Development of Methods for the Determination of pKₐ Values

Jetse Reijenga, Arno van Hoof, Antonie van Loon and Bram Teunissen

Department of Chemical Engineering and Chemistry, Eindhoven University of Technology, Eindhoven, The Netherlands.
Corresponding author email: j.c.reijenga@tue.nl

Abstract: The acid dissociation constant (pKₐ) is among the most frequently used physicochemical parameters, and its determination is of interest to a wide range of research fields. We present a brief introduction on the conceptual development of pKₐ as a physical parameter and its relationship to the concept of the pH of a solution. This is followed by a general summary of the historical development and current state of the techniques of pKa determination and an attempt to develop insight into future developments. Fourteen methods of determining the acid dissociation constant are placed in context and are critically evaluated to make a fair comparison and to determine their applications in modern chemistry. Additionally, we have studied these techniques in light of present trends in science and technology and attempt to determine how these trends might affect future developments in the field.

Keywords: review, history, dissociation constant, pKₐ, pH
Introduction

History

The centennial of the concept and of the quantitative measurement of pH was celebrated not long ago.1–3 By now, accurate and precise determination of pH seems to have few secrets left. The related concept of the acid dissociation constant (pK\textsubscript{a}) as a substance property is recognized as being among the most commonly used parameters in modern-day chemistry. Both pH and pK\textsubscript{a} are essential for understanding the behavior of chemical substances in everyday life. This realization came gradually and under different names. The first notion of acids comes from ancient Greece, where people noticed that some substances tasted sour. This is also where the word “acid” comes from; it is derived from the Greek word “oxein” which in Latin is “acere,” meaning “to make sour.” It was also noted that acids and bases could color certain substances.

The first description of an equilibrium constant came from Guldberg and Waage in 1864 in their “law of mass action.” With the help of van’t Hoff’s work on osmotic pressures, Ostwald formulated his “dilution law” for solutions. Measurements of osmotic pressures and conductivity of solutions gave insight into the degree of dissociation.

It was in 1907 that Henderson published a paper first describing the relation between the hydrogen ion [H\textsuperscript{+}] and the composition of a buffer.5 In 1909, Sörensen6 proposed the more convenient pH and pK\textsubscript{a} terms as the negative logarithm of [H\textsuperscript{+}] and the equilibrium constant, K, respectively. Although Henderson defined K in terms of a concentration ratio in 1908, it was not until 1916 that Hasselbalch7 proposed their now famous equation (1), which remains the most commonly used equation to calculate pK\textsubscript{a} values: the Henderson-Hasselbalch equation. It relates pH and pK\textsubscript{a} to the equilibrium concentrations of dissociated acid [A\textsuperscript{-}] and non-dissociated acid [HA] respectively:

\[
\text{pH} = \text{pK}_a + \log \left( \frac{[A^-]}{[HA]} \right)
\] (1)

In many experimental methods to determine pK\textsubscript{a} values, a certain parameter is measured as a function of pH. This results in a characteristic sigmoid curve (Fig. 1) from which the pK\textsubscript{a} may be determined by locating the inflection point. Generally speaking, for acidic components X ranges from a bulk property of a solution of only non-dissociated acid to the situation where only dissociated acid is present. A more specific example is represented if parameter X denotes the degree of dissociation \(\alpha\) ranging between 0 and 1, the inflection point will be at \(\alpha = 0.5\), where pH equals pK\textsubscript{a}. The degree of dissociation \(\alpha\) for acids is defined as:

\[
\alpha = \frac{[A^-]}{([HA] + [A^-])}
\] (2)

Combining Equations 1 and 2 leads to (for anions):

\[
\log \left( \alpha/(1 - \alpha) \right) = \text{pH} - \text{pK}_a
\] (3)

However, for concentrated solutions or pK\textsubscript{a}’s near the extremes of the pH scale, it has been shown that there are significant differences between the predicted and actual concentration of the hydrogen ion.8

Since the Henderson-Hasselbalch equation only gives accurate results for dilute acids in aqueous solutions, another formula for the quantification of acid strength was developed by Hammett.9 Instead of measuring the concentration of the species present in solution, Hammett described the strength of an acid as the relation between the hydrogen ion activity (a) and the activity coefficients (f) of the various species in solution. However, difficulty associated with accurate determination of the parameters in this model has kept it from being as widely used as the Henderson-Hasselbalch equation.
Influences on pKₐ

In spite of being universally referred to as a constant, the dissociation constant pKₐ is not in fact truly constant; it depends on temperature (T), ionic strength (I), and the solvent dielectric constant (ε).

The temperature dependence of the pKₐ values is sometimes fitted to a van’t Hoff type equation:

\[ \frac{d \ln K_a}{dT} = \frac{\Delta H}{RT^2} \]  \hspace{1cm} (4)

Here \( \Delta H \) is the enthalpy change of dissociation and R is the gas constant. The value of \( \Delta H \) is often negative. In the case where \( \Delta H \) is independent of temperature, plotting pKₐ versus 1/T will result in a linear plot. This is commonly used to interpolate to other temperatures.\(^{10,11} \)

Extrapolation of experimental data is, of course, not advised. We can understand this by realizing that \( \Delta H \) can be re-written as:

\[ \Delta H = \Delta G + T\Delta S \]  \hspace{1cm} (5)

Here \( \Delta G \) is the Gibbs free energy change and \( \Delta S \) is the entropy change. The linear relation mentioned only holds if \( \Delta H >> T\Delta S \). Moreover in some cases \( \Delta S \) also depends on temperature, resulting in even more non-linear dependence of pKₐ on 1/T. An example of the pKₐ temperature dependency is shown in Figure 2.

Measured pKₐ values also depend on the ionic strength of the solution under investigation. The ionic strength is defined as the sum of concentrations (c) of all ionic species, corrected for their charge number (z):

\[ I = \frac{1}{2} \sum z^2 \cdot c \]  \hspace{1cm} (6)

At extreme values of the pH scale, the contribution of H⁺ or OH⁻ should also be included. Activity coefficients (γ) of different ionic species in solution strongly depend on ionic strength (Debye-Hückel theory). Because pKₐ depends on the activity coefficients, the ionic strength will also influence pKₐ, especially at higher charge numbers (z). An example of this dependency is shown in Figure 3.

Because the acid-base equilibrium occurs in solution, the solvent composition can also influence the pKₐ values (Fig. 4). Measuring pH of mixtures of organic solvents and water is in itself far from straightforward, and are beyond the scope of the present contribution. When regarding an acid dissociation reaction, three thermodynamic steps are considered: (1) the dissolution of the acid from the solvent into the gas phase, (2) the dissociation of the acid into the ions, and (3) the solution step of the ions into the solvent. In the first and the last step, the solvent is involved. When considering the influence of the solvent, the difference between the solvation energies of

---

**Figure 2.** Various pKₐ values as function of temperature. Each component has an own dependency. Values in water at infinite dilution, data from Everaerts et al.\(^ {12} \)

**Figure 3.** The dependency of the pKₐ of acetic acid on the ionic strength, at 18 °C in water. Data from Cohn et al.\(^ {13} \)
the acid and the dissociated acid influences the final pKₐ value. Hence the pH range of one solvent may differ from that of another solvent.

It must be stressed that when performing pKₐ measurements, all parameters mentioned will have to be kept constant in order to produce a meaningful result.

It is often overlooked that this is also the case with pH measurements. Before use, pH meters should be calibrated under the same conditions of temperature, ionic strength, and solvent. Reporting pKₐ values in literature also require that exact conditions of temperature, ionic strength, and solvent be stated. If these details are omitted, it cannot be assumed they were measured in water, at room temperature, and extrapolated to infinite dilution.

Many of the techniques mentioned in the subsequent section measure solutions in which not only the analyte is present, but also various other components for buffering. One has to be sure that there is no ionic or other interaction between the analyte and these other components.

Complications and overview
Depending on the sample and matrix under investigation, the choice of technique can be a difficult one, even for the case of monovalent ions, to which this paper is limited to. For multivalent components, matters are more complicated as the pKₐ differences are smaller. This is because all methods, except perhaps for nuclear magnetic resonance (NMR), require curve fitting in addition to the normal calculation procedure for the respective technique. Investigating both acidic and basic pKₐ of amphoteric compounds also requires curve fitting in a much broader range. For such compounds, especially peptides and proteins, the isoelectric point is often relevant for identification purposes but beyond the scope of this paper.

Figure 5 displays an overview of the first time these techniques where used for this purpose.

Techniques
Potentiometry
The simplicity and low cost of potentiometric titration has made it one of the most commonly used methods for pKₐ determination. In a potentiometric titration, a known volume of reagent is added stepwise to a solution of analyte. The change in potential (E) upon reaction is consequently measured with the use of two electrodes, an indicator, and a reference electrode. These are often integrated in what is now commonly called a combined pH electrode.

Plotting the potential versus volume subsequently gives rise to a sigmoid curve, where the inflection point gives the potential at equilibrium. With the use of standards with known pH, this potential can be linearly converted into a pH, equaling pKₐ. Sigel et al have, however, correctly noted that there are multiple

Figure 4. The dependency of pKₐ of different Benzoic-acids on the solvent composition, methanol in water at 25 °C and I = 0.02 M. Data from Sarmini and Kenndler.¹⁴

Figure 5. Timeline of the first notion of the various techniques to determine pKₐ (dissociation constant, acid strength).
Acid strength-models in use, each giving a different H⁺ concentration for a given pH.¹⁵

Increasing understanding of electrochemical processes in the late 19th century gave rise to the first potentiometer. This consisted of a platinum working electrode and a reference electrode and used a H₂/H⁺ equilibrium to determine its standard reduction potential, which is by definition 0 V. The first description of the use of a setup to determine equilibrium constants was made by Denham in 1908.¹⁶

It was not long until the cumbersome hydrogen electrode was replaced by the familiar glass electrode. Completely automated and self-adjusting pH-measuring equipment by Keeler is known from as early as 1928.¹⁷ Because of difficulty in obtaining reliable results, the first working pH-meter was only constructed in the 1930’s.¹⁸

Although the glass electrode has become widely used, it has been proven that at higher temperatures or more extreme pH’s the results deviate from theoretical predictions. The “alkaline-error” is certainly the most widely known. Because temperature influences not only the measurement but also the pKₐ itself, it is of vital importance to conduct the titration at constant temperature. A good review about the various errors of the electrode itself is given by Gardiner¹⁹ while Benett²⁰ discusses the various ways to determine pKₐ from the measured potential.

Traditional potentiometric titrations have a lower concentration limit of about 10⁻⁴ M, however various methods where developed to extend this range. Another practical complication is the pKₐ-measurement of substances with a low water-solubility. One example is extrapolation of measurements in solvent mixtures.²¹ These require, however, a relatively high co-solvent concentration, and are inherently inaccurate and time consuming. An alternative approach using surfactants is proposed²² which avoids tedious extrapolating while giving the same level of accuracy (± 0.2 pKₐ value).

A precise determination of the pH from the titration slope has also been difficult in the earlier period of its use. However, over the years various software programs have become available to minimize most of the previous mentioned errors. Potentiometric titration requires a relatively large amount of sample compared with separation methods such as high performance liquid chromatography (HPLC) and capillary electrophoresis (CE).

Completely automated potentiometric pH meters for a wide range of applications and with complicated calibration software are widely available. Because of this and the simplicity and relatively low costs associated with the potentiometric pH-meter, it will probably remain in use in the foreseeable future. This of course only holds for those analytes that are available in sufficient quantity and purity.

**Conductometry**

The determination of acid dissociation constants by conductometry relies on the assumption that strong electrolytes are completely dissociated at all concentrations, while weak electrolytes only attain complete dissociation at infinite dilution. A measurement of the conductivity of a sample yields a value that is the sum of the independent contributions of all ions present in the solution:

\[
\Lambda = \sum \lambda_i \tag{7}
\]

Here, \(\Lambda\) represents the equivalent conductance and \(\lambda_i\) represents the specific conductivity contribution of ionic species i. The equivalent conductance is dependent on the number of ions in solution, and reaches its limiting value \(\Lambda_0\) at infinite dilution, where, by definition, activity coefficients are unity.

\(\Lambda\) is a measurable quantity and \(\Lambda_0\) can be obtained from extrapolation in a plot of \(\Lambda\) versus the square root of the analytical concentration as shown in Figure 6. It can also be seen from Figure 6, however, that this linear relationship does not hold for all substances.

![Figure 6. Plots of \(\Lambda\) versus \(\sqrt{c}\) required for pKᵦ determination of formic acid at room temperature in water. Data from Saxton and Darken²⁴ and Landolt-Börnstein et al.²⁵](image-url)
extrapolation does not hold for weak acids (or bases). This is because for these electrolytes, the assumption that all ions are independent of their counter ions does not hold as these species are not fully dissociated.

Though the limiting conductance of a weak electrolyte cannot be directly extrapolated, their value can still be obtained quite readily. Even for weak electrolytes, the Kolhrausch law\(^23\) of the independent migration of ions holds. This law can be stated as meaning that at infinite dilution, each ion makes a specific contribution to the conductivity regardless of the ions associated with it. It is not possible to measure the limiting conductance of ions directly, but \(\Lambda^0\) for a salt may be expressed as a linear combination of its ionic components.

Expressing the limiting conductance of a salt in terms of ionic contributions is useful as it allows the calculation of the limiting conductance of a weak electrolyte. In order to do this, the limiting conductance of other relevant salts of a strong electrolyte is extrapolated. For a weak acid (formic acid) relevant plots are shown in Figure 6. One would obtain the ionic contributions for the limiting conductance of the acid from the salt of its conjugate base and from hydrochloric acid, subtracting the value for sodium chloride to eliminate the ionic contributions of the sodium and chlorine counter ions in the reference compounds. This yields the sum shown in Equation 8:

\[
\Lambda_{HA}^0 = \Lambda_{NaA}^0 + \Lambda_{HCl}^0 - \Lambda_{NaCl}^0 \tag{8}
\]

Once the limiting conductance is known, the degree of dissociation \(\alpha\) for a weak electrolyte is given by \(\alpha = \Lambda^0/\Lambda^0\). An apparent dissociation constant \(K'\) can be obtained directly from the degree of dissociation using Ostwald’s dilution law by expressing it in terms of a concentration quotient, as shown in Equation 9.\(^26\)

In order to obtain the true thermodynamic dissociation constant \(K_{a^*}\) one has to correct for the activity of the ions:

\[
K' = \Lambda^2 \cdot c/(\Lambda^0 \cdot (\Lambda^0 - \Lambda)) \tag{9}
\]

Equation 9 yields the dissociation constant for a given analytical concentration \(c\) and a series of measurements would yield the dissociation constant as a function of ionic strength. It is worth noting that this determination does not require knowledge of the pH of the solution, making it easily applicable to non-aqueous systems where such a measurement of pH would be impracticable. It also means that the method, unlike many others which express their measured quantity as a function of pH, is not constrained by the precision of the pH electrode.

The conductometric method offers a relatively fast and reliable method of determining the \(pK_a\) and is also capable of attaining a high degree of precision with \(pK_a\) deviations as small as \(\pm 0.01\)–\(0.03\) units.\(^27\) The main downside of the method is that conductometric measurements are nonspecific, ie, different ions cannot be measured separately. This requires working with pure compounds.

Foundational work that enabled the development of the conductometric method was undertaken by Friedrich Kohlrausch. Among the key ideas he developed was the use of alternating current to prevent electrolysis during conductivity measurements. His work examining a variety of electrolyte solutions led to the law of independent migration of ions that is ascribed to him. Building on this early work, and further contributions by Ostwald and Arrhenius, the method reached maturity during the late 1930s, and the accuracy of the method approached that of modern methods. The incorporation of Debye-Hückel activity coefficients enabled the calculation of true thermodynamic \(pK_a\) values, and the calculation of these coefficients for a range of ions was published during this period.\(^28\) In contrast to the earlier period, which focused mainly on obtaining thermodynamic dissociation constants, experiments performed after this period were additionally concerned with correlating this data with the structure of the organic acids being studied.\(^29\)

Work on the conductometric method came to a virtual standstill after the outbreak of World War II, and further research was practically abandoned after the end of the war. Renewed activity would not come until the 1970s, when two new conductance equations were published. These equations enabled the study of asymmetrical electrolytes, and even mixtures of electrolytes.\(^30\)–\(^33\)

Since then, it has been remarked that the spectacular interest for the method that existed during its early period has waned with time, leaving the subject a somewhat unfashionable topic of research. With the developments made over its history, however, the method has achieved a high degree of precision.\(^26,34,35\)
Voltammetry

In voltammetry, a changing potential is applied over a sample solution and the resulting current is measured. When the potential reaches the reduction potential of the analyte this will give rise to an increase in current, followed by a decrease due to depletion of the molecule. In the case of cyclic voltammetry, for example, this will lead to results much like those shown in Figure 7.

A typical voltammetry setup usually consists of 3 electrodes: reference, working, and auxiliary electrode. The working and auxiliary electrodes act as the anode and cathode, respectively, while the reference electrode acts as a fixed point to measure the applied voltage. Modern-day voltammetric methods can be extremely sensitive; with specific techniques it is possible to accurately measure very low concentrations.36,37

The earliest use of voltammetry was the use of a dripping mercury electrode in 1922 by Jaroslav Heyrovsky to develop the first polarograph.38 As early as 1941, voltammetric methods were deployed to determine local pH at the electrode surface.39 However, the use of voltammetry to specifically determine bulk pKₐ values came into use in the 1960s.40

When the pKₐ of a substance is being determined voltammetrically, one could in principle measure the electrochemical response of the molecule itself.41 However, accurate knowledge of the electrochemical behavior of the sample substance is required and therefore a reference with known characteristics is usually added. The shift of the peaks of the reference upon addition of the acid is then used to determine the pKₐ value.42,43 Care has to be taken since the reference will influence the ionic strength of the solution. Additionally, its concentration should be in the same order as that of the analyte to give optimal sensitivity.

More recently, advanced techniques have been developed such as those measuring the surface pKₐ of self-assembled monolayers.43 Voltammetry has not been used extensively for the measurement of pKₐ values. The reason for this is the need for an electro-active molecule which is soluble in a conductive solvent. Also, it is often necessary to recalibrate the apparatus when different samples are used. Besides these “operational” drawbacks, polarography also requires the use of mercury electrodes.

On the other hand, voltammetry has some advantages. It is of particular use in measurements of pKₐ values in less polar solvents, something which is often difficult to do accurately with the use of techniques such as potentiometric titration.41 When optimized, it is usually not very time-consuming and relatively cheap in comparison to other techniques such as NMR, separation methods, and spectrometry.

Another advantage over other techniques is that it is able to conduct a quantitative measurement of different oxidation states.44

Calorimetry

All calorimetric methods work by the same principle: a physical or chemical process takes place in a sample and the amount of heat evolved is measured. For the measurement of pKₐ values, a technique called Isothermal Titration Calorimetry (ITC) has been used. Here, a regular acid-base titration is carried out inside the calorimeter while the energy needed to keep the temperature constant is measured. It is also one of the oldest analytical techniques. The first recorded model was made by Lavoisier and Laplace in 1783.45

In recent years, the ITC-method has been used to measure the dissociation constants of peptides and the influence of binding on the specific ionizable groups. This method also calculates the pKₐ indirectly from a measured enthalpy change ΔH.46

A related technique was developed which measures the pKₐ directly: Isothermal Titration
Microcalorimetry (ITM). Here the reagent for the ionizable groups is added in equivalent amounts and at once. The resulting heat released upon reaction is measured in buffer solutions with different pH values (Fig. 8). By plotting the minima or maxima versus pH, a sigmoid curve is obtained from which the pKₐ can be determined from the inflection point. Uncertainties are in the range of 0.05–0.15 pKₐ units depending on the calorimetric technique.

Nuclear magnetic resonance

Before NMR was used to determine acid dissociation constants, the technique was already applied to determine the site of deprotonation of an acid, or the site of protonation of a base. In these cases, the temperature was decreased to such extent, that the acid-base equilibrium was slow on the NMR time-scale, so that two separately peaks would be observed for HA and A⁻.

In 1957, Grunwald et al. used NMR to determine the pKₐ of mono-, di- and trimethyl-amine, thus determining the chemical shift of the triplet from the protons in the CH₃ group(s) as a function of pH. A linear correlation was found between the chemical shift and the acid-base ratio. Experiments could be carried out in water by using a reference measurement. A sigmoid curve is obtained from which the pKₐ was calculated.

Pioneering work was performed by Lee et al., who determined the pKₐ values of a wide range of functional groups with good concurrence towards existing literature. Based on his work, further knowledge of the pH-chemical shift relation was described and published.

When the pH dependent acid-base equilibrium is fast on the NMR time scale, a mean chemical shift of the HA and A⁻ groups can be measured. Because the equilibrium is pH dependent, the chemical shift will also change with the pH level. For this situation, the pKₐ can be written as Equation 10:

\[
pK_a = pH + \log \left[ \frac{(\delta_A - \delta_{obs})}{(\delta_{A^-} - \delta_{HA})} \right] \tag{10}
\]

The pKₐ value can be determined by plotting the δ-component shown above versus the pH. This yields a familiar sigmoid curve where the pKₐ is located at the inflection point.

Instead of performing a continuous titration, a known amount of a strong acid or base is added to different samples (constant volume titration), from which the pH is consequently calculated. This however neglects the influences of the target molecule on the pH and the risk of a systematic error is added.

The main advantage of the NMR technique is that it is possible to measure mixtures, even when impurities are present, because mole fractions are observed instead of the total acid concentration, in contrast to potentiometric titrations. When observing the chemical shift of one characteristic group, no other groups are involved, so even the pKₐ values of diprotic acids with pKₐ values close together can be observed separately, provided that the chemical shifts do not overlap. These individual constants are referred to as “microscopic pKₐ values” and their determination is one of the most promising applications of this technique. Also, performing NMR with ²H, ¹³C, ³¹P or other nuclei with an electromagnetic moment can be used to measure the pKₐ.

With this characterization method, even more complex molecules, such as enzymes can be fully characterized. So, the pKₐ of individual acid sites on complex molecules can be determined, making this a promising technique for further development. This was discovered by Rabenstein and Sayer in 1976 who determined microscopic dissociation constants for polyprotic acids by means of curve fitting.

The main errors for the NMR method are found to be caused by imprecision of the chemical shift and the pH level, which is calculated and not measured.
The pK_a can be measured precisely up to ±0.05 log units. For all NMR measurements, a reference compound is required to acquire a ‘lock’ for the apparatus; this process is called shimming. Because an internal reference can interfere with the acid-base equilibrium, an external reference is recommended, either with an extra measurement or a second co-axial sample tube.

Control of temperature in NMR is often not a problem, but when performing NMR the fact that energy dissipation can occur must be taken to account; locally, at the vibrating nucleus of interest the temperature can rise.

Glaser et al presented the first fully-automated NMR apparatus for measuring the pK_a. This method also has good concurrence with available data in literature. With this automation and stronger magnets, the NMR technique is a promising technique to measure multiple enzymes at specific (de-)protonation sites within a reasonable time frame. For less complex acids and bases with only one protonation site, however, this technique is rather expensive.

**Electrophoresis**

In electrophoresis, charged species are separated under the influence of an electric field, migrating with a velocity proportional to their size-to-charge ratio. The ratio of the linear velocity v_i and field strength E is defined as the electrophoretic mobility m_i:

\[ m_i = \frac{v_i}{E} \]  

(11)

The relation with previously discussed conductometric methods and electrophoretic ones is important: in the latter we measure individual mobilities m_i (Equation 11) whereas in the former we measure the sum of mobilities of all ions together (Equation 7). They are interrelated on the basis of individual ions using the Faraday constant F:

\[ m_i = \lambda_i/F \]  

(12)

The use of electrophoresis for the determination of the pK_a value depends on the differing mobilities of the protonated and deprotonated forms of the analyte. As the two forms exist in fast equilibrium, a net mobility is measured that can be related to the degree of dissociation (α) of the analyte. When the degree of dissociation for acids is expressed in terms of mobility, one obtains the relation shown in Equation 13:

\[ m_{\text{eff}} = (1 - \alpha) \cdot m_b + \alpha \cdot m_d \]  

(13)

Here, m_eff is the effective mobility, m_d is the mobility of the fully dissociated species, and m_b the mobility of the non-dissociated species (which equals zero). For bases, a similar equation is derived. A sigmoid curve is obtained by plotting m_eff vs. pH, with the inflection point of \( \alpha = 0.5 \) at pH = pK_a.

Model equations can be derived for weak acids and bases with any number of ionizable centers by rewriting Equation 13. These models for species with up to three ionizable centers were summarized in a 2004 review article by Poole. The pK_a may be determined by numerically fitting the resulting relations to a plot of mobility versus pH through nonlinear regression, as shown in Figure 9 for the case of 2-aminopyridine.

The shape of these curves depends only upon m_d and the position depends only upon pK_a, therefore this regression directly yields the pK_a value(s) of interest. Remarkably, few data points are needed to provide an accurate fit for these models, and a precision of ± 0.03–0.08 can be achieved depending on the analyte, placing the method roughly on par with potentiometry in terms of precision.

The method has a number of key advantages in comparison to more traditional alternatives.
Firstly, only a very small amount of the sample is required for a measurement, on the order of microlitres in terms of volume, and with detection limits in the \(10^{-9}\) M range. This allows for the processing of poorly soluble species without much difficulty. Since electrophoresis is a separation technique, impure samples can be readily processed and as the molecules are measured directly, exact knowledge of sample concentrations is not required. In addition, commercially available equipment is capable of automatic operation without requiring modification, allowing large numbers of measurements to be conducted at speed. This makes the method quite suitable for screening applications.\(^{56}\) A potential problem of the method is the requirement of preparing buffer solutions for each pH to be measured, since the buffer compounds must be carefully chosen to avoid undesirable interactions between buffer and analyte.

The relationship between the electrophoretic mobility of an acid or base and the pH of the background electrolyte was already considered when Consden and Martin\(^{56}\) published their paper on ionophoresis in 1946, which discussed the separation of two analytes based on a difference in their \(pK_a\) values. This relation was explicitly applied to the estimation of acid dissociation constants by Waldron in the 1950s\(^{57}\) and by Kiso et al in the 1960s\(^{58}\) using a paper strip as a supporting medium. However, despite the known advantage of requiring only minute amounts of sample, both methods were found to be impractical in application. Improvements over the next decades would eventually lead to the introduction of the fused silica capillaries.

By the end of the 1980s, isotachophoresis was considered a viable method for the determination of dissociation constants. While the method showed reduced precision in comparison to potentiometric or conductometric methods, this was offset by its faster measurement times and lower sample requirements.\(^{59}\) However, determining \(pK_a\) values by isotachophoresis was not without disadvantages which are discussed in detail by Beckers et al.\(^{60}\) It was remarked that the calculations required are quite laborious as the zones formed during the separation all have different parameters (pH, ionic strength, and even temperature). To avoid the complexities and limitations of isotachophoresis, it was proposed that the simpler method of capillary zone electrophoresis be used specifically for the determination of mobilities and \(pK_a\) values. The potential sensitivity of the method had already been demonstrated\(^{61}\) and further refinements of the technique would be made over the next few years. A general methodology for the determination of \(pK_a\) values by CE was proposed in 1992, and further refined by the addition of terms concerning the rate of electroosmotic flow and a method of handling potential discontinuities between buffer electrolytes.\(^{62,63}\) An important step was made by Ishihama\(^{64}\) when he studied multivalent compounds and derived relationships between \(pK_a\) and zone mobility that were more easily applicable than the earlier equation proposed by Kiso.\(^{58}\)

Developments over the next decade would include further examinations of the \(pK_a\) equations, comparing different regression techniques, and better experimental methods.\(^{65}\) Improvements included using charged polymer coatings of the capillary wall to influence the rate, influencing the electroosmotic flow to enable measurements at a lower pH,\(^{66}\) and the use of pressure applied over the capillary to shorten measurement times for screening purposes.\(^{67}\) Recent work includes efforts undertaken by Fuguet et al\(^{68–70}\) to improve the internal standard method first proposed by Gluck and Cleveland.\(^{65}\)

**High performance liquid chromatography**

The first observation that the time of elution can be changed by adjusting the pH level was made by Singhal.\(^{71}\) By changing the pH, the performance of the HPLC was optimized, in this case ion-exchange chromatography.

With the advance of reversed-phase HPLC in the late 1970s, advanced models were made relating the capacity factor \((k)\) to the degree of dissociation \((\alpha)\). An excellent overview of the principle is presented by Horváth,\(^{72}\) who presented a generally applicable equation for acidic components:

\[
k = (1 - \alpha) \cdot k_0 + \alpha \cdot k_{-1}
\]  

(14)

in which \(k_0\) and \(k_{-1}\) are the capacity factors on the non-ionic and the ionic species respectively, where in reversed-phase HPLC \(k_0 \gg k_{-1}\). Plotting \(k\) vs. pH gives a sigmoid curve with the inflection point of \(\alpha = 0.5\) at \(pH = pK_a\). The similarity of Equations 13 and 14 are immediately obvious.
Development of pK\textsubscript{a} measurements

A solid theory was formulated by Foley\textsuperscript{73,74} where the pH dependence of the capacity factor follows the dissociation curve. Again, some recommendations and optimizations were formulated to improve selectivity, retention, and efficiency.

As long as the analyte has a chromophore for detection, this method works fine, even with samples that are not 100% pure, as HPLC is a separation method. On the other hand, the full range of $\alpha$ from 0 to unity cannot always be utilized, because $k_0$ is often unacceptably large. Of course, the addition of organic modifiers decreases all $k$ values but hugely complicates matters if we desire aqueous pK\textsubscript{a} values.

Partition and distribution

The first investigation into the division of a substance between two immiscible solvents was carried out by Berthelot and Jungleisch in 1872. They came to the important conclusion that the ratio of the concentration was a constant, e.g., it does not depend on the relative amounts of solvents used. Following up on this research, Nernst concluded in 1891 that the partition coefficient ($P$) was only a constant if a single substance was considered.\textsuperscript{75}

Later insight further revealed that for ionizable components, the partition coefficient depended on the pH of the aqueous phase. In the limiting case where the ionization is completely suppressed by pH (for bases, for example, at high pH) a distribution coefficient ($D$) can be defined mathematically (for bases):

$$D = [B]_o/[B]_w$$ (15)

$$P = [B]_o/([B]_w + [BH]^+)$$ (16)

Here B is the uncharged and BH$^+$ the charged species, subscript o refers to the organic and subscript w, the water phase. Several derivations for pK\textsubscript{a} from P and D are found in literature,\textsuperscript{76,77} but all of them result in roughly the same expression (for bases) as they combine Equations 1, 15, and 16:

$$pK_a = \log ((P - D)/D) + pH$$ (17)

These are usually determined by adding a known amount of sample to an organic solvent/water mixture, followed by the measurement of the concentration in one of the phases.

There are two main techniques used to determine log P. One is the “shake-flask” technique\textsuperscript{78} where an octanol/water mixture together with the sample is shaken in a separation funnel, after which the concentration in one or both of the phases is measured.

Another is the so called Filter probe\textsuperscript{79} which was later improved to the “filter chamber technique.”\textsuperscript{80} These are automated separating funnels connected to a pump which directs the layer of choice to an analytical device (usually a spectrophotometer) and back.

In some specific cases, the technique used to determine pK\textsubscript{a} by P is largely unaffected by the choice of solvent.\textsuperscript{77} Although it might seem that log P is a relatively simple quantity to determine, it has been proven that there are still many sources of errors,\textsuperscript{78} one of which being the case of mutual miscibility of the two phases.

The use of partition coefficients to determine pK\textsubscript{a} is not used extensively. Partition coefficients are however still very much in use in drug development as they give information about the uptake of a certain drug in various parts of the body.

A particularly illuminating example of this follows. In some surgeries, the body temperature and blood pH values deviate from normal values. This naturally affects the log P value and drug efficacy. Thurlkill et al\textsuperscript{81} reported an additional aspect for the case of Fentanyl, a local anesthetic. It was found that its pK\textsubscript{a} significantly depends on temperature, which might lead to further complications in drug administration, in other words a temperature and blood gas dependent bioavailability.

Solubility

In 1945, Krebs\textsuperscript{82} described the relationship between pH, pK\textsubscript{a}, and the solubility of sparingly soluble weak acids and bases, where a derivation of the Henderson-Hasselbalch equation was used to describe the behavior. A drawback of this method at that time was the solubility of the non-ionized analyte had to be known, which was not often the case.\textsuperscript{82}

This theory was further expanded by Zimmerman et al\textsuperscript{83} who made mathematical derivations by which it was possible so determine the solubility of the neutral analyte and the pK\textsubscript{a}. By these means, the solubility of the neutral compound was no longer required and the use of solubility data to determine the pK\textsubscript{a} was much more applicable.\textsuperscript{83}
A derivation of the Henderson-Hasselbalch equation allows us to determine the \( pK_a \) from solubility data, the graphical representation is shown in Figure 10.

\[
\log S = \log S_0 + \log (10^{pK_a - pH} + 1) \tag{18}
\]

Here \( S_0 \) is equal to the intrinsic solubility. When \( pH >> pK_a \) or \( pH << pK_a \) assumptions can be made and linear \( \log S/pH \) functions are obtained. By extrapolating these two functions and calculating the intercept, the \( pK_a \) can be calculated:

\[
\log S = (\log S_0 - pK_a) + pH \tag{19}
\]

In the later years, \( pK_a \) values for zwitterionic compounds were determined and a solid method for the bi-functional bases and acids was formulated. Avdeef found a special way of determining the \( pK_a \). Under certain conditions the \( pH \) does not change under addition of more titrant. This \( pH \) is known as the Gibbs \( pK_a \).

Nowadays, the solubility data are used to determine \( pK_a \) values for a wide range of drugs, where the \( pK_a \) value is of great interest. The power of this technique is that poorly soluble drug can be analyzed at very low concentrations, in the order of \( \mu M \), and with a precision up to \( \pm 0.5 \) in log units. A drawback is that for most solubility determinations, a time frame of 1–24 hours is required where a measurement with spectroscopy only takes a few minutes.

Therefore, solubility measurements for \( pK_a \) determinations can be used for sparingly soluble compounds which have a chromophore near the ionization center.

**UV/Vis spectrometry**

Well before 1900, it was already known that a change in acidity could lead to color changes of natural substances. Spectrometry with visible light made it possible to measure \( pK_a \) values of acid/base indicators and this in turn was extended to the use of UV light to measure \( pK_a \)'s of other components.

A requirement for this UV/pH measurement is the presence of a chromophore close to the ionization site in the molecule. If this is fulfilled, then the spectra of the dissociated and the non-dissociated form can be expected to differ. In principle any wavelength can be used for the determination of \( pK_a \), except at the isosbestic point at which wavelength of both forms have the same molar absorptivity. The best choice however is a wavelength at which the molar absorbivities are as different as possible.

The method was further improved by measuring the absorption of two different wavelengths at a variable \( pH \). The ratio in absorption at those two wavelengths is plotted against the \( pH \). In this way, a sigmoid curve is obtained and the \( pK_a \) can be determined from the inflection point as normal. One of the wavelengths has to be assigned to the chromophore and the other wavelength should be invariant under change of \( pH \) (if this is possible). By using a 2nd wavelength as reference, change in total concentration will not affect the final result and activity/concentration issues and assumptions are bypassed.

This method was introduced by Holmes and Snyder in 1925 when the “decomposition” constant of a dye was measured. This was then further elaborated by Flexser et al in 1935 by determining different ionization constants. In the 1960s, Wigler et al were the first to determine \( pK_a \) values of di-protic compounds.

Up to this point, the \( pK_a \) values calculated required prior knowledge of experimental data, such as the absorption coefficients of the neutral and ionized compound. By measuring over a whole wavelength range, Allen et al were able to determine the \( pK_a \) values without this prior knowledge. The measurements could also be done much faster. This method...
showed good concurrence with previous single wave-
length methods\textsuperscript{93} and was later highly automated by
Saurina et al.\textsuperscript{94}

Fluorometry

It can be argued that fluorometry is a specific form
of spectrometry as any fluorescence is the result of
light absorption, while the reverse is not the case.

The use of fluorescence spectroscopy to determine
pK\textsubscript{a} values depends on the difference in the fluores-
cence spectrum between a free acid or base and its
conjugated form. While fluorometry can potentially
be more sensitive and selective than conventional
spectrometry, it has the disadvantage of being only
applicable to fluorescent analytes. Additionally, it is
known that the pH dependence of fluorometry often
does not agree with those obtained from spectrom-
etry or other methods. The reason for this is that the
former depends on excited state proton exchange
as well as the ground-state equilibrium. This causes
problems because the pK\textsubscript{a} for the ground state and
pK\textsubscript{a}* for the excited state can differ quite strongly,
and the position of the inflection point in a fluo-
rometric titration will depend on both values as
well as the kinetics of the excited state proton
transfer.\textsuperscript{95} Another problem may occur when buf-
fer components lead to pH-dependent fluorescence
quenching.

In spite of these challenges, a successful determi-
nation of the ground-state pK\textsubscript{a} values of sparingly
soluble N-heterocyclic bases was accomplished by
Rosenberg et al.\textsuperscript{96} They made use of dilute solutions
of a strong acid or base to perform a pH titration;
strong buffers were not used in order to prevent
reactions of the buffer ions with the highly reactive
excited state forms of the aromatic analyte. By care-
fully selecting the excitation wavelength, they were
able to obtain a titration curve in which the inflec-
tion point corresponded to the ground-state pK\textsubscript{a}.
The more precise of the two methods suggested involved
choosing an excitation wavelength such that only the
conjugate acid can absorb, meaning that no excited-
state proton transfer can occur to the free base, in
order for the fraction of conjugate acid to be mea-
sured directly. The bottom line is that the linear
relationship between fluorescence intensity and the
degree of dissociation is not as common as in absorp-
tion spectrometry.

Polarimetry

Determination of an acid dissociation constant by
polarimetry involves the measurement of the optical
rotation of plane polarized light by the sample solu-
tion as a function of pH. This method depends on the
difference in optical rotation between the ionized and
non-ionized forms of an analyte.

Only very few examples of polarimetry were
found. A determination of the pK\textsubscript{a} values of tartaric
acid was performed by Katzin and Gulyas\textsuperscript{97} using this
method. They expressed the optical rotation of the
sample as the sum of the optical rotation of the ionic
fractions derived from the analyte.

When the fractions are expressed as a function
of the acid dissociation constants and the solvated pro-
ton concentration, the pK\textsubscript{a} values may be determined
by curve fitting of a plot of the optical rotation against
the pH, where small differences in pK\textsubscript{a} inhibit accu-
rate determination.

While polarimetry was shown to be a reason-
ably sensitive method to determine the pK\textsubscript{a} values,
the method has a number of significant drawbacks.
Firstly, the method can only be applied to optically
active analytes, and samples must be relatively pure
enantiomers as the presence of the opposite enantiom-
ers will cause a reduction in the sensitivity of the
measurement with a corresponding drop in precision.
Secondly, the determinations are carried out at high
concentrations meaning that relatively large amounts
of sample are required.

Kinetic method

The kinetic method of determining pK\textsubscript{a} values
depends on measuring the rate of reaction of a con-
tral reaction that is influenced by the pH of its reac-
tion medium. While determinations based on reaction
kinetics are rarely encountered in literature, Bunnett
and Nudelman\textsuperscript{98} demonstrated a method by which
the principle might be applied systematically. Their
method involved a reference reaction, the reaction of
thiophenoxide ion with 2,4-dinitrofluorobenzene to
form a conjugated double ring system, that could be
easily monitored using absorption spectrometry.

The rate of this reaction is determined by the dis-
ociation of thiophenol to yield the free ion, and this
dissociation is repressed by an increase in the solvated
proton concentration. When the dissociation constant
of thiophenol is known, performing the reaction in a
buffering solution of the analyte allows for the calculation of the $pK_a$. The assumption is made that the solvated proton concentration, and thus the position of the thiophenol/thiophenoxide equilibrium, is entirely controlled by the unknown buffer, and that the reaction rate thus depends only on the buffer composition and the $pK_a$ of the buffering acid.

The method as described seemingly has several advantages. Firstly, no exact knowledge of the pH is required since the solvated proton concentration is directly linked to the analytical composition of the buffering solution and it in turn is directly linked to the thiophenoxide concentration. A second advantage is that the $pK_a$ can, in principle, be determined in a single measurement. In spite of this, the method still remains complicated and not generally applicable.

**Computational method**

Finally, we consider the method of computational chemistry. This method is unusual when compared to those previously described in that it is a mathematical method rather than an experimental one. Computation requires no sample and is in principle unrestrained in terms of physical conditions, but the accuracy of the values obtained depends entirely upon the model used to perform the calculations.

Since the science of computational chemistry began to take shape in the early 1970s with the development of efficient software for the calculation of molecular orbitals and the development of the first molecular mechanics methods, many different methods have been developed to computationally estimate molecular properties. Several of these methods have been evaluated for the determination of acid dissociation constants.

The most precise results available are generally obtained from ab initio calculations, meaning that these calculations tend to converge to an exact mathematical solution. However, since the equations used to describe the system are entirely derived from theoretical principles, any flaw in the model will still produce a result that may deviate from its physical value. For instance, considerable difficulties exist when attempting to calculate the $pK_a$ value in a solvated or aqueous phase, since the problem of how to accurately take solvent-analyte interactions into account remains largely unsolved. An article by da Silva et al. published in 1999 compares several methodologies. It compares the results obtained by their own method with those published by Schüürman et al. a year earlier. It is remarked that the methodology used in describing the molecules in the system and the computational methods used to determine the properties can drastically influence the outcome of the calculation, illustrating the difficulty in deriving ab initio systems. Another major downside of these calculations compared to other computational techniques is their enormous cost, making their application in any context where large numbers of molecules must be investigated impractical.

Semi-empirical quantum-mechanical (QM) methods are based on the same formalisms as the ab initio calculations, but make use of further approximations and obtain many parameters from experimental data. A good treatment of these methods was given by Tehan. The method works by taking information from a large database of known molecules and using this information to obtain approximate QM parameters for the calculation. While these methods are much faster than ab initio calculations, the results of the calculation are only reliable if the molecule being computed is similar enough to those stored in the database. More recently, there has been much work on the development of quantum structure-property relationship (QSAR) based methods. These may be considered a further development of the semi-empirical methods described above in the sense that empirical and ab initio data are correlated with the contribution of certain fragments within a molecule. This dataset is then used for further calculations. The most recent methods make use of Molecular Tree Structured Fingerprints to describe the chemical neighborhood of an ionizable center.

In terms of the accuracy of their predictions, computational methods suffer where multiple competing models exist, since the choice of parameters and starting assumptions can dramatically affect the outcome of the calculations. In addition, there are often significant deviations from literature values as shown in Figure 11.

Despite these problems, there is still considerable interest in computational $pK_a$ determination methods in such fields as drug discovery, where the method might be used to reduce uncertainty of the physical/chemical properties of (modifications of) molecules without even having to synthesize all of them. However, for the time being the method is purely of
interest as an estimator, since the reliability of the results leaves much to be desired. It may be expected that the method will find more use in the future as the computational power available increases and better models are derived, but it shall be some time before computational methods are able to compete with actual measurement in terms of accuracy. There are several software packages available (ACD/labs was one of the first) and five of these are compared in a recent review by Manchester et al.\textsuperscript{104}

### Table 1. Overview of the strong (++) and weak (−−) points of the methods discussed. Precision based largely on Xie et al.,\textsuperscript{47} Manchester et al.,\textsuperscript{105} Barbosa et al.,\textsuperscript{106} and Beltrain et al.\textsuperscript{107} T, I and ε denotes effects of Temperature, Ionic Strength and organic modifier, respectively.

| Method           | Amount/conc | Restrictions | pKa range | Costs/time | Precision | T, I and ε |
|------------------|-------------|--------------|-----------|------------|-----------|------------|
| Potentiometry    | −           | +            | −         | ++         | +         | +          |
| Conductometry    | −           | ++           | −         | +          | +         | −          |
| Voltammetry      | −           | +            | +         | +          | +         | +          |
| Calorimetry      | −           | ++           | ++        | +          | +         | +          |
| NMR              | −           | −            | ++        | +++        | ++        | +          |
| Electrophoresis  | ++          | +            | +         | +          | ++        | ++         |
| HPLC             | ++          | +            | −         | +          | +         | +          |
| Solubility       | −           | −            | +         | −−         | +         | −          |
| Spectrometry     | +           | +            | +         | ++         | ++        | +          |
| Fluorimetry      | +           | −            | +         | −          | +         | +          |
| Polarimetry      | +           | −            | +         | +          | +         | +          |
| Kinetic          | −           | −            | −         | −−         | −         | +          |
| Computational    | ++          | −            | ++        | −          | −−        | −          |

**Comparison and Discussion**

Comparing the incomparable is usually difficult and can only be done in a qualitative way as it depends on many factors, among which those specific for each analyte and experimental facilities available. This is even more so as the introduction and application of the different methods described in this paper cover many decades. As such, they also provide a fascinating historical cross-section of chemical experimentation. We are, however, able to make a tabulated comparison of the methods discussed, see Table 1.

The amount and concentration of analyte available are the most obvious factor for method selection. If only minute amounts in organic micro-synthesis are available for characterization, most of the methods cannot be used. Other sample restrictions include, for example, overlapping pK\textsubscript{a}’s, very low solubility, lack of a chromophore, etc. If the available sample has impurities, only separation methods such as HPLC and particularly electrophoresis can be used successfully. Measuring in the extremes of the pH scale is sometimes impossible. Financial factors must also be taken into consideration. It is not fiscally responsible to invest in NMR equipment for the purpose of determining only a few pK\textsubscript{a} values annually.

In regards to precision, for a very rough estimate (± 1 pK unit), the only thing needed is a software package,\textsuperscript{104,108} however experienced chemists are often able to estimate this from the structural formula. Additionally, if the pure analyte is available as an acid and as a salt of a strong base, a 1:1 solution of both

![Figure 11. Plot of calculated versus experimentally observed pK\textsubscript{a} for a set of 143 aliphatic carboxylic acids, data from Schüürmann et al.\textsuperscript{101}](image-url)
will by definition have a pH equal to the $pK_a$ within the range $4 < \text{pH} < 10$.

On the other hand, precisions claimed in literature generally refer only to specific analytes under investigation, and as we all know, are sometimes brightly colored. Three decimals is impossible and two decimals is considered very good. The last column in Table 1, in our view, is a most important one for reasons mentioned in section 1.2. In most cases, $pK_a$ are measured under conditions imposed by the method of choice. These are not always the conditions relevant for the eventual application of the analyte. As already indicated in the Fentanyl example, $pK_a$ values of drugs inherent to their application are those in water at $37 \, ^\circ\text{C}$ and with an ionic strength of $0.9\% \text{NaCl (154 mM)}$. Thermodynamic values at $25 \, {^\circ}\text{C}$ and infinite dilution are irrelevant in this area. In a recent 700 page compilation of aqueous $pK_a$ values of drugs, only half are listed with details of temperature and ionic strength.

The most versatile method able to accurately measure under these conditions appears to be CE. The theoretical basis is straightforward and well understood and the experiments can use a high degree of automation. In the world of drug characterization and analysis, the method is well established. The $pK$ range in which reliable values can be determined is expanding to very acidic regimes unavailable to other techniques.

**Conclusion and Recommendations**

Being able to actually measure under the conditions relevant for the application is a great advantage, but alas often impossible due to limitations of the technique used. Extrapolation to the desired conditions is often possible at the cost of accuracy and precision inherent to all extrapolations.

Depending on the sample purity, a choice can be made between a spectrometric method and a separation method. HPLC is time consuming in the case of small dissociation constants ($\alpha$) whereas CE is faster and cheaper. In the absence of a chromophore for optical detection, CE has the additional advantage over chromatography in that it is possible to apply indirect detection.

Whichever method is used, matrix effects can always occur, especially ionic interaction between analyte and buffer ions. These are checked with analyte behavior in two different buffers of same pH but different buffering constituents. Ionic strength dependence is checked by adding sodium chloride buffer made with the analyte under investigation. Temperature dependence is most easily checked by measuring a buffer of the analyte at different temperatures.

A laboratory where accurate $pK_a$ determinations are essential would be wise to invest in equipment and experience of CE, complemented with spectrometry, pH, and conductivity meters. All of the former should be well thermostated.

Besides those, it would be advised to monitor future developments in $pK_a$ estimation software, especially those programs that can be fed with experimental data for more accurate prediction (rather than estimation) of similar structures.

**Author Contributions**

Entire literature search and first draft versions of manuscript chapters: AHM van Loon, AJF van Hoof, AJP Teunissen. Jointly developed the final structure, illustrations and revised chapters of the paper: AHM van Loon, AJF van Hoof, AJP Teunissen, JC Reijenga. Made critical revisions and approved final version: JC Reijenga. All authors reviewed and approved of the final manuscript.

**Funding**

Author(s) disclose no funding sources.

**Competing Interests**

Author(s) disclose no potential conflicts of interest.

**Disclosures and Ethics**

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to
References

1. Camoës MF. The quality of pH measurements 100 years after its definition. Accad QuaI Assur. 2009;14(10):521–3.
2. Camoës MF. A century of pH measurement. Chem Internet. 2010;32:2.
3. Beluytina AA. The centenary of glass electrode: from Max Cremer to F.G.K. Bouche. Journal of Solid State Electrochemistry. 2011;15:47.
4. Brock WH, editor. The Fontana History of Chemistry. Fontana press; 1992.
5. Henderson LJ. Concerning the relationship between the strength of acids and their capacity to preserve neutrality. Am J Physiol. 1908;21:173.
6. Sörenson SPL. Enzymstudien. II: Mitteilung. Über die Messung und die Bedeutung der Wasserstoffonenkonzentration bei enzymatischen Prozessen. Biochemische Zeitschrift. 1909;21:131–200.
7. Hasselbalch KA. Die Berechnung der Wasserstoffzahl des Blutes aus der freien und gebundenen Kohlensäure desselben, und die Sauerstoffbindung des Blutes als Funktion der Wasserstoffzahl. Biochemische Zeitschrift. 1916;78:112–44.
8. Po HN, Senozan NM. Henderson—Hasselbalch equation: its history and limitations. J Chem Educ. 2001;78:1499–503.
9. Hammett LP. The theory of acidity. J Am Chem Soc. 1928;50:2666.
10. Monzýk C, Crumblis AL. Acid dissociation constants (Ka) and their temperature dependencies (ΔH, ΔS) for a series of carbon- and nitrogen-substituted hydroxydimic acids in aqueous solution. J Org Chem. 1980;45:4670.
11. Poth-Brink C, Crumblis AL. Temperature-dependent acid dissociation constants (Ka, DELTA,DELTA,DELTA,SA) for a series of nitrogen-substituted hydroxydimic acids in aqueous solution. J Org Chem. 1982;47(7):1171–6.
12. Everaerts FM, Beckers JL, Verheggen TEM. Isotachophoresis, Theory, Instrumentation and Applications. Elsevier Scientific Publishing Co.; Amsterdam; 1976.
13. Cohn EH, Heyrov FF, Menkin MF. The dissociation constant of acetic acid and the activity coefficients of the ions in certain acetate solutions. J Am Chem Soc. 1928:50:696–714.
14. Sarmini K, Kenndler E. Capillary zone electrophoresis in mixed aqueous-organic media: effect of organic solvent on actual ionic mobilities, acidity constants and separation selectivity of substituted aromatic acids. I. Methanol. J Chromatogr A. 1998;806:325–35.
15. Sigel H, Zuberbuhler AD, Yamauchi. Comments on potentiometric pH titrations and the relationship between pH-meter reading and hydrogen ion concentration. Anal Chem Acta. 1991;225:63–72.
16. Denham HG. The electrometric determination of the hydrolysis of salts. J Chem Soc Trans. 1908:93:41–63.
17. Stock JT. Early industrial pH measurement and control. Bull Hist Chem. 1991;10:31–4.
18. Beckman AO, Fracker HE. United States patent 2085761. 1936.
19. Gardiner WC, Sanders HL. Errors of the glass electrode. Induct Eng Chem. 1937;9:274–8.
20. Benet LZ, Goyan JE. Potentiometric determination of dissociation constants. J Pharm Sci. 1967;56:665–80.
21. Avdeef A, Corner JEA, Thomson SJ. pH-Metric log P. 3. Glass electrode calibration in methanol-water, applied to pKa determination of water-insoluble substances. Anal Chem. 1993;65(1):42–9.
22. Ravichandiran V, Devajaran V, Masilamani K. Determination of ionization constant (pKa) for poorly soluble drugs by using surfactants: a novel approach. Der Pharmacia Lettre. 2011;13(4):183–92.
23. Kohlrausch F, Göttinger Nachrichten. Über das Leitungsvermögen der in Wasser gelösten Elektrolyte in Zusammenhang mit der Wanderung ihrer Bestandteile. 1876;13:213–4.
24. Saxton B, Darken LS. The ionization constants of weak acids at 25° from conductance measurements. A method of extrapolating the data. J Am Chem Soc. 1940;62(4):846–52.
25. Landolt-Börnstein, Zahlenwerte und Funktionen. 6. Auflage, II. Band, 7. Teil, Elektrische Eigenschaften II. Springer Verlag, Berlin; 1960.
26. Apelblat A. Dissociation constants and limiting conductance of organic acids in water. J Mol Liq. 2002:95-99.
27. Gehr RJ. Conductometric determination of pKa values. Oxalic and squaric acids. Anal Chem. 1971;43(8):1110–3.
28. Kießlind J. Individual activity coefficients of ions in aqueous solutions. J Am Chem Soc. 1937;59:1675–8.
29. Dippy JFJ. The dissociation constants of monocarboxylic acids; their measurement and their significance in theoretical organic chemistry. Chem Rev. 1939;25:151.
30. Quint J, Viallard A. The electrophoretic effect for the case of electrolyte mixtures. J Solution Chem. 1978;7(7):525–31.
31. Lee WH, Wheaton RJ. Conductance of symmetrical, unsymmetrical and mixed electrolytes. Part 1.—Relaxation terms. J Chem Soc, Faraday Trans II. 1978;74:743–66.
32. Lee WH, Wheaton RJ. Conductance of symmetrical, unsymmetrical and mixed electrolytes. Part 2.—Hydrodynamic terms and complete conductance equation. J Chem Soc, Faraday Trans II. 1978;74:1456–82.
33. Lee WH, Wheaton RJ. Conductance of symmetrical, unsymmetrical and mixed electrolytes. Part 3.—Examination of new model and analysis of data for symmetrical electrolytes. J Chem Soc, Faraday Trans II. 1979;75:1128–45.
34. Apelblat A. Representation of electrical conductances for polyanion electrolytes by the quint-viardall conductivity equation. Part 1. Symmetrical 2:2 type electrolytes. Dilute aqueous solutions of alkaline earth metal sulfates and transition metal sulfates. J Solution Chem. 2011;40(7):1209–33.
35. Krofčí A, Apelblat A, Belter-Rogáč M. Dissociation constants of para-benzenes and limiting conductances of their ions in water. J Phys Chem B. 2012;116(4):1385–92.
36. Kalvoda R, Fresenius J. Review of adsorptive stripping voltammetry-assessment and prospects. Anal Chem. 1994;349:565.
37. Zuman P. Current status of potrolography and voltammetry in analytical chemistry. Anal Lett. 2000;33(2):163–74.
38. Bard AJ. The rise of voltammetry: from potrolography to the scanning electrochemical microscope. J Chem Educ. 2007;84(4):644–50.
39. Kolthoff IM, Orlenem EF. The Use of the dropping mercury electrode as an indicator electrode in poorly poised systems. J Am Chem Soc. 1941;63(3):664–7.
40. Breslow R, Babasubramanian K. The pK_a of triphenylcyclopropene. Electrochemical determination of an inaccessible equilibrium constant. J Am Chem Soc. 1969;91:5182.
41. Barette WC Jr, Johnson HW Jr, Sawyer DT. Voltammetric evaluation of the effective acidities (pK_a) for Brönsted acids in aprotic solvents. J Am Chem Soc. 1984;56:1890.
42. Kim H-S, Chung TD, Kim H. Voltammetric determination of the pKa of various acids in polar aprotic solvents using 1,4-benzoquinone. J Electroanal Chem. 2001;498(1–2):209–15.
43. Gupta N, Linschitz H. Hydrogen-bonding and protonation effects in electrochemistry of quinones in aprotic solvents. J Am Chem Soc. 1997;119(27):6384–91.
44. Zhao J, Luo L, Yang X, Wang E, Dong S. Determination of surface pKa of SAM using an electrochemical titration method. Electroanalysis. 1999;11(15):1108–13.
45. Heinze J. Cyclic Voltammetry—Electrochemical spectroscopy”. Ang Chem. 1984;23:831–47.
46. Fenby DV, Heat: its measurement from Galileo to Lavoisier. J Am Chem Soc. 2011;136(4):1385–92.
47. Xie D, Gulnik S, Collins L, Gustchina E, Suvorov L, Erickson JW. Dissection of data for symmetrical 2:2 type electrolytes. Dilute aqueous solutions of alkaline earth metal sulfates and transition metal sulfates. J Solution Chem. 2011;40(7):1209–33.
48. Shin YJ, Fedotov AO, Koris MF, Fung HY, Cui B, Jiao L, Murphy SA, Zhang H. Determination of surface pKa of SAM using an electrochemical titration method. Electroanalysis. 1999;11(15):1108–13.
49. Heinze J. Cyclic Voltammetry—Electrochemical spectroscopy”. Ang Chem. 1984;23:831–47.
50. Xie D, Gulnik S, Collins L, Gustchina E, Suvorov L, Erickson JW. Dissection of the pH dependence of inhibitor binding energetics for an aspartic protease: direct measurement of the protonation states of the catalytic aspartic acid residues. Biochemistry. 1997;36(51):16166–72.
51. Tajc SG, Tolbert BS, Basavappa R, Miller BL. Direct determination of thiol pKa by isothermal titration microcalorimetry. J Am Chem Soc. 2004;126(34):10508–9.
52. Grunwald E, Loewenstein A, Meiboom S. Rates and mechanisms of protonation of diethylammonium ion in aqueous solution studied by proton magnetic resonance. J Chem Phys. 1957;27:641.
50. Lee DG, Cameron R. The basicity of aliphatic ethers. Can J Chem. 1972;50(3):445–8.
51. Popov K, Rönkönäkki H, Lajanen LH. Guidelines for NMR measurements for determination of high and low pK values (IUPAC Technical Report). Pure Appl Chem. 2006;78(3):663–75.
52. Rabenstein DL, Sayer TL. Determination of microscopic acid dissociation constants by nuclear magnetic resonance spectroscopy. Anal Chem. 1976;48:1141–6.
53. Glaser J, Henriksen U, Klassen T. A 28Si NMR titration study of the complex formation between Ti(II) and Cl– in aqueous solution. Act Chem Scand. 1986;A40:344.
54. Poole SK, Patel S, Dehring K, Workman H, Poole CF. Determination of Acid Dissociation Constants by Capillary Electrophoresis. J Chromatogr A. 2004;1037(1-2):445–54.
55. Gluck SJ, Cleveland JA Jr. Investigation of experimental approaches to the determination of pKa values by capillary electrophoresis. J Chromatogr A. 1994;680(1):49–56.
56. Babš S, Horvat AJM, Pavlović DM, Kaštelan-Macan M. Determination of pKa values of active pharmaceutical ingredients. Trends Anal Chem. 2007;26(11):1043–61.
57.Consden R, Martin AJP. Ionophoresis in silica jelly: A method for the separation of amino-acids and peptides. Biochem J. 1946;40(1):33–41.
58. Waldron-Edward D. The micro determination of acid and base dissociation constants by paper electrophoresis. J Chromatogr. 1965;20:556.
59. Kiso Y, Kobayashi M, Kitaoka Y, Kawamoto K, Takeda J. A theoretical study on the zone mobility–pH curve in paper electrophoresis of low molecular weight compounds with a dissociable proton and its application to phosphorus compounds. J Chromatogr. 1968;36:215.
60. Pospichal J, Gebauer P, Boček P. Measurement of mobilities and dissociation constants by capillary isoelectric focusing. Chem Rev. 1989;89(2):419–30.
61. Beckers JL, Everaerts FM, Ackermans MT. Determination of absolute mobilities, pK values and separation numbers by capillary zone electrophoresis. J Chromatogr A. 2004;1037(1-2):445–54.
62. Deardcn JC, Bresnen GM. The measurement of partition coefficients. Quant Struct–Act Relat. 1988;7:113–44.
63. Gluck SJ, Cleveland JA Jr. Investigation of experimental approaches to the determination of pKa values by capillary electrophoresis. J Chromatogr A. 1994;680(1):49–56.
64. Cai J, Smith JT, El Rassi Z. Determination of the ionization constants of weak acids and bases. J Phys Chem. 1992;96(2):3353–65.
65. Consden R, Martin AJP. Ionophoresis in silica jelly: A method for the separation of amino-acids and peptides. Biochem J. 1946;40(1):33–41.
66. Waldron-Edward D. The micro determination of acid and base dissociation constants by paper electrophoresis. J Chromatogr. 1965;20:556.
67. Kiso Y, Kobayashi M, Kitaoka Y, Kawamoto K, Takeda J. A theoretical study on the zone mobility–pH curve in paper electrophoresis of low molecular weight compounds with a dissociable proton and its application to phosphorus compounds. J Chromatogr. 1968;36:215.
68. Pospichal J, Gebauer P, Boček P. Measurement of mobilities and dissociation constants by capillary isoelectric focusing. Chem Rev. 1989;89(2):419–30.
69. Beckers JL, Everaerts FM, Ackermans MT. Determination of absolute mobilities, pK values and separation numbers by capillary zone electrophoresis. J Chromatogr A. 2004;1037(1-2):445–54.
98. Katzin LI, Gulyas E. Dissociation constants of tartaric acid with the aid of polarimetry. *J Phys Chem.* 1960;64:1739–41.

99. Bunnett JF, Nudelman NS. An Independent Kinetic Method for Determining Acid Dissociation Constants in Methanol. *J Org Chem.* 1969;34:2043.

100. da Silva CO, da Silva EC, Nascimento MAC. Ab Initio calculations of absolute pKa values in aqueous solution I. Carboxylic Acids. *J Phys Chem A.* 1999;103(50):11194–9.

101. Schüürmann G, Cossi M, Barone V, Tonasi J. Prediction of the pKa of carboxylic acids using the ab initio continuum-solvation model PCM-UAHF. *J Phys Chem A.* 1998;102:6706.

102. Tehan BG, Lloyd EJ, Wong MG, et al. Estimation of pKa using semiempirical molecular orbital methods. Part 1: Application to phenols and carboxylic acids. *QSAR.* 2002;21:457–72.

103. Chaudry UA, Popelier PL A. Estimation of pK(a) using quantum topological molecular similarity descriptors: Application to carboxylic acids, amines and phenols. *J Org Chem.* 2004;69(2):233–41.

104. Xing L, Glen RC, Clark RD. Predicting pK(a) by molecular tree structured fingerprints and PLS. *J Chem Inf Comput Sci.* 2003;43(3):870–9.

105. Manchester J, Walkup G, Rivin O, You Z. Evaluation of pKa estimation methods on 211 druglike compounds. *J Chem Inf Model.* 26 2010; 50(4):565–71.

106. Barbosa J, Barron D, Jimenez-Lozano E, Sanz-Nebot V. Comparison between capillary electrophoresis, liquid chromatography, potentiometric and spectrophotometric techniques for evaluation of pKa values of zwitterionic drugs in acetonitrile—water mixtures. *Anal Chim Acta.* 2001;437:309–21.

107. Beltran JL, Sanli N, Fonrodona G, Barron D, Ozkan G, Barbosa J. Spectrophotometric, potentiometric and chromatographic pKa values of polyphenolic acids in water and acetonitrile-water media. *Anal Chim Acta.* 2003;484(2):253–64.

108. Babić S, Horvat AJM, Mutavdžić Pavlović D, Kaštelan-Macan M. Determination of pKa values of active pharmaceutical ingredients. *Trends Anal Chem.* 2007;26:10431061.

109. ACD/pKa: Predict accurate acid/base dissociation constants from structure—the industry standard. Available at: http://www.acdlabs.com/products/percepta/predictors/pka/. Accessibility verified Nov 2012.

110. Brittain HG, editor. *Profiles of Drug Substances, Excipients and Related Methodology.* Vol 33. Academic Press; 2007.

111. Li SFY. *Capillary Electrophoresis: Principles, Practice, and Applications.* Amsterdam: Elsevier; 1992.

112. Atria KD, editor. *Capillary Electrophoresis Guidebook: Principles, Operation, and Applications.* Totowa NJ, USA: Humana Press; 1996.

113. Foret F, Boček P. *Capillary Electrophoresis: Instrumentation and Methodology.* VCH Weinheim; 1996.

114. Ahuja S, Jimida MI. *Capillary Electrophoresis Methods for Pharmaceutical Analysis.* Amsterdam: Elsevier; 2008.

115. Včeláková K, Zusková I, Kenndler E, Gaš B. Determination of cationic mobilities and pKa values of 22 amino acids by capillary zone electrophoresis. *Electrophoresis.* 2004;25(2):309–17.

116. Zusková I, Novotná A, Včeláková K, Gaš B. Determination of limiting mobilities and dissociation constants of 21 amino acids by capillary zone electrophoresis at very low pH. *J Chromatogr B.* 2006;841:129.

117. Bruin GM, van Asten AC, Xu X, Poppe H. Theoretical and experimental aspects of indirect detection in capillary electrophoresis. *J Chromatogr.* 1992;608:97.

118. Doble P, Macka M, Haddad PR. Use of dyes as indirect detection probes for the high-sensitivity determination of anions by capillary electrophoresis. *J Chromatogr A.* 1998;804(1–2):327–36.