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GUIDELINES FOR NMR MEASUREMENTS FOR DETERMINATION OF HIGH AND LOW pK_a VALUES

(IUPAC Technical Report)

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Guidelines for NMR measurements for determination of high and low pK_a values

(IUPAC Technical Report)

Abstract: Factors affecting the NMR titration procedures for the determination of pK_a values in strongly basic and strongly acidic aqueous solutions ($2 \geq pH \geq 0$ and $14 \geq pH \geq 12$) are analyzed. Guidelines for experimental procedure and publication protocols are formulated. These include: calculation of the equilibrium H^+ concentration in a sample; avoidance of measurement with glass electrode in highly acidic (basic) solutions; exclusion of D_2O as a solvent; use of an individual sample isolated from air for each pH value; use of external reference and lock compounds; use of a medium of constant ionic strength with clear indication of the supporting electrolyte and of the way the contribution of any ligand to the ionic strength of the medium is accounted for; use of the NMR technique in a way that eliminates sample heating to facilitate better sample temperature control (e.g., ^{1}H-coupled NMR for nuclei other than protons, GD-mode, CPD-mode, etc.); use of Me_4NCl/Me_4NOH or KCl/KOH as a supporting electrolyte in basic solution rather than sodium salts in order to eliminate errors arising from NaOH association; verification of the independence of the NMR chemical shift from background electrolyte composition and concentration; use of extrapolation procedures.

Keywords: NMR titration; dissociation constants; acidity constants; chemical shift dependence on medium; high and low pK measurement; IUPAC Analytical Chemistry Division.

INTRODUCTION

Numerical data for acid–base equilibria (lg K_a values) have contributed significantly to the theoretical foundation of modern organic and inorganic chemistry [1,2]. In particular, the ligand acid dissociation constants (pK_a) correlate strongly with complex stability for many classes of ligands [3]. The related linear Gibbs energy relationships may be used for prediction of metal complex stability constants K_{ML} in cases where their direct experimental measurement is difficult or impossible [2,4,5].

Many important acid–base equilibria take place in highly basic or highly acidic aqueous solutions. For strongly acidic aquametal ions (e.g., Tl^{III}, Bi^{III}, Ti^{IV}, Th^{IV}, Be^{II}, Pd^{II}), the measurement of stability constants frequently requires solutions of low pH ($pH \leq 2$) [6], while complex formation frequently involves ligands with very small pK_a values. By contrast, many technologies and complexation reactions require $pH \geq 12$ [7] and ligands that are strongly basic (e.g., phosphonates, anionic forms of sugars, hydroxybenzoates, polyamines). In both cases, the application of glass electrode-based potentiometry does not give reliable results [8].

In recent years, a variety of new techniques have been developed as alternatives to the classical potentiometric titration procedure. Among these is nuclear magnetic resonance (NMR), which has a unique application for microscopic acid dissociation constant measurements [9] as well as for work in highly basic and highly acidic media [1,6–8]. Although early reports on the use of the NMR technique were not promising [1], later work revealed good concurrence with potentiometric results for compounds with pK_a values in the range $11 \geq pK_a \geq 3$ [10–11]. Recently, fully automated pH-NMR titration equipment for protonation studies has been reported [10a,10c,12–14]. However, the pK_a values estimated from NMR measurements in strongly basic (acidic) solutions often differ significantly from

those obtained by potentiometry. The higher reliability of equilibrium data based on NMR measurements in the ranges $2 \geq$ pH and pH ≥ 12 is widely recognized [7,8b,8c,8d,12,13].

At the same time, diverse experimental conditions have been used for protonation and stability constant measurements by NMR. This affects the reliability and the comparison of the resulting equilibrium constants. Further, many authors have not used a standard approach to the chemical shift reference application, preparation of samples, pD/pH corrections, ionic strength control, etc. [11,14–17]. This in turn has resulted in a considerable disparity among the calculated constants. The present report is therefore focused on general recommendations for the application of NMR spectroscopy to the determination of protonation (dissociation) constants in aqueous solution, with an emphasis on titration procedures in highly acidic or highly basic media ($2 \geq$ pH ≥ 0 and $14 \geq$ pH ≥ 12). At the same time, it provides some guidelines for the critical treatment of the NMR-based pK_a values published earlier.

FACTORS AFFECTING THE ACCURACY OF NMR TITRATIONS

Acid dissociation constants can be expressed in terms of activity (thermodynamic constants) or concentrations (concentration, conditional constants). In the former case, the activity constant $K_a = a_H a_L / a_{HL} a^o$, or a mixed activity-concentration constant $K_a = a_H[L]/[HL]c^o$ are considered, where $c^o = 1$ mol dm^{-3} is the standard amount concentration; a^o is the corresponding activity; a_H, a_L, a_{HL} represent activities; and [H], [L], [HL] amount concentrations of H^+, L^-, and HL species, respectively. IUPAC recommends for solution equilibrium studies the determination of concentration-based constants $K_a = [H][L]/[HL]c^o$ [18,19]. In the present paper, the term pK_a always indicates the concentration constant valid for a particular ionic strength I and temperature, while pH corresponds to the concentration p[H] scale (p[H^+]); i.e., we define p[H] = –lg {[H^+]/c^o} unless otherwise is stated. In a similar way, p[D] should correspond to –lg {[D^+]/c^o}. This requires either calibration of a pH meter by solutions with known [D^+] at a particular I, or the direct calculation of [D^+] in strongly basic (acidic) solutions when the concentration of L can be neglected. However, this ideal condition is seldom if ever fulfilled, and the common practice is based on the "pH meter readings" in D_2O solutions after the pH meter was calibrated in H_2O buffer solution [8a] (see eqs. 5–7 and further discussion). Obviously, this approach gives some value of pD as unclear function of activity a_D and cannot be recommended for work in concentrated (>0.1 mol dm^{-3}) solutions of bases (acids).

For the dissociation equilibrium of the protonated ligand HL (charge numbers are neglected):

$$HL \leftrightharpoons L + H \tag{1}$$

the acidity constant K_a is defined at a particular ionic strength I as $K_a = [L][H]/[HL]c^o$. Then pK_a = – lg K_a = p[H] + lg ([HL]/[L]) and at half-neutralization p[H] becomes a reasonable estimate of pK_a as [HL] = [L] and lg ([HL]/[L]) = 0.

However, many research groups use the NMR technique for D_2O solutions and therefore operate with measurements of pD in terms of activity as indicated earlier. The corresponding mixed activity-concentration constant is denoted here as $K_a(D_2O) = a_D[L]/[DL]c^o$. Then the $K_a(D_2O)$ values are recalculated by means of some empirical and very arbitrary equations (see further discussion) into some equilibrium activity-concentration constant $K_a(H_2O)$, which is supposed to indicate $K_a = a_H[L]/[HL]c^o$ for H_2O solutions, although there is no rigorous background for that supposition.

From the p[H] dependence of the chemical shift, the pK_a can be determined, using ^{1}H, ^{13}C, ^{14}N, ^{15}N, ^{31}P, ^{19}F, or any other NMR-active nucleus in a ligand [9a]. Since proton dissociation from HL changes the electron density, species HL and L reveal different chemical shifts, denoted as δ_{HL} and δ_L. Most acids in aqueous solutions are characterized by rapid proton-transfer reactions on the NMR timescale. Thus, the observed chemical shift of any one nucleus represents the single concentration-weighted average δ_{obs} of the chemical shifts for the nucleus of each chemical species in the equilibrium:

$$\delta_{obs} = x_{HL}\delta_{HL} + x_L\delta_L \quad (2)$$

where x_{HL} and x_L denote the mole fractions of equilibrium species HL and L. The dynamically averaged chemical shift δ_{obs} provides a good measure for the degree of ionization (proton dissociation):

$$x_L = (\delta_{obs} - \delta_{HL})/(\delta_L - \delta_{HL}) \quad (3)$$

The mole fractions can be expressed in terms of p[H] and pK_a [1,13]:

$$pK_a = p[H] + \lg [(\delta_L - \delta_{obs})/(\delta_{obs} - \delta_{HL})] \quad (4)$$

It is easy to demonstrate from eq. 4 that: (a) for an acid HL, a plot of δ_{obs} vs. p[H] has the shape of a titration curve lying between the asymptotes δ_L and δ_{HL}, with a point of inflection at p[H] = pK_a, $\delta_{obs} = (\delta_L + \delta_{HL})/2$; (b) the titration curve is symmetrical about the inflection point (Fig. 1), which gives a possible simple method of estimating pK_a, δ_L, and δ_{HL}.

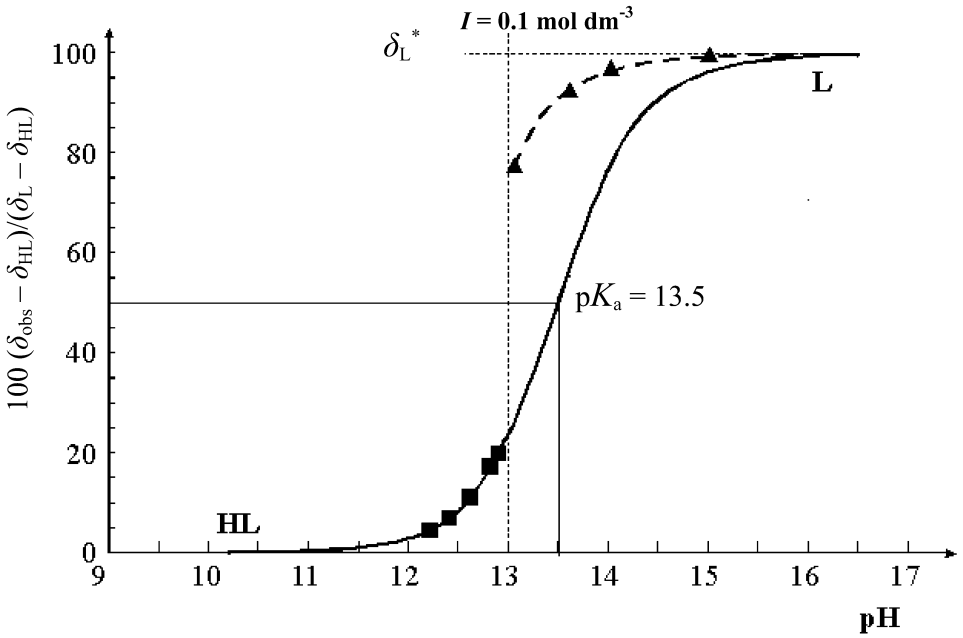

Fig. 1 Simulated NMR titration curve for the hypothetical 0.001 mol dm^{-3} acid HL with pK_a =13.5 at I = 0.1 mol dm^{-3} (solid line), plotted by SPECIES [35]. Squares refer to hypothetical experimental NMR-titration points at I = 0.1 mol dm^{-3}. Their range is limited by the ionic strength I (high pH limit) and by the requirement [OH$^-$] >> [HL] (low pH limit). Dashed line and triangles refer to a simulated NMR titration of the same acid at I = 1.0 mol dm^{-3}, which provides the value δ_L* directly or via extrapolation.

The normal procedure for a NMR titration is based on the dependence of the chemical shift δ_{obs} on p[H], with subsequent treatment of experimental data via routine software. Therefore, three constants are to be found from the δ_{obs} vs. p[H] data by computer analysis of this nonlinear equation, and preliminary values can often be found directly from the plot. A significant advantage of the NMR technique is associated with the possibility of titrating a mixture of ligands, including impurities, if the total concentration of the ligands (and therefore of impurities associated with the ligands) is much less then the base (acid) concentration.

It is important to stress that for classical potentiometry with a glass electrode the inflection point can be observed only if pK_a is close to $(pK_w)^{1/2}$ [1]. For a NMR titration, the situation is completely

different. As far as only mole fractions, instead of the total acid concentration, are involved in the data evaluation, NMR facilitates pK_a measurement outside the range of potentiometry if high or low p[H] are determined by means other than glass electrode readings [13]. Thus, the main sources of errors in NMR-based pK_a determinations are the accuracy and precision of δ_{obs} and p[H] values.

CHEMICAL SHIFTS

General conventions for chemical shifts are comprehensively considered in recent IUPAC recommendations [20]. In the present paper, we will focus only on specific problems associated with NMR-based pK_a determinations, bearing in mind that many research groups involved in solution chemistry equilibria still do not have modern NMR equipment and have to work with routine spectrometers. It is essential that the chemical shift measurement being made for each datum point is reliable. Another important requirement is to obtain from the set of chemical shifts such a pK_a value that can pass comparison with other equilibrium constants.

As described in ref. [20], there are three types of referencing method that could reasonably be applied in titrations: *internal referencing*, *substitution method*, and *external referencing*. These methods all have various advantages and disadvantages in relevance to NMR titration.

Internal referencing may lead to intermolecular interactions between ligand, solvent, and reference compound. Further, in many spectrometers the sample must normally include a deuterium-containing molecule for magnetic field stabilization ("lock"). For many purposes, all these interactions can be safely ignored, but for NMR-based titrations at high and low p[H] a considerable caution is needed. The use of D_2O (as the "lock") instead of H_2O as a solvent, and the addition of uncontrolled "small" amounts of a reference compound like sodium 3-(trimethylsilyl)propane-1-sulfonate (DSS), dimethyl sulfoxide (DMSO), *tert*-butylalcohol or 1,4-dioxane inside a sample (internally), became common practice for ^{1}H and ^{13}C NMR [12,15,16]. In some cases (^{13}C NMR), the added reference compound is itself deuterated (e.g., ($^2H_6$)DMSO or DSS, deuterated at the CH_2 positions), thus providing the lock signal as well. Modern NMR techniques give the possibility to work with very low concentrations of DSS. Therefore, it gives a negligible contribution to ionic strength and to solution properties. It is demonstrated to be effective at p[H] 0–1 [12]. At the same time, little is known about the properties of internal references at elevated p[H]. Nevertheless, any internal substance can potentially participate in association processes either with the cation under complex formation study or with the background electrolyte and is therefore generally undesirable from the point of view of equilibrium studies.

For ^{1}H NMR titrations, the use of D_2O as a solvent instead of H_2O is a common procedure. This is usually done to eliminate masking of a substrate peak by the H_2O resonance [15–17]. The use of D_2O internally raises the problems of how to effect pD measurement with a standard glass electrode, as well as the relationship between pK_a(H_2O) and pK_a(D_2O). The proposed simple empirical eq. 5 derived for ionic strength I = 0.001–0.01 mol dm^{-3} and 25 °C [21] to obtain values on the conventional pD scale from glass electrode readings is widely accepted, although it is frequently used far outside of the originally intended ionic strength and temperature limits:

$$\text{pD} = \text{pH-meter reading}^* + 0.40 \quad (5)$$

Some authors, however, use eqs. 6 or 7 [22,23]:

$$\text{pD} = \text{pH-meter reading} + 0.44 \ (22\ ^\circ\text{C}, I = 0.01 \text{ mol dm}^{-3}) \quad (6)$$

$$\text{pD} = \text{pH-meter reading} + 0.50 \ (25\ ^\circ\text{C}, I = 0.1 \text{ mol dm}^{-3}) \quad (7)$$

*pH-meter reading for solutions in D_2O when the pH electrodes are calibrated with standard aqueous buffers.

Although the difference between pD values calculated by different equations is not large, it is a substantial contribution to systematic error, even for low ionic strength and room temperature, but particularly for high ionic strengths and high temperatures.

There is an even greater diversity of relationships between $pK_a(H_2O)$ and $pK_a(D_2O)$. Both quantities are ionic strength-dependent. The proposed empirical equations yield significantly different results and seem very arbitrary: relationships depend on the nature and number of compounds studied [24–26]. It is observed that the activity coefficient products undergo significant changes when one goes from light to heavy water [27]. It is obvious that at present a correct extrapolation of $pK_a(D_2O)$ to an aqueous phase $pK_a(H_2O)$ is not possible, and that the systematic errors for calculated values are outside the accepted uncertainty for $pK_a(H_2O)$ values derived directly from NMR measurements with external D_2O. Besides, $pK_a(D_2O)$ values can hardly pass comparison with other equilibrium constants measured in H_2O, and their use for complex formation equilibria in H_2O is very doubtful. Assuming the above difficulties, the use of internal D_2O is not recommended.

For ^{31}P NMR, the use of internal referencing at high and low p[H] is difficult, and external reference application is widely used and recommended [7c,7d,8b,8c,11a,11b,16,17a,17b].

Substitution method uses measurement of sample and reference spectra in two separate experiments. It became feasible due to implementation of stable, internally locked spectrometers. In this procedure, the sample and reference materials are not mixed. This benefits the equilibrium study. If locking is not used, the magnet should not be reshimmed between running the sample and reference solution, since this changes the applied magnetic field [20]. This can become a disadvantage for time-consuming ^{13}C NMR-based equilibrium experiments because the ligand concentrations have to be small.

External referencing involves sample and reference contained separately in coaxial cylindrical tubes. A single spectrum is recorded, which includes signals from both the sample and the reference. It is also an ideal situation for equilibrium study as far as both reference and "lock" substances are separated from the ligand solution. The external reference procedure generally requires corrections arising from differences in bulk magnetic susceptibility between sample and reference [20]. This is important for precise chemical shift measurements, but for the relative change of δ_{obs} between δ_L and δ_{HL} for a series of nearly identical aqueous solutions in a narrow pH range (either p[H] 0–2 or 12–14) with constant ionic strength and a constant sample volume it is insignificant. Numerous measurements of ^{13}P NMR-based pK_a values revealed no influence from this factor [7c,7d,8b,8c,11a,11b,16,17a,17b]. Alternatively, magic-angle spinning could be used. Therefore, such a technique seems to be the preferable choice.

p[H] values and titration procedure

An important source of error in NMR-based pK_a determinations is the accuracy and precision of the p[H] values. Determining extreme values of p[H] requires special attention, since glass electrodes cannot be used reliably [8a,8c,12,13]. Therefore, the traditional single-sample NMR titration is recommended [8c,13,27,29,30]. A set of individual samples with constant monoprotic acid HL (or ligand L) concentration (e.g., 0.01 mol dm^{-3}), constant ionic strength (e.g., 1 mol dm^{-3}) and varying p[H] value are prepared one-by-one ("constant volume titration") in such a way that a strong acid or a strong base added for desired p[H] adjustment is taken in a significant excess over HL or L (e.g., 0.1–1.0 mol dm^{-3}). This permits the equilibrium p[H] value to be calculated reliably as it is equated to the total amount of a strong acid (strong base) added to the sample [27]. Since each sample is prepared individually from stock solutions, the ionic strength can be very precisely controlled [12]. The use of "lock" and reference substances externally excludes their undesirable influence on the equilibrium system. Alternatively, in an approach developed by Hägele [13], the glass electrode can be completely avoided by adding an indicator molecule to the sample for in situ p[H] monitoring. However, this method is primarily based on the procedure stated above.

The proposed method is equally valid for both strongly acidic (pH < 2) and strongly basic (pH > 12) solutions, although some peculiarities do exist in the latter. For the acidic medium, p[H] is directly derived from the total strong acid concentration. In the case of highly basic solutions, the initially calculated p[OH^-] values have to be converted to the p[H] scale, using appropriate pK_w values to allow calculation of the corresponding pK_a values. Some important issues that restrict the application of the above method and influence the data quality should also be considered.

Titration procedure and titration curve treatment

A full-scale NMR titration for a single proton equilibrium 1 will provide values of δ_{HL}, δ_L, and some intermediate chemical shift values applicable to a particular pH at a constant *I*. Ideally, a titration spans over 4 pH units with the half-neutralization point in the middle of this pH range. For extremely high or low pK_a, this condition is not achievable: for $pK_a = 13.5$, the value for δ_L has to be measured at pH 15.5, while for $pK_a = 0.5$ a direct observation of δ_{HL} requires pH = – 1.5.

If the ionic strength is 1.0 mol dm^{-3} (NaCl/NaOH), then the highest pH attainable at 25 °C (pH_{max}) is less than 13.72 (pK_w for 1 mol dm^{-3} NaOH[‡]), while for $I = 0.1$ mol dm^{-3} NaCl/NaOH $pH_{max} < 12.78$ (limitation due to pK_w and *I*). Thus for $pK_a = 13.5$ at $I = 1.0$ mol dm^{-3} (NaCl/NaOH) only about 80 % of the titration curve is accessible, providing a value for δ_{HL} and a half-neutralization point, but not for δ_L. In the case of $I = 0.1$ mol dm^{-3} (NaCl/NaOH), about 30 % of the full curve can be obtained experimentally, but excluding the half-neutralization point and δ_L, Fig. 1 (square points). Although comprehensive software (SigmaPlot, WinEQNMR) permits calculation of pK_a and δ_L values for very weak acids on the basis of data at different pH values below that for the half-neutralization point, the corresponding constants have a large error. But in some cases, the programs fail to produce results and experimental measurement of high pK_a at low ionic strength becomes impossible. This can be illustrated by the last dissociation step of nitrilotris(methylenephosphonic acid) (NTPH, H_6ntph) and ethylenediaminetetra(methylenephosphonic acid) (EDTPH, H_8edtph)[‡‡], Table 1.

Table 1 Dissociation constants pK_a for $Hedtph^{-7}$ and $Hntph^{-5}$ derived from ^{31}P NMR measurements by SigmaPlot data treatment.[a]

Ligand	*I*/(mol dm^{-3})	*t*/°C	pK_a	pK_a^*	Reference
$Hedtph^{-7}$	0.1 (KNO_3)	25	Calculation failed	13.29 ± 0.07	[28]
	0.15 (NaCl)	37	13 ± 1	12.86 ± 0.07	[28]
$Hntph^{-5}$	0.1 (KNO_3)	25	12.2 ± 0.3	12.9 ± 0.1	[29]

[a]pK_a and pK_a^* represent constants calculated without δ_L^* and with δ_L^* values, respectively; see text for other explanations.

On the other hand, if the initial δ_{HL} experimental value and the subsequent 30–40 % of a complete titration curve are supported by at least one final high pH titration point to provide δ_L, then the precision of the pK_a calculation becomes sufficiently high and the measurement becomes feasible.

For those nuclei with chemical shift poorly dependent on the ionic strength and nature of the supporting electrolyte (the case of ^{31}P and ^{13}C), δ_L can be obtained by titration of the same system at a higher or even uncontrolled ionic strength until the "plateau" is reached (triangle points, Fig. 1). The resultant δ_L^* is very close to δ_L, e.g., δ_L ($I = 0.1$ mol dm^{-3}) ~ δ_L^* ($I = 1.0$ mol dm^{-3}). Then the fol-

[‡]Reliable values for pK_w are measured only for some common supporting electrolytes, e.g., NaCl, $NaClO_4$, KNO_3, etc. For 1 mol dm^{-3} NaOH, the pK_w value found for 1 mol dm^{-3} NaCl is valid as far as the difference in corresponding activity coefficients is negligible. The same situation is observed for the 0.1 mol dm^{-3} NaCl/NaOH system. However, it is not the case for a complete substitution of 1 mol dm^{-3} NO_3^- for OH^- or of 1 mol dm^{-3} K^+ (Na^+) for H^+ (acidic solutions).

[‡‡]The PINs (preferred IUPAC names) for NTPH and EDTPH are: [nitrilotris(methylene)]tris(phosphonic acid) and ethane-1,2-diyldinitrilotetrakis(methylene)tetrakis(phosphonic acid).

lowing two-step procedure is recommended. The first step involves the titration of a ligand at a sufficiently high ionic strength, e.g., 1.0 mol dm^{-3} NaCl (triangles in Fig. 1) or 1.5 mol dm^{-3} NaCl, etc., rather than in 0.1 mol dm^{-3} NaCl. This gives two advantages. The first is that the pH_{max} is shifted from 12.78 to ca. 14. The second derives from the fact that sodium ion forms weak complexes with L (e.g., phosphonic acids). Therefore, the whole titration curve is shifted to a lower pH range as the total sodium concentration is increased. Both of these factors facilitated the direct observation of a "plateau" corresponding to δ_L^* (I = 1 mol dm^{-3}).

Due to the fact that δ_L (0.1 mol dm^{-3} NaCl) is practically equal to δ_L^* (1 mol dm^{-3} NaCl), then the δ_L^*-value could be used instead of δ_L along with experimental points obtained for I = 0.1 mol dm^{-3} (square points, Fig. 1). Therefore, within the second step, δ_L^* is assigned to a conventional pH = 16 or pH = 17, where the titration curve definitely has a plateau. A titration is repeated for 0.1 mol dm^{-3} NaCl solutions, a δ_L^* point is added to the experimental data set, and a pK_a^* value is calculated. The subsequent treatment of the united data reveals a significant increase in the accuracy of pK_a. This can be seen from Table 1, where both constants pK_a (calculated without δ_L^*) and pK_a^* (calculated with a δ_L^* point) are represented. If δ_L is significantly dependent on ionic strength, then the extrapolation procedure proposed by Popov, Lajunen, and Rönkkömäki [28,29] could be applied.

Ligand concentration

Calculation of p[H] from the acid stoichiometry requires a low ligand concentration: for a monoprotic acid $C_{HL} < 0.01\ I$. Recent developments of the NMR technique make it possible to now work with very dilute solutions. In case of the organophosphonates, concentrations $C_L \sim 0.001$ mol dm^{-3} are quite suitable for ^{31}P NMR titrations [28,29].

By contrast, for ^{13}C NMR titrations, the ligand concentration has to be rather high (about 0.1 mol dm^{-3}) in order to perform the titration in a reasonable time. Therefore, the equilibrium [OH^-] cannot be equal to the total [OH^-] added to the system. For this case (e.g., 0.1 mol dm^{-3} HL), another two-step procedure reported for sucrose dissociation constant measurements [31] is recommended. In the first step, the equilibrium [OH^-] is taken as equal to the total [OH^-] added, and the full titration curve is plotted, mathematically treated, and the pK_a, δ_L, and δ_{HL} values are calculated. The difference between δ_L and δ_{HL} chemical shifts defines the linear scale of OH^- consumption by the ligand: 0 mol dm^{-3} (δ_{HL}) and 0.1 mol dm^{-3} (δ_L) for a 0.1 mol dm^{-3} solution of L. Within the second step, all the experimental values δ_{obs} are treated again with redefined values of p[OH], and an improved value of pK_a is calculated. As indicated in Table 2, the correction due to the second step reveals a systematic error of 0.1 in pK_a.

Table 2 Dissociation constant of 0.1 mol dm^{-3} sucrose (HL) from ^{13}C NMR titration at 60 °C in 1 mol dm^{-3} NaCl/NaOH [31].

Procedure	δ_L/ppm	δ_{HL}/ppm	pK_a	R
One-step data treatment	103.00	101.98	12.40 ± 0.05	0.999
Two-step data treatment	102.96	101.98	12.30 ± 0.05	0.999

Background electrolyte and ionic strength

To date, the background electrolyte effect on chemical shifts has been inadequately studied. Equilibrium concentration products are ionic strength-dependent, yet numerous NMR titration experiments have been performed without ionic strength control [14c,16c], and have produced pK_a values in reasonable agreement with potentiometric results. In part, this arises from the fact that the chemical shifts depend on concentrations, rather than the activities of various species in solution [32]. The best agreement has been demonstrated for systems studied by ^{13}C and ^{31}P NMR [28,29,33]. For the ^{13}C and ^{31}P NMR resonances in alkylcarboxylic and alkylphosphonic acids, the chemical shifts correlate linearly with the background electrolyte concentration. However, this effect is normally negligible in comparison with

that associated with a ligand dissociation or complex formation. This fact offers a unique possibility to use ^{13}C and ^{31}P NMR chemical shifts, δ_L, of a ligand, measured at high pH and high ionic strength, for calculations of pK_a at low ionic strength [28,29]. General observation reveals that the chemical shift depends on both the nature of the nucleus and its position in the ligand. The ^{31}P nuclei in phosphonic ($–PO_3H_2$, $–PO_3H^-$, $–PO_3^{2-}$) as well as ^{13}C nuclei in carboxylate or methylenic groups ($–CO_2^-$, $–CH_2–$, $–CH_3$) are relatively isolated from solution by oxygen or hydrogen atoms. Thus, their chemical shifts are mostly sensitive to the substrate intramolecular processes (deprotonation/protonation, complex formation), while the solvent changes give the least contribution. On the other hand, the nuclei that contact the solvent directly, e.g., $^{133}Cs^+$, $^{35}Cl^-$, are more affected by medium effects. Therefore, a NMR titration under variable ionic strength is not desirable, unless the independence of chemical shift δ on ionic strength I is demonstrated.

Among the supporting electrolytes for $14 \geq pH \geq 12$, the use of 1.0 mol dm^{-3} Me_4NCl/Me_4NOH is recommended as there is no reported evidence for Me_4NOH self-association. In the case of KOH and NaOH, corrections for base self-association could be needed. The uncertainty is associated with imprecise knowledge of the MOH stability constants. Table 3 represents the estimation of errors if the MOH stability constants recommended by Baes and Mesmer [34] are used. Table 3 also demonstrates that for NaOH solutions the pH scale has to be corrected, while for KOH no correction is needed. However, it should be mentioned that Martell [35] gives significantly higher stability constants for MOH ion pairing. Thus, the corresponding corrections could be larger.

Table 3 Calculated –lg {[H^+]/mol dm^{-3}} for MOH solutions in 0.1 and 1.0 mol dm^{-3} MCl/MOH.[a]

MOH	Total [OH^-], mol dm^{-3}	Free[a] [OH^-], mol dm^{-3}	pH calculated without correction for MOH association	pH corrected for MOH association[a]	ΔpH
NaOH	0.1000	0.0947	12.75	12.73	0.02
	1.00	0.69	13.75	13.54	0.21
KOH	0.1000	0.0998	13.15	13.15	0.00
	1.00	0.91	14.15	14.11	0.04

[a]Free [OH^-] is calculated with the SPECIES software [36] using MOH stability constants lg K_1 from [34] (for ionic strength 1.0 mol dm^{-3} lg K_1 = –0.5 for NaOH and –0.8 for KOH), [H^+] is calculated from [OH^-] using pK_w = 13.75 for 1 mol dm^{-3} NaCl and 14.16 for 1 mol dm^{-3} KCl [37].

Another important issue for NMR titration is the need for a clear indication as to whether the contribution of the ligand to the total ionic strength is considered or not. For monobasic acids, this contribution could be negligible, but it is not the case for polyprotic substrates such as EDTPH. In basic 0.01 mol dm^{-3} solutions of EDTPH, the ligand contribution to the total ionic strength constitutes 0.25 mol dm^{-3} for $Hedtph^{-7}$ and 0.33 for $edtph^{-8}$.

Special care should be taken over supporting electrolyte purity. Indeed, in 1 mol dm^{-3} Me_4NCl/Me_4NOH medium, the concentration of Ca^{2+} impurities in the supporting electrolyte can be comparable with the ligand content in the system [8c].

Another important peculiarity of the titration procedure at high and low pH arises from a complete substitution of either cation or anion. Indeed, within the constant background electrolyte concentration, e.g., 1 mol dm^{-3} at 25 °C, the ionic strength can change significantly. For example, the complete substitution of 1 mol dm^{-3} KNO_3 for 1 mol dm^{-3} KOH induces the change of mean activity coefficient from 0.444 to 0.733. In the same way, a substitution of 1 mol dm^{-3} KNO_3 for 1 mol dm^{-3} HNO_3 results in a change of activity coefficient from 0.444 to 0.730. At the same time for 1 mol dm^{-3}

NaCl/NaOH system, the corresponding change is negligible (0.657 and 0.674)*. Therefore, a proper choice of supporting electrolyte, or clear indication of corresponding corrections, is needed.

Temperature

Dissociation constants, as well as pK_w, are temperature-dependent [35]. A temperature variation of 20–30 °C can result in a change of 0.2–0.3 in pK_a (or more). Especially critical are the high pK_a – values. For example, for $Hntph^{-5}$ dissociation $\Delta H = -38.8$ kJ mol^{-1} for I = 0.1 mol dm^{-3} and 25 °C ([36], Mini Database). Therefore, pK_a = 13.30 at 25 °C and 12.98 at 40 °C. The difference in 0.1 pK_a unit per 5 °C is significant for dissociation constant. The temperature dependence of pK_w additionally affects all the measurements in basic solutions. For example, in 0.51 mol dm^{-3} NaCl solutions, pK_w changes from 13.71 (25 °C) to 12.96 (50 °C). In this respect, the noise associated with ^{1}H-decoupling widely used in early NMR measurements might have led to some errors in pK_a values due to significant energy dissipation and therefore to a sample heating. Although modern multipulse decoupling methods (GD-mode, CPD-mode) dissipate less energy, some caution is needed to control the process. In some cases, ^{1}H-coupled spectra are the better choice.

GUIDELINES

Recommendations for NMR titrations in solutions of high and low pH ($2 \geq pH \geq 0$ and $14 \geq pH \geq 12$) are intended to be a supplement to the IUPAC guidelines for the determination of stability constants [19] and to a standard format for the publication of stability constant measurements [38] considering the peculiarities of NMR spectroscopy mentioned above. Some of these requirements are also valid for the range $12 \geq pH \geq 2$.

1. Within the NMR titration procedure at high and low pH solutions ($2 \geq pH \geq 0$ and $14 \geq pH \geq 12$), the equilibrium H^+ concentration should be calculated from solution stoichiometry, not measured with a glass electrode. For this reason, the ligand concentration has to be ≤0.001 mol dm^{-3}. For higher ligand concentrations, the titration is possible, but corrections for strong base (strong acid) consumption by a ligand are necessary.
2. Arrangement of a titration procedure. Sets of samples should be prepared in such a way that the concentration of the ligand and the total ionic strength remain constant, while the supporting electrolyte composition is varied from sample to sample to provide different concentrations of OH^- or H^+. For highly basic solutions, the total concentration of added base should be much greater than the ligand concentration ($[OH^-] >> [L]$). For highly acidic media, the same requirement applies to $[H^+]$ ($[H^+] >> [L]$). Thus, the total concentration of added base (acid) can be treated as the equilibrium concentration (i.e., the OH^- or H^+ consumption by the substrate can be neglected). This circumvents the problems associated with pH measurements with the glass electrode. An additional advantage of such an approach is that the protonation constants are derived in terms of concentration, not activity.
3. A medium of constant ionic strength should be used, with clear indication of the supporting electrolyte and of the way the contribution of the deprotonated ligand and of the change in a background electrolyte composition (e.g., change of $[Cl^-]$ for $[OH^-]$ or $[Cl^-]$ for $[H^+]$) to medium ionic strength is taken into account.
4. The supporting electrolytes Me_4NCl/Me_4NOH or KCl/KOH should be used in basic solution rather than NaCl/NaOH of LiCl/LiOH in order to eliminate errors arising from NaOH and LiOH association. A clear indication of the pK_w used is necessary.

*Mean activity coefficients are taken from *CRC Handbook of Chemistry and Physics*, 82nd ed., R. Lide (Ed.), CRC Press, Boca Raton, FL (2001–2003).

5. External reference and "lock" compounds should be used to eliminate any possible interactions with the ligand and additional changes of the medium.
6. Water should be used as a solvent rather then D_2O or H_2O/D_2O mixtures. This eliminates the need for pD/pH corrections and makes the pK_a values obtained comparable and compatible with values derived from potentiometric measurements performed in H_2O.
7. An NMR procedure should be selected, and described clearly, that will minimize possible sample heating (e.g., ^{1}H-coupled NMR, GD-technique, etc.) and provide confidence in temperature control.
8. The calculation of pK_a requires the chemical shift value for the free ligand L (δ_L) and for the protonated species HL (δ_{HL}) along with a number of intermediate experimental values. This is seldom possible for high (low) pH range. In those cases where the δ_{HL} or δ_L value is not available due to ionic strength (and pH) limitations, it should be derived either directly from higher ionic strength measurements (for ionic strength-independent resonance) or by an extrapolation of high ionic strength values to the lower *I* used in the experiment (for ionic strength-dependent resonance).

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