeling of bases), or chromatic properties. 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. Arrhenius’ seminal work is at the basis of the modern definition of acids and bases given by Bronsted and Lowry: 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−:

\[ HA \rightleftharpoons A^- + H^+. \]  

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:

\[ 2H_2O \rightleftharpoons H_3O^+ + OH^- . \]

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

\[ HA + H_2O \rightleftharpoons A^- + H_3O^+. \]  

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 Sorensen 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:

\[ \text{pH} = -\log_{10} [H^+], \]

where [H+] is the activity of the hydronium ions on the molal scale. This definition holds for pH ranging between 2 and 12, and for ionic strength below 100 mM. This in this paper, we will assume this definition is equivalent to the measurement given by a (calibrated) Harned cell pH-meter. The activity αX can be viewed as an effective concentration of the species X. It is defined from the chemical potential \( \mu_X \) as:

\[ \mu_X = R T \ln a_X. \]
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.12,13 We define the molar scale activity coefficient $\gamma_X$ as $a_X = \gamma_X c_X$ where $c_X = 1$ M.13 As is common practice, we will omit $c_X$ in such activity relations and replace it by its value so the quantity $c_X$ is written simply as $c_X$. $\gamma_X$ 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$^-$:

$$K_a = \frac{a_H a_X}{a_A a_{H^+}} = \frac{c_H c_A}{c_{HA}} = \frac{\gamma_H \gamma_A}{\gamma_{HA}}$$

(6)

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

$$\text{pH} = \text{p}K_a + \log_{10} \frac{\gamma_A c_A}{\gamma_{HA} c_{HA}}$$

(7)

where $pK_a = -\log_{10} K_a$

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, 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$^-$:

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including \( \text{PO}_4^{3-} \) (i.e., \( X^{-3} \)), \( \text{HPO}_4^{2-} \) (\( X^{-2} \)), \( \text{H}_2\text{PO}_4 \) (\( X^{-1} \)), and \( \text{H}_3\text{PO}_4 \) (\( X^0 \)). For each reaction from valence \((z+1)\) to \(z\), we can write generally the acid dissociation constant \( K_{X,z} \) as:

\[
K_{X,z} = c_{X,z}c_{\text{H}^+}c_{X,z+1},
\]

where \( c_{\text{H}^+} \) is the hydronium ion concentration and \( c_{X,z} \) and \( c_{X,z+1} \) 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, \( pK_{X,z} \) 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 \( \text{H}^+ \) on the right hand side and \( c_{\text{H}^+} \) in the numerator of the expression of \( K \)). Respectively, the dissociation of a weak acid (acetic acid \( \text{CH}_3\text{COOH} \)), a weak base (ammonia \( \text{NH}_3 \)), strong acid (hydrochloric acid \( \text{HCl} \)), and a strong base (sodium \( \text{Na}^+ \)) can be written as

\[
\text{CH}_3\text{COOH} \rightleftharpoons \text{CH}_3\text{COO}^- + \text{H}^+
\]

\[
\text{NH}_4^+ \rightleftharpoons \text{NH}_3 + \text{H}^+
\]

\[
\text{HCl} \rightleftharpoons \text{Cl}^- + \text{H}^+
\]

\[
\text{Na}^+ + \frac{1}{2}\text{H}_2\text{g} \rightleftharpoons \text{Na} + \text{H}^+
\]

We note that salts are strong electrolytes, but their dissociation does not involve the acceptance or donation of a proton, and may be written as \( Y_zX_n(s) \rightleftharpoons p_zY^- + p_nX^- \) (e.g., as in potassium chloride \( \text{KCl}(s) \rightleftharpoons \text{K}^+ + \text{Cl}^- \), or disodium phosphate \( \text{Na}_2\text{HPO}_4(s) \rightleftharpoons 2\text{Na}^+ + \text{HPO}_4^{2-} \)). 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 \( \text{NaCH}_3\text{COO}(s) \rightleftharpoons \text{Na}^+ + \text{CH}_3\text{COO}^- \) mathematically introduces two associated \( pK_a \) 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 general equations from law of mass action, mass balance, and electroneutrality (a.k.a. charge balance). Consider a simple weak electrolyte \( A \) whose acid form dissociates as \( \text{HA} \rightleftharpoons \text{A}^- + \text{H}^+ \). The relevant equilibrium reactions have dissociation constants \( K_a = c_{\text{HA}}c_{\text{A}^-}/c_{\text{A},0} \) (recall that, in our notation, \( c_{\text{A},0} = c_A \) and \( c_{\text{A},0} = c_{\text{HA}} \) for this case). The total concentration of \( A \) (a.k.a. the analytical concentration) is \( c_A = c_{\text{A},0} + c_{\text{A},0} \), 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 \( c_{\text{A},0} + c_{\text{OH}^-} = c_{\text{H}^+} \) (see below). These four equations can be combined to yield an implicit third order polynomial in \( c_{\text{H}^+} \):

\[
c_{\text{H}^+} + K_{\text{A},0}c_{\text{H}^+} = c_{\text{H}^+}(K_{\text{A},0}c_A + K_a) - K_{\text{A},0}K_a = 0.
\]

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 \( c_{\text{H}^+} \). We can plot the solution to both of these polynomials in a single plot known as Flood’s diagram. Fig. 1 shows Flood’s diagram of log\(_{10} C_X\) 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 pH 7 are for acids \( X \) of various \( pK_a \) values; while those above pH 7 are for bases \( X \) and their associated \( pK_a \) values. We see that at sufficiently high analytical concentrations of weak acid, there is a linear relation between \( c_{\text{H}^+} \) 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 \( pK_a \) 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 pH = -log\(_{10} C_X\). pH always decreases with addition of weak acid, but more and more weakly as their \( pK_a \) increases. Similarly, weak bases deviate from the effect of strong base on pH (pH = 14 + log\(_{10} C_B\)). For the same base concentration, the pH of a stronger base (higher \( pK_a \)) is higher than for a weak base.](image-url)
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 $r_X = p_X - n_X$. For a species $X$ considered, we have $2 + r_X$ unknowns (including $c_{1H}$), 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 $K_{X,z} = c_{X,z}c_{1H}/c_{X,z+1}$) 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 $c_{OH}c_{1H} = K_w$. 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 $c_{1H}$ 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. 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:

$$c_X = \sum_{z=-n_X}^{p_X} c_{X,z}. \tag{11}$$

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:

$$\sum_{X} p_X c_{X,z} + c_{1H} - c_{OH} = 0. \tag{12}$$

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

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., the concentration of each ionic state can be related to the concentration of the neutral state, $c_{X,0}$, and the hydronium concentration, $c_{1H}$. For example, multiplication of all $K$ values from $z' = 0$ to $z' = z - 1$ yields:

$$K_{X,0}K_{X,1}K_{X,2} \ldots K_{X,z-1} = \frac{c_{X,0}c_{X,1} \ldots c_{X,z-1}}{c_{1H}^z} = \frac{c_{X,0}}{c_{X,z}}c_{1H}^z. \tag{13}$$

Generalizing this result for both positive and negative ionic states yields

$$c_{X,z} = c_{X,0}L_{X,z}c_{1H}, \tag{14}$$

where

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

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

$$c_{X,z} = c_{X,0}L_{X,z}c_{1H} \sum_{z=-n_X}^{p_X} L_{X,z'}c_{1H}^z. \tag{16}$$

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 $c_{1H}$:

$$\sum_{X=1}^{N} \sum_{z=-n_X}^{p_X} z c_{X} L_{X,z} c_{1H}^z + c_{1H} = \frac{K_w}{c_{1H}} = 0. \tag{17}$$

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 $c_{1H}$. However, more conveniently, since only integer powers appear in the expression, additional algebraic manipulation of this equation results in a polynomial form:

$$\sum_{X} c_{X} P_X + Q = 0, \tag{18}$$

where

$$Q = \prod_{Y} Q_Y, \tag{19}$$

$$P_X = c_{1H} \prod_{Y} \left( \sum_{z=-n_Y}^{p_Y} L_{Y,z'} (1 + (z' - 1)\delta_{XY}) c_{1H}^{z'-n_Y} \right), \tag{20}$$

$$Q_0 = (c_{1H} - K_w). \tag{21}$$
\[ Q_T = \prod_{Y} \left( \sum_{Z} L_{Y,Z} c_H^{Z-Y} \right), \]  

(22)

d}\_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, Simul, and Spresso, 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-expansible 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 hydroxyl ions. In this regime, it is useful to assume a “moderate pH”, 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

\[ \sum_{X} \sum_{Z} z_{XZ} c_X = 0 \quad \text{(moderate pH).} \]  

(23)

In other words, here we 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
of a weak acid can be a strong base, a weak base with \( pK_u \sim 2 \) 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 acetate 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.\(^\text{26,27}\)

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:

\[
HA \rightleftharpoons A^- + H^+
\]

\[
BH^+ \rightleftharpoons B + H^+
\]

The relevant acid dissociation constants are \( K_{A,0} = c_{A,-1}c_{H}/c_{A,0} \), \( K_u = c_{H}c_{A^-} \), and \( K_{B,0} = c_{B,0}c_{H}/c_{B,1} \). With our convention, the value of the latter is very low (smaller than \( 10^{-12} \)) so that \( c_{B,0} = 0 \). The conservation equations are \( c_A = c_{A,-1} + c_{A,0} \) and \( c_B = c_{B,0} + c_{B,1} \) (i.e., full dissociation), while electroneutrality (assuming moderate \( pH \)) can be written as \( c_{A,-1} \equiv c_{B,1} \). 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 \( c_H \),

\[
c_H = K_{A,-1} (c_A/c_B - 1). \tag{24}\]

Taking the negative logarithm:

\[
pH = pK_{A,-1} + \log_{10} \frac{c_B}{c_A} \tag{25}\]

We use this relation to show the titration of a weak acid by a strong base about its \( pK_u \) 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 \( c_A = 0.2 \) \( c_B \). When the titrant concentration approaches half of the weak electrolyte concentration, \( pH \) is more robust to addition of titrant; and, at \( c_A = 0.5 \) \( c_B \), \( pH = pK_{weak} \), 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 \( c_{titrant}/c_{weak} = 1.5 \) are due to further addition of titrant.

### Table 1  Common buffer classifications and a few examples of titrant

<table>
<thead>
<tr>
<th>Buffer type</th>
<th>Weak electrolyte example</th>
<th>Titrant examples</th>
<th>Weak acid/base</th>
<th>Strong acid/base</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak acid</td>
<td>Phosphoric Acid, HEPES, MES, MOPS</td>
<td>(Mono/bi/tri) sodium phosphate</td>
<td>Tris, Bis-Tris</td>
<td>NaOH, KOH</td>
</tr>
<tr>
<td>Weak base</td>
<td>Acetic acid, Tris, Bis-Tris</td>
<td>Sodium acetate, Tris-HCl</td>
<td>Boric acid, acetic acid</td>
<td>HCl</td>
</tr>
</tbody>
</table>

\[2009, 9, 2437–2453 | 2443\]
Again, we can arrange this into polynomial form as
\[ (c_A - c_A/2) c_H - K_{A-1} c_A c_B = 0, \]
so that
\[ c_H = \frac{K_{A-1}}{2} \left( \frac{c_A}{c_B} - 1 \right) + \sqrt{\left( \frac{c_A}{c_B} - 1 \right)^2 + 4 \frac{K_{B,0} c_A}{K_{A-1} c_B}}. \]

In Fig. 4 we show the titration of 50 mM Tris (a weak base with \( pK_a = 8.1 \)) by acetic acid (a weak acid with \( pK_a = 4.8 \)). 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 \( pK_a \) 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:

\[ \beta = \frac{\partial c_B}{\partial pH} \quad \text{(addition of strong base)}, \]
\[ \beta = -\frac{\partial c_A}{\partial pH} \quad \text{(addition of strong acid)}, \]

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

\[ \frac{\partial c_H}{\partial c_B} = -\frac{c_A}{c_H} \frac{K_{A-1}}{c_B^2}. \]

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):

\[ \beta = \frac{\partial c_B}{\partial pH} = 2.30 \left( c_A c_H K_{A-1}/(c_H + K_{A-1})^2 + c_H + K_a/c_H \right). \]

(32)

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

\[ \beta = -\frac{\partial c_A}{\partial pH} = 2.30 \left( c_B c_H K_{B,0}/(c_H + K_{B,0})^2 + c_H + K_a/c_H \right). \]

(33)

As a rule of thumb, buffers are most effective when \( c_H \) is roughly equal to the \( pK_a \) of one of the buffering species, i.e., pH \( \approx pK_a \). 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 pH \( \approx pK_a \). Conversely, a small amount of titrant dramatically changes pH when pH is far from the buffer \( pK_a \). 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 \( c_H = K_{A-1} \)).

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 \( pK_a \) 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 pH \( \approx pK_a \). 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 \( pK_a \)), or in buffers with multiple weak electrolytes, is more complex. For instance, if two \( pK_a \)’s are within about 2 pH units of each other, then the pH (or pH’s) at which buffer...
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.\(^\text{10}\)

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,\(^\text{12}\) 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:\(^\text{13,14}\)

\[
I = \frac{1}{2} \sum_X \sum_z z^2 c_{Xz}.
\]

(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 coefficient \(\gamma_\pm\) of an ion pair \(p_+Y^-; p_-,X^+\) by \((\gamma_\pm)^{Y--} \equiv (\gamma_\pm)^{Y-}\) (from electroneutrality, valences \(z_\pm\), and the number of ions \(p_\pm\), we see that \(z_+p_+ + z_-p_- = 0\)). For example the mean activity coefficient of a magnesium chloride salt MgCl\(_2\) in solution is \(\gamma_{\pm}^1 = \gamma_+^1\gamma_-^1\) (i.e., \(\gamma_\pm = (\gamma_+\gamma_-)^{1/2}\)). Or for a weak acid HA which dissociates into H\(^+\) and A\(^-\), \(\gamma_\pm^1 = \gamma_{HA}\). The acid dissociation constant (6) therefore becomes:

\[
K_a = \frac{\epsilon_{HA}c_{HA}}{\gamma_{HA}}\gamma_+^1.
\]

(35)

While the treatment of the activity coefficient of the neutral species is rather simple,\(^\text{12,13}\) the physics involved in the mean activity coefficient for charged species are complex.\(^\text{12}\) Debye and Hückel\(^\text{11}\) 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 \(I\):

\[
\log_{10}\gamma_\pm = -\frac{A_z^1|z_\pm|\sqrt{I}}{1 + aB\sqrt{I}}.
\]

(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 \(B = 0.328\text{ Å}^{-1}\text{ M}^{-1/2}\)), \(a\) is the ionic diameter of the ion in Å. A good approximation for small ions is to assume \(a = 3\text{ Å}\), which yields a product \(aB\) of approximately unity.\(^\text{13}\)

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.\(^\text{11}\) 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.\(^\text{12}\) Davies\(^\text{14}\) proposed an improvement over the Debye–Hückel equation which shows better agreement with experimental values of mean activity coefficients:\(^\text{13,14}\)

\[
\log_{10}\gamma_\pm = -0.51z_\pm^1\sqrt{I}
\]

(37)

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

\[
\log_{10}\gamma_{Xz} = -0.51z_\pm^2\left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3I\right).
\]

(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.\(^\text{13}\) 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 \(\gamma_{HA}\) is approximately unity.\(^\text{13,14}\) so that starting with equations of the form of (7) and (38) we can derive:

\[
pK'_a = pK_a + 2\log_{10}\gamma_\pm.
\]

(39)

\(^\text{14}\) The coefficient of the linear term is here taken equal to 0.3 \textit{versus} 0.2 as initially reported by Davies.\(^\text{16}\) 0.3 shows better agreement with experimental data.\(^\text{14}\)
where $2 \log_{10} \gamma_\pm (e.g. , \text{from eqn (37)})$ 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 $\text{HA}'$ of valence $z$ as

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

(40)

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

$$pK_a' = pK_{a, I=0} + \Delta pK_a.$$  

(41)

The Davies equation is a rather popular relation,\textsuperscript{33,34} 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 fluorescein fluorescent dye with pH and ionic strength.

The acid dissociation constant $K$ is related to the enthalpy of acid dissociation through the Van‘t Hoff equation:\textsuperscript{34}

$$\frac{d \log K}{dT} = \frac{\Delta H^0}{2.3RT^2}$$

(42)

where $\Delta H^0$ is the standard enthalpy of reaction. $\Delta H^0$ 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:\textsuperscript{33}

$$pK_a = \frac{\lambda_1}{T} + \frac{\lambda_2}{T^2} + \frac{\lambda_3}{T^3} + T + ..., \quad (43)$$

where the coefficients $\lambda_i$ are typically determined from fitting experimental data. For example, Harned and Ehlers\textsuperscript{33} fitted the measurement of the temperature dependence of acetic acid $pK_a$ as follows:

$$pK_{\text{CH}_3\text{COOH}} = \frac{1501}{T} - 18.67 + 6.5 \log_{10} T + 0.007T.$$  

Over small temperature differences, $pK_a$ dependence on temperature is approximately linear,\textsuperscript{29} and so it is common to report the temperature coefficient $d pK_a/dT$. 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,\textsuperscript{34,35} and are widely known as “Good’s buffers”.\textsuperscript{33,35} 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.\textsuperscript{29} As we mention later, both primary and secondary amine groups can react with dissolved CO₂ (e.g., atmospheric CO₂) 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,\textsuperscript{36} binding constants,\textsuperscript{37} Joule heating,\textsuperscript{38} biomolecular adsorption characteristics,\textsuperscript{39} 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 electrosoretic 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).\textsuperscript{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 electrosoretic 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).\textsuperscript{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.\textsuperscript{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...
<table>
<thead>
<tr>
<th>Name</th>
<th>Formula</th>
<th>(pK_a^a)</th>
<th>(dpK_a/dT) in (°C)</th>
<th>(Valence, mobility)</th>
<th>Amine group (^f)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>C(_6)H(_7)O(_7)</td>
<td>3.13 (−0.1)</td>
<td>(−1,28.7)</td>
<td>Tertiary/Secondary</td>
<td>Tertiary</td>
<td>Corrosive, fumes are an irritant. (^{29})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.76 (−1.2)</td>
<td>(−2,54.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.40 (−2.3)</td>
<td>(−3,74.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinic acid</td>
<td>C(_6)H(_8)O(_4)</td>
<td>4.21 (0.1)</td>
<td>(−1,33.0)</td>
<td>Tertiary/Secondary</td>
<td>Tertiary</td>
<td>May complex with protonated amines. (^{40,47})</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>C(_2)(_2)COOH</td>
<td>4.76 (0.1)</td>
<td>(−1,42.4)</td>
<td>Primary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>C(_6)H(_5)(_2)N(_2)O(_4)</td>
<td>4.83 (+1,0)</td>
<td>(+1,37.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MES</td>
<td>C(_9)H(_19)NO(_4)S</td>
<td>6.21 (0.1)</td>
<td>(−1,26.8)</td>
<td></td>
<td>Tertiary/Secondary</td>
<td></td>
</tr>
<tr>
<td>Bis-tris Carbonate</td>
<td>C(_9)H(_19)NO(_5)S</td>
<td>6.35 (+1,0)</td>
<td>(−1,26.8)</td>
<td></td>
<td>Tertiary</td>
<td>p(K_a) has high temperature dependence. (^{29})</td>
</tr>
<tr>
<td></td>
<td>H(_2)CO(_3)</td>
<td>6.35 (0.1)</td>
<td>(−1,46.1)</td>
<td></td>
<td>Tertiary</td>
<td>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})</td>
</tr>
<tr>
<td></td>
<td>10.3 (−1.2)</td>
<td>−0.009</td>
<td>(−2,71.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADA</td>
<td>C(_6)H(_10)N(_2)O(_3)</td>
<td>6.96 (−1.2)</td>
<td>(−1,26.7)</td>
<td></td>
<td>Tertiary/Secondary</td>
<td>Contributes strongly to ionic strength due to its valence. Can bind metal ions. (^{20,29})</td>
</tr>
<tr>
<td>ACES</td>
<td>C(_9)H(_10)N(_2)O(_4)S</td>
<td>6.99 (0.1)</td>
<td>(−1,31.1)</td>
<td>Secondary</td>
<td></td>
<td>Low UV absorbance at 260−280 nm. (^{29})</td>
</tr>
<tr>
<td>MOPS</td>
<td>C(_9)H(_12)N(_2)O(_4)S</td>
<td>7.0 (0.1)</td>
<td>(−1,26.6)</td>
<td></td>
<td>Tertiary</td>
<td></td>
</tr>
<tr>
<td>TAPSO</td>
<td>C(_9)H(_19)NO(_5)S</td>
<td>7.14 (0.1)</td>
<td>(−1,28.7)</td>
<td></td>
<td>Tertiary</td>
<td></td>
</tr>
<tr>
<td>Imidazole Phosphate</td>
<td>H(_2)PO(_4)</td>
<td>2.15 (0.1)</td>
<td>(−1,52.0)</td>
<td></td>
<td>Tertiary</td>
<td>Reactive and unstable. (^{46}) Can interfere with biological reactions, and precipitate divalent metal ions. (^{29,46}) Can complex with some organic compounds and lead to poor reproducibility. (^{46,50})</td>
</tr>
<tr>
<td>Imidazole Phosphate</td>
<td>H(_2)CO(_3)</td>
<td>7.21 (−1,2)</td>
<td>+0.044</td>
<td></td>
<td>Tertiary</td>
<td>Has a reactive primary amine, (^{44}) and can damage electrodes. (^{29}) Reacts with some metal cations. (^{45}) Can interact with carbohydrates. (^{29}) Often used with Tris and EDTA as the electrophoresis buffer. Weakly toxic. Complexes with many organic compounds, including deprotonated amines and vicinal diols. (^{34,46,50,51,54}) Use with caution. (^f)</td>
</tr>
<tr>
<td>Serine</td>
<td>C(_8)H(_7)N(_2)O(_3)</td>
<td>9.33 (0.1)</td>
<td>(−1,34.3)</td>
<td></td>
<td></td>
<td>Low UV absorbance at 260−280 nm. (^{29})</td>
</tr>
<tr>
<td>CHES</td>
<td>C(_8)H(_12)N(_2)O(_4)S</td>
<td>9.41 (0.1)</td>
<td>(−1,25.1)</td>
<td></td>
<td>Secondary/Secondary</td>
<td></td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>C(_8)H(_10)NO</td>
<td>9.0 (1.0)</td>
<td>(−1,44.3)</td>
<td></td>
<td>Secondary/Secondary</td>
<td></td>
</tr>
<tr>
<td>CAPS</td>
<td>C(_9)H(_19)NO(_5)S</td>
<td>9.71 (0.1)</td>
<td>(−1,29.5)</td>
<td></td>
<td>Secondary/Secondary</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>C(_8)H(_12)N(_2)O(_4)S</td>
<td>3.25 (+1,0)</td>
<td>(−1,32.9)</td>
<td></td>
<td>Secondary/Secondary</td>
<td></td>
</tr>
<tr>
<td>Tricine</td>
<td>C(_8)H(_11)N(_3)O(_3)</td>
<td>8.06 (+1,0)</td>
<td>(−1,25.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bicine</td>
<td>C(_8)H(_12)NO(_4)</td>
<td>8.46 (0.1)</td>
<td>(−1,26.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAPS</td>
<td>C(_8)H(_11)N(_3)O(_3)</td>
<td>8.51 (0.1)</td>
<td>(−1,22.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammediol</td>
<td>C(_8)H(_11)N(_2)O(_2)</td>
<td>8.78 (+1,0)</td>
<td>(−1,29.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bis-tris propane</td>
<td>C(_8)H(_19)N(_2)O(_6)</td>
<td>6.8 (+2,1)</td>
<td>(−1,21.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borate</td>
<td>H(_2)BO(_3)</td>
<td>9.23 (0.1)</td>
<td>(−1,32.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) \(pK_a\) at infinite dilution at 25 °C, taken from Beynon and Easterby unless otherwise noted. Column also lists valences corresponding to the \(pK_a\) values reported here. \(^b\) From Beynon and Easterby. \(^c\) Fully ionized electrophoretic mobilities, in \(10^{-9}\) \(\text{m}^2\ \text{V}^{-1}\ \text{s}^{-1}\). \(^d\) As noted in the text, both primary and secondary amine groups can react with dissolved CO\(_2\) for form carbamate. \(^e\) One of the original buffers designed by Good \(et\ al\). Zwitterionic buffer. As noted by Good \(et\ al\), 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 pKᵦ (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 pKᵦ (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.³¹ 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.¹⁷,⁴²

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 pKᵦ),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 x TAE or 0.5 x 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)methyl] amino}-ethanesulfonic acid).

**Water and impurities**

“Pure” water as an electrolyte

Water ionizes with the dissociation 2H2O ⇌ H3O+ + OH−. The chemical kinetics associated with this equilibrium are relatively fast (k ∀ = 1.4 × 1011 M−1 s−1, kfwd = kfwd/Kw = 1.4 × 1025 M−1 s−1). A general equilibrium constant can be defined as Kw = cH3O− cOH− = 10−14 at 25°C (see Robinson and Stokes32 for data on the temperature dependence of Kw). This yields theoretically minimum ion densities of cH3O− = cOH− = 0.1 μM, yielding pH = 7.0, ionic strength of 0.1 μM, and Debye length of approximately 1 μm. 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 CO2,(gas) from the atmosphere to form carbonic acid H2CO3, which dissociates to successively form bicarbonate (HCO3−) and carbonate (CO32−) ions.65 Typical, practical laboratory water chemistry at 25°C therefore includes at least the following equilibrium reactions:

$$H_2O \rightleftharpoons H^+ + OH^- \quad K_w = c_{H^+}c_{OH^-} = 10^{-14} \quad (\text{ref. 12})$$

$$CO_{2(g)} \rightleftharpoons CO_{2(aq)} \quad HCO_3^- = c_{CO_2}/p_{CO_2} = 3.5 \times 10^{-2} \quad (\text{ref. 67})$$

$$CO_{2(aq)} + H_2O \rightleftharpoons HCO_3^- + CO_2(aq) \quad K_{a1} = c_{HCO_3}/c_{CO_2} = 2.6 \times 10^{-3} \quad (\text{ref. 68})$$

$$HCO_3^- \rightleftharpoons H^+ + CO_3^{2-} \quad K_{a2} = c_{H^+}c_{CO_3}/c_{HCO_3} = 4.7 \times 10^{-11} \quad (\text{ref. 12})$$

where the equilibrium constants are expressed in the dilute approximation. $HCO_3^-$ is simply the Henry’s law coefficient for carbon dioxide, and $pCO_2$ is the partial pressure of carbon dioxide in the atmosphere. The latter is currently ~39 × 10^{-5} atm (390 ppm69) (note CO2 is respectively 30 and 50 times more soluble in water than oxygen and nitrogen11). 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 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).65 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.58,56 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 CO2 concentration). For pH < 5.5, the dissociation of CO2,a is negligible and...
so \(c_{\text{HCO}_3}\) and especially \(c_{\text{CO}_2}\) are negligible compared to the total carbonic acid concentration \(c_{\text{CO}_2}\). For \(pH > 5.5\), \(c_{\text{HCO}_3}\) and \(c_{\text{CO}_2}\) can increase to significant quantities. In Fig. 6, we plot \(c_{\text{HCO}_3}\) and \(c_{\text{CO}_2}\) on a log scale versus solution pH. The contribution of carbonate to ionic strength can grow to 0.4 mM at pH = 8.1 (e.g., in a typical Tris buffer), and to 8.3 mM at 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 pH = 9, the concentration of carbonate species reaches 3.6 mM (cf. Fig. 6). Intuitively, a 1 mM buffer around 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 CO2) and ideal conditions (without CO2). We obtained the non-ideal case curves from resolution of the full acid–base problem, using the general formulation we presented 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 vary, and pH changes accordingly with the contribution from atmospheric CO2. From left to right, the curves respectively correspond to \(c_{\text{buffer}} = 11\; c_{\text{buffer}}, 0.5\; c_{\text{buffer}}\) and \(0.9\; c_{\text{buffer}}\) (so that the pH values in the absence of CO2 effects are \(pK_a - 1, pK_a\), and \(pK_a + 1\), 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 \(pK_a + 1\), 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 CO2), 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 \(10^3\), 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, CO2, 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:

\[
\text{RNH}_2 + \text{CO}_2 \rightleftharpoons \text{RNHCOO}^- + \text{H}^+ 
\]

where “R” denotes an arbitrary functional group. Typically, the forward reaction has a characteristic time scale of \(\sim 0.1\) s and the equilibrium constant a typical value of \(10^{-4}\). 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 isotachophoresis.

On the use of barium hydroxide to mitigate CO2 effects

We note that one method for reducing dissolved CO2 (and therefore carbonate and in some cases bicarbonate and carbonate ions and their effects on pH) is to add barium hydroxide (\(\text{Ba(OH)}_2\)) to a buffer. Barium hydroxide is a moderately soluble strong base; 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, $\text{BaCO}_3$. $\text{BaCO}_3$ is practically insoluble in water$^{76}$ 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 isotachophoresis.$^{78}$ 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.$^{79}$ Khurana and Santiago introduced $\text{Ba(OH)}_2$ 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 $\mu$M 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 (i.e., 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 $pK_a$.

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 $pK_a$ and its associated valence, chemical formulas, and temperature dependence of $pK_a$. 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 $\mu$M 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 equilibriums 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
Table 3 Summary of properties for a three electrolyte example formulation

<table>
<thead>
<tr>
<th>Number</th>
<th>Component</th>
<th>Valences</th>
<th>$pK_a$</th>
<th>$n_X$</th>
<th>$p_X$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glycine</td>
<td>$[-1 0 + 1]$</td>
<td>9.8 2.3</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Carbonic acid</td>
<td>$[-2 -1 0]$</td>
<td>10.3 6.4</td>
<td>-2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Tris</td>
<td>$[0 + 1]$</td>
<td>8.08</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

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

$$Q = \left( -10^{-14} + c_{\text{H}}^1 \right) \left( 10^{-8.08} + c_{\text{H}} + 10^{-2.08} c_{\text{H}}^2 \right) \left( 10^{-16.7} + 10^{-6.4} c_{\text{H}} + c_{\text{H}}^2 \right) \left( 1 + 10^{8.1} c_{\text{H}} \right)$$

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:

$$Q = [-10^{-14}, 0, 1][10^{-8.08}, 1, 10^{-2.08}][10^{-16.7}, 10^{-6.4}, 1][1, 10^{8.1}, 0]$$

The result is a vector of size $1 \times 8$, 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

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

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

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

$$\hat{M}_4 = \begin{bmatrix} 10^{-9.8} & 1 & 10^{2.3} \\ 10^{-16.7} & 10^{-6.4} & 1 \end{bmatrix}$$

respectively, where the last row in each matrix represents the multiplication by $c_{\text{H}}$ 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).

Acknowledgements

Rob D. Chambers was supported by a Stanford School of Engineering graduate fellowship and is currently supported by a Kodak fellowship. We gratefully acknowledge the support of the DARPA MTO sponsored SPAWAR Grant N66001-09-1-2007. We thank Thomas Zangle and Moran Bercovici for insightful discussions and comments.

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