DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS
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UNCERTAINTY ESTIMATION OF POTENTIOMETRICALLY MEASURED pH AND pK_a VALUES

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LIST OF ORIGINAL PUBLICATIONS

This thesis consists of three articles listed below and a review. The articles are referred in the text by Roman numerals I–III. The review summarizes and supplements the articles.

I. Leito, I., Strauss, L., Koort, E., Pihl, V. Estimation of uncertainty in routine pH measurement. *Accred Qual Assur* (2002) 7:242–249. DOI: 10.1007/s00769-002-0470-2
II. Koort, E., Herodes K., Pihl V., Leito I. Estimation of uncertainty in pK_a values determined by potentiometric titration. *Anal Bioanal Chem* (2004) 379: 720–729. DOI: 10.1007/s00216-004-2586-1
III. Koort, E., Herodes K., Gans P., Pihl V., Leito I. Acidity Constant in different media I=0 and I=0.1M KCl: from the Uncertainty Perspective. *Accepted for publication in Anal Bioanal Chem on 28.04.2006.*

Author's contribution

Paper I: One of the two main contributors to writing the text. Performed all calculations and part of the experimental work.

Paper II: Main person responsible for planning and writing. Performed all experimental work.

Paper III: Main person responsible for planning and writing. Performed all experimental work.

ABBREVIATIONS

pK_a	Negative common logarithm of the acidic dissociation constant. The simple notation is used when no distinction is necessary between the standard states and in section 3 to denote the pK_a value having zero ionic strength as the standard state.
pK_a(I=0)	pK_a value having zero ionic strength as the standard state (also known as the thermodynamic pK_a value)
pK_a(I=0.1M KCl)	pK_a value having 0.1 M potassium chloride solution as the standard state (also known as the concentrational pK_a value)
EMF	Electromotive Force
ESM	Electronic Supplementary Information
ISO	International Organization for Standardization
GUM	The Guide to the Expression of Uncertainty in Measurement
LJP	liquid junction potential

INTRODUCTION

In recent years the quality of results of chemical measurements – Metrology in Chemistry (traceability of results, measurement uncertainty, etc) – has become an increasingly important topic. It is reflected by the growing number of publications, conferences, etc.[1–4] One of the main points that is now widely recognized is that every measurement result should be accompanied by an estimate of uncertainty – a parameter of the result that characterizes the dispersion of the values that could reasonably be attributed to the measurand.[2,3]

One of the most widespread measurements carried out by analytical laboratories is determination of pH. Huge amount of work has been published on pH measurement[5–9] including the assessment of uncertainty[10,11] and traceability[12] of pH measurements. The methods for uncertainty estimation that have been published, however, are applicable mostly to high-level pH measurements,[8,11] not to the routine laboratory measurement. Estimation of uncertainty of pH is very important when estimating uncertainties of many other physicochemical quantities (pK_a values, complexation constants, etc.) that depend on pH. Methodologically the pH measurement is not easy: pH is a highly unstable parameter. Thus, preparation of reference solutions sufficiently stable for an extended period is difficult.

The dissociation constant K_a or its negative logarithm pK_a value is one of the most important physicochemical characteristics of a compound which has acidic (or basic) properties. Reliable pK_a data are indispensable in analytical chemistry, biochemistry, chemical technology, etc.

A huge amount of pK_a data has been reported in the literature and collected into several compilations.[13–16] There is one additional aspect that is stressed less often than it should be: in broad terms the pK_a data that are available are divided into two categories:

1) pK_a values having infinite dilution as the standard state. Sometimes these are also called thermodynamic pK_a values. Their measurement involves either extrapolation of the values found at finite ionic strengths to zero ionic strength or (more often) calculation of the activity coefficients using some model approach, such as the Debye-Hückel theory,[5] and expressing the pK_a via activities. In this work these values are denoted as pK_a (I=0).
2) pK_a values having 0.1 M KCl solution as the standard state. These are determined in solutions containing 0.1 M KCl background electrolyte that generates constant and sufficiently high ionic strength to the solution. Sometimes these are called concentrational pK_a values as opposed to thermodynamic ones. In reality they are also thermodynamic, but with a standard state that is not infinite dilution but 0.1 M KCl solution. In this work these are denoted as pK_a (I=0.1M KCl)

Both types of pK_a values have their advantages and ample data on both is available in the literature and most importantly – these values cannot be directly compared to each other. It is unfortunate that the same symbol pK_a is used for both values. Hence it is sometimes difficult to distinguish which of the two has been used.

Potentiometric titration methods using the glass electrode is the most widely used group of methods for pK_a determination. Numerous methodologies have been described, starting from those described in the classic books [17–18] and finishing with the modern computational approaches (for example MINIPOT[19], PHCONST[19], HYPERQUAD[20], SUPERQUAD[21], PKPOT[22], MINIGLASS[23] etc) for calculation and refinement of pK_a values from potentiometric data.

Efforts have also been devoted to investigating the sources of uncertainty of pK_a values. The various computer programs mentioned above are very useful in this respect. They can be used in the search of systematic errors, because many parameters are adjustable. Standard errors of the parameters are obtained by weighted or unweighted non-linear regression and curve-fitting.[19–26] The influence of various uncertainty sources in pH and titrant volume measurements on the accuracy of acid-base titration has been studied using logarithmic approximation functions by Kropotov.[27] The uncertainty of titration equivalence point (predict values and detect systematic errors) was investigated by a graphical method using spreadsheets by Schwartz.[28] Gran plots can also be used to determine titration equivalence point[29] and they are useful to assess the extent of carbonate contamination in the alkaline titrant. The various sources of uncertainty have thus been investigated quite extensively.

However, what seems to be almost missing from the literature is such approach whereby all uncertainty sources of pH and pK_a value are taken into account and propagated (using the corresponding mathematical model) to give the combined uncertainty, which takes simultaneously into account the uncertainty contributions from all the uncertainty sources. This combined uncertainty, which is obtained as a result, is a range where the true value remains with a stated level of confidence. In addition, the full uncertainty budget gives a powerful tool for finding the bottlenecks and for optimizing the measurement procedure because it shows what the most important uncertainty sources are.

The aims of this work were:

1. To systematically study the sources of uncertainty in pH measurement using the most widespread potentiometric equipment (glass electrode and reference electrode with liquid junction) and to develop a generally applicable procedure for calculating the uncertainty of such pH measurement based on ISO GUM.[2]
2. To systematically study the sources of uncertainty in measurement of pK_a values (having I=0 and I=0.1 M KCl as the standard states) of acids using the same level of equipment and to develop generally applicable procedures for calculating the uncertainty of such pK_a measurement based on ISO GUM.

3. To compare the uncertainty budgets of the pK_a values having I=0 and I=0.1 M KCl as the standard states.

The uncertainty estimation procedures are based on mathematical models of pH and pK_a measurements and involve identification and quantification of individual uncertainty sources according to the ISO GUM approach,[2] that was subsequently adapted by EURACHEM and CITAC for chemical measurements.[3] Two different software packages – MS Excel and GUM Workbench are used in order to realize the uncertainty calculations.

1. CONCEPTS

1.1. pH and calibration in hydrogen ion activity scale

1.1.1. The definition of pH

The pH is from fundamental point of view defined as the relative activity of hydrogen ions in solutions:

$$\mathrm{pH} = -\log a_{\mathrm{H}^+} = -\log(a_{\mathrm{H}^+} / a_{\mathrm{H}^+}{}^0) \qquad (1)$$

where a_{H+} is the relative (molarity basis) activity of the solvated hydrogen ion and $a_{H+}{}^0$ is the hydrogen ion activity 1 mol/L. The quantity pH is intended to be a measure of the activity of hydrogen ions in solution. However, since it is defined in terms of single ion activity – a quantity that cannot be measured by a thermodynamically valid method, without involving extra-thermodynamic assumptions – eq 1 can be only a *notional definition* of pH. For practical measurements pH is defined via a primary method of measurement which is EMF measurement using the Harned cell according to a pre-defined procedure, described in ref 9.

1.1.2. Traceability and pH standards

pH by itself is not measured in terms of a fundamental (or base) unit of any measurement system. It is however possible to link pH to the SI system if pH measurement results can be traced to measurement results obtained by a "primary measurement method". The accepted definition of a primary measurement procedure is given in VIM (*International vocabulary of basic and general terms in metrology*).[30] The essential feature of such a method is that its physical basis of operation must be fully understood and that it must be possible to model the procedure by a well-defined measurement equation in which all of the variables can be determined experimentally in terms of SI units. Any limitation in the determination of the experimental variables, or in the theory, must be included within the estimated uncertainty of the method if traceability to the SI is to be established. It has been shown[9] that measurement of electromotive force of the Harned cell fulfils the definition of a primary method for the measurement of the acidity function, $p(a_H\gamma_{Cl})$, and subsequently of the pH of a buffer solution.
The cell without transference using the hydrogen gas electrode defined by

$$\mathrm{Pt} \mid \mathrm{H_2} \mid \text{buffer S}, \mathrm{Cl^-} \mid \mathrm{AgCl} \mid \mathrm{Ag} \qquad \text{Cell I}$$

is known as the *Harned cell*.[9] For primary measurement of pH by the Harned cell the solution of unknown pH is mixed with different quantities of potassium

chloride in the cell and the electromotive force of the cell containing such mixtures is measured. From the data of the measurements with different amount of KCl added the quantity $p(a_H\gamma_{Cl})$ is calculated as follows:

$$p(a_H\gamma_{Cl}) = -\log(a_H\gamma_{Cl}) = (E_I - E_0)/[(RT/F)\ln 10] + \log(m_{Cl}/m^\circ) \qquad (2)$$

The $p(a_H\gamma_{Cl})$ is extrapolated to zero chloride molality and the activity coefficient of chloride ion γ_{Cl} is found according to the Bates-Guggenheim convention by the Debye-Hückel theory.[9] The $p(a_H)$ of the unknown solution found this way approaches the true negative common logarithm of hydrogen ion activity. Detailed explanations can be found in ref 9.

The primary measurements using the Harned cell are time-consuming and require high competence. They are therefore not practical as a routine method. It is instead common to use the Harned cell for assigning pH values to primary standard solutions. The primary standard solutions are thereafter used to assign pH values to secondary standard solutions by comparison measurement. The latter are used for calibration of routine pH measurement equipment in everyday pH measurements. Although several designs of experimental setup are available for comparing pH values of two solutions, none of these can be considered as primary since they all involve liquid junctions. This invokes liquid junction potential contribution to the measured potential difference. The liquid junction potentials (LJPs) vary with the composition of the solutions forming the junction (e.g. with pH), hence the liquid junction potential will differ if one solution is replaced by another. The LJPs are also affected by the geometry of the liquid junction device. Hence, the measurement equation contain terms that are not quantifiable and the methods are secondary. These methods involve cells that are practically more convenient than measurements with Harned cell, but have greater uncertainties associated with the results.

This hierarchical approach to primary and secondary measurements facilitates the availability of traceable buffers for calibrations in laboratory.

1.1.3. pH measurements in laboratory

In practice, the glass electrode cells (Cell II) are the most common:

Reference electrode | KCl ($c \geq 3.5$mol dm^{-3}) ‖ solution (pH_x) | glass electrode Cell II

Nowadays the glass electrode and the reference electrode are very often designed as a single probe (so-called combination electrode). The potential difference E_x consists of the potentials of the glass and reference electrodes, E_{glass} and E_{ref}, and the liquid junction potential, E_j. It is expressed as follows:

$$E_x = E_{glass} - E_{ref} + E_j \qquad (3)$$

pH measurement is often considered a simple measurement but in reality it is quite complex as many effects are operational:

- The slope of the glass electrodes is often lower than the theoretical value derived from the Nernst equation $k = (RT/F)\cdot\ln 10$. This effect is called sub-Nernstian response and instead of the k the so-called practical slope k' is used, which is determined experimentally.
- The k' may vary with time. The variation is dependent on the samples measured and the storage conditions. It is thus necessary to recalibrate the electrode. The frequency of recalibration depends on the desired accuracy and the extent of variation.
- The potential of the glass electrode E_{glass} is influenced by temperature. Temperature should therefore be controlled when calibrating the system or making measurements. If the temperature of the sample is different from the calibration temperature this should be taken into account. Modern pH meters normally have temperature sensors and enable to correct for this temperature automatically.
- The E_j depends on many parameters of the system: ionic composition of the measured solution, liquid junction geometry, etc (see section 1.1.6). In addition it is usually influenced by hydrodynamic effects e.g. stirring. The stirring effect is in fact another manifestation of the liquid junction potential. E_j is dependent on stirring because stirring speed influences the shape of the diffusion gradient and thus the E_j value.

Most of these effects have unknown magnitude but the magnitude can be estimated and taken into account in the uncertainty budget.

There are three different calibration procedures in common use: single-point calibration, two-point calibration and multi-point calibration. Two- and multi-point calibration approaches are mostly used in every-day measurements. Single-point calibration assumes use of the theoretical slope and gives thus results of very low accuracy and can only be used for the simplest indicative measurements. In this work the two-point and multi-point calibrations are used.

1.1.4. Two-point calibration

In the majority of practical applications (for routine pH measurements), glass electrode cells (Cell II) are calibrated by two-point calibration (also known as bracketing) procedure using two standard buffer solutions, with pH values pH_1 and pH_2 that bracket the unknown pH_x. Bracketing is sometimes assumed to mean that the pH values pH_1 and pH_2 of the buffers selected should be such that they are immediately above and below pH_x. This may not be appropriate in all situations and choice of a wider range may be better, especially if measurement over a wider range is desired.

The dependence of the potential of the electrode system E on the pH of the measured solution is described by the Nernst equation:

$$E = E_0 - \frac{RT}{nF} \log a_{H^+} \tag{4}$$

where E_0 is called the standard electrode potential, R is the gas constant, T the temperature/K, F the Faraday constant, n = 1 in the case of hydrogen ions and log stands for logarithm to base 10. The Nernst equation shows the electrode response to be linear in hydrogen ion activity as indicated by a_{H+}.

In practice various more specialized equations, based on the Nernst equation, are used. In this work the most convenient is the one that includes the coordinates of the isopotential point and the slope:[5,6]

$$E_x = E_{is} - s \cdot (1 + \alpha \cdot \Delta t)(pH_x - pH_{is}) \tag{5}$$

E_x is the electromotive force (EMF) of the electrode system, pH_x is the pH of the measured solution, E_{is} and pH_{is} are the coordinates of the isopotential point (the intersection point of calibration lines at different temperatures), s is the slope of the calibration line, α is the temperature coefficient of the slope[6] and Δt is the difference between the measurement temperature and the calibration temperature. When two-point calibration is used then the isopotential pH and the slope can be expressed as follows:

$$pH_{is} = pH_1 + \frac{E_1 - E_{is}}{s} \tag{6}$$

$$s = \frac{E_2 - E_1}{pH_1 - pH_2} \tag{7}$$

pH_1 and pH_2 are the pH values of the standard solutions used for calibrating the pH meter. E_1 and E_2 are the emf of the standard solutions.

Based on equation 5, the pH of an unknown solution pH_x is expressed as follows:

$$pH_x = \frac{E_{is} - E_x}{s \cdot (1 + \alpha \cdot \Delta t)} + pH_{is} \tag{8}$$

1.1.5. Multipoint-calibration

Normally up to five buffer solutions are used in multi-point calibration. Use of more than five buffer solutions does not decrease the uncertainty noticeably[9].

In this work two different forms of the Nernst equation, with and without the isopotential point, are used:

$$E_x = E_{is} - s \cdot (1 + \alpha \cdot (t_{meas} - t_{cal})) \cdot (pH_x - pH_{is}) \quad (9)$$

$$E_x = E_0 - s \cdot (1 + \alpha \cdot (t_{meas} - t_{cal})) \cdot pH_x \quad (10)$$

where E_x is the electromotive force (EMF) of the electrode system in the measured solution, pH_x is the pH of the measured solution, E_{is} and pH_{is} are the coordinates of the isopotential point of the electrode system (the intersection point of calibration lines at different temperatures), E_0 is the standard potential of the electrode system, s is the slope of the calibration line, α is the temperature coefficient of the slope[6], t_{meas} and t_{cal} are the measurement temperature and the calibration temperature, respectively. The slope s and the isopotential pH_{is} are found by calibrating the system using standard solutions with known pH values.

Based on equations 9 and 10, the pH of an unknown solution pH_x is expressed respectively as follows:

$$pH_x = \frac{E_x - E_{is}}{s \cdot (1 + \alpha \cdot (t_{meas} - t_{cal}))} + pH_{is} \quad (11)$$

$$pH_x = \frac{E_x - E_0}{s(1 + \alpha(t_{meas} - t_{cal}))} \quad (12)$$

1.1.6. Liquid junction potential

The liquid junction potential is caused by the separation of two solutions by a boundary (permeable membrane) which allows the permeation of ions but at the same time prevents the solutions from mixing. The liquid junction of the reference electrode enables the electronic contact between the reference electrolyte and the test solution. The liquid junction has to be chemically inert and have a low rate of leaking the electrolyte into the measured solution. Also, it has to have a low resistance to ion mobility. If there is a concentration difference in the solutions separated by the junction, then the ions diffuse from the more concentrated solution to the less concentrated solution. The liquid junction potential (or diffusion potential) is formed across the membrane because different ions have different mobilities.

The liquid junction potential has to remain constant when the calibration solution is replaced by the unknown solution if accurate pH measurement is required. In real situation this cannot be fully achieved, especially at low and high pH values. The liquid junction potential is the higher the higher are the mobilities of the ions in the solutions. The highest hydrogen and hydroxyl have the highest mobilities and therefore the liquid junction is especially problematic for pH measurements outside the pH range 3 to 11, where the diffusion potential may be appreciably different from that in the standard buffer solutions.

The difference, or the so-called residual liquid-junction potential is then quite high and causes an error in the pH measurement. A possibility for estimating the residual liquid junction potential is compare the pH values obtained for the same solution using measurements made by the primary method and using the system with liquid junction.

1.2. pH_c and calibration in hydrogen ion concentration scale

1.2.1. pH_c

As mentioned above, the quantity pH, defined as $-\log a_{H+}$ (see eq 1), is not an explicitly measurable quantity, because it is not possible to determine the activity of a single positive or negative ion. However, it is possible to measure the concentration of a single ion – concentration of hydrogen ion $[H^+]$ in this case. This is possible if the ionic strength is kept constant by using an inert supporting electrolyte. This way the activity coefficients are essentially constant and the relationship between the potential of the electrode system and $\log[H^+]$ is linear. The linearity holds also for systems with liquid junction if the supporting electrolyte remains as the dominant ionic conductor. The relationship between measured EMF and hydrogen ion concentration is given by modified Nernst equation:

$$E = E_0 + s \cdot \log[H^+] \tag{13}$$

where E is a measured electrode potential, E_0 and s are the standard electrode potential and slope and $[H^+]$ represents the hydrogen ion concentration. This equation is usable only in the range $\log[H^+] = 2 \,..\, 12$ where junction potential is negligible.

In order to make clear the distinction between pH and $\log[H^+]$, in this work pH_c is used to denote the negative common logarithm of hydrogen ion concentration. The appropriate form of Nernst equation for pH_c measurement is expressed as follows:

$$\mathrm{pH_c} = \frac{E_x - E_0}{s(1 + \alpha(t_{meas} - t_{cal}))} \tag{14}$$

where α is the temperature coefficient of the slope[6]

1.2.2. Calibration in terms of hydrogen ion concentration

The most reliable sources of accurate concentrations of hydrogen ions are strong acids and bases. Therefore calibration of the glass electrode in terms of $\log[H^+]$ is usually carried out by a titration of strong acid with strong base.[31] Both solutions are prepared such a way that the concentration of the supporting electrolyte (normally KCl) in the solutions is 0.1M. Titration is carried out and a plot of meter reading (mV) vs. pH_c is used as calibration curve. The calibration parameters are found by least squares minimization to fit the modified Nernst eq 13. The pH_c values used for calibration is restricted to approximately the following ranges 2.5–4.0 and 10.7–11.3. These ranges are chosen taking into account the following:

1. At low pH value the liquid junction potentials cause the graph to deviate from linearity because the supporting electrolyte fails to be the main conductor when high concentration of hydrogen ions is present in the solution.
2. Near the equivalence-point region of the titration curve the calculation of the hydrogen ion concentration is unreliable because of the negligible buffer capacity of the solution.
3. At high pH values the supporting electrolyte fails to be the main conductor due to the highly mobile hydroxyl ions and in addition the so-called "alkaline error" of the glass electrode will come into operation.

1.3. pK_a measurements

1.3.1. pK_a values having zero ionic strength as the standard state

The dissociation of a Brønsted acid HA in aqueous medium refers to the simplified equation:

$$HA \rightleftarrows H^+ + A^- \tag{15}$$

This equilibrium is described by the equilibrium constant K_a and its negative common logarithm pK_a as follows:

$$K_a = \frac{a(H^+) \cdot a(A^-)}{a(HA)}, \tag{16}$$

$$pK_a = -\log K_a \tag{17}$$

1.3.2. pK_a values having 0.1 M KCl as the standard state

pK_a values having 0.1 M KCl as the standard state, denoted as pK_a (I=0.1M KCl), are determined in solutions containing 0.1 M KCl background electrolyte. The equilibrium constant K_a (I=0.1M KCl) and its negative decadic logarithm pK_a(I=0.1M KCl) are expressed in terms of ion concentrations as follows:

$$K_a(\mathrm{I}=0.1\mathrm{M\ KCl}) = \frac{[\mathrm{H}^+]\cdot[\mathrm{A}^-]}{[\mathrm{HA}]} \tag{18}$$

$$\mathrm{p}K_a(\mathrm{I}=0.1\mathrm{M\ KCl}) = -\log K_a(\mathrm{I}=0.1\mathrm{M\ KCl}) \tag{19}$$

where $[H^+]$, $[A^-]$ and [HA] are the concentrations of the hydrogen ion, the anion and the undissociated acid molecules, respectively.

1.3.3. Experimental methods for pK_a determination

1.3.3.1. Potentiometric titration

The method consists of titration of a given amount of an acid HA solution of known concentration with a solution of strong base MOH (M can be Na or K) with known concentration. From the potentiometric pH measurements and the amounts and concentrations of the solutions the $a(H^+)$ and the ratio $a(A^-)/a(HA)$ can be calculated and a K_a (and pK_a) value can be calculated for every point of the titration curve.

The pK_a value for that titration point is calculated as follows:

$$\mathrm{p}K_a = \mathrm{pH}_x - \log\frac{[\mathrm{A}^-]\cdot f_1}{C_a - [\mathrm{A}^-]} \tag{20}$$

where

$$\log f_1 = \frac{-A\cdot\sqrt{I}}{1+B\cdot a\cdot\sqrt{I}} \tag{21}$$

C_a is the total concentration of the acid HA in the titration cell, $[A^-]$ is the equilibrium concentration of the anion A^- and f_1 is the activity coefficient for singly charged ions (found from the Debye-Hückel theory)[5], where A and B are constants, a is the mean distance of closest approach of the ions (ion size parameter) and I is the ionic strength of the solution. pH_x is the pH value at a given titration point, calculated according to eq 11 or 12.

For pK_a values having 0.1 M KCl as the standard state the pK_a(I=0.1M KCl) value for that titration point is calculated as follows:

$$pK_a(I = 0.1M\ KCl) = pH_c - \log\frac{[A^-]}{C_a - [A^-]} \quad (22)$$

C_a is the total concentration of the acid HA in the titration cell, $[A^-]$ is the concentration of the anion and pH_c is negative common logarithm of hydrogen ion concentration, calculated according to eq 14.

1.3.3.2. Other methods for determination of pK_a values

Conductometric method bases on evaluation of the limiting equivalent conductivity of an appreciably dissociated electrolyte from the dependence of equivalent conductivity from concentration. These measurements are generally made with very dilute solutions. The method is best for acids with pK_a values between 1.9 and 5.2. In practice, this method is more laborious than the potentiometric method, and more calculations are needed.

UV-VIS spectrophotometry is used to study the dissociation of weak electrolytes whose molecular and ionized species have sufficiently different absorptivities at some experimentally suitable wavelength (generally above 220 nm). Spectrophotometry has advantages with compounds that have limited solubility (so that only very dilute solutions can be used), or that have either very low or very high pK_a value.

Several other experimental methods have found application, for example Raman and magnetic resonance spectroscopy. They have been used mainly for pK_a values lying outside the range 2–11.

1.4. Uncertainty

1.4.1. Terms and Definitions

According to the ISO GUM (The Guide to the Expression of Uncertainty in Measurement)[2], the *uncertainty* is a parameter associated with the result of measurement that characterizes the dispersion of the values that could be reasonably be attributed to the measurand.

$$Result = Value \pm Uncertainty$$

If the uncertainty is expressed at standard deviation level (that is the uncertainty limits are estimated to encompass 68% of the possible values of the result) then it is termed *standard uncertainty*.

The model of the measurement procedure is a functional relation between directly measured input quantities X_1 .. X_n and the output quantity Y (measurand)

$$Y = f(X_1, X_2, ..., X_n)$$

The uncertainty estimates of the input quantities can be obtained in the course of the current measurement (Type A, *statistical analysis of series of observations*) or brought into the measurement from external sources (Type B, *previous experiments, literature data, information from manufacturer*).

The estimated standard deviation associated with the measurement result (output quantity) that takes into account the uncertainty contributions from all input quantities is termed *combined standard uncertainty*. It is determined from the estimated standard uncertainties of the input quantities.

In the case of uncorrelated input quantities the combined standard uncertainty $u_c(y)$ of a value y is calculated using the following equation[2]:

$$u_c(y) = \sqrt{\left(\frac{\partial y}{\partial x_1}u(x_1)\right)^2 + \left(\frac{\partial y}{\partial x_2}u(x_2)\right)^2 + \cdots\cdots + \left(\frac{\partial y}{\partial x_i}u(x_i)\right)^2} \qquad (23)$$

where $\partial y/\partial x_i$ is the partial differential of y with respect to x_i (sensitivity coefficient). This equation can be used if all the input quantities are statistically independent.

If there is correlation between some of the input quantities, then the combined standard uncertainty is found as follows:

$$u_c(y) = \sqrt{\sum_{i=1,n} c_i^2 u(x_i)^2 + 2 \sum_{\substack{i,k=1,n \\ i \neq k}} c_i c_k \cdot u(x_i, x_k)} \qquad (24)$$

where the c_i are the sensitivity coefficients evaluated as $c_i = \partial y/\partial x_i$ and $u(x_i,x_k)$ is the covariance between x_i and x_k. The covariance is related to the correlation coefficient r_{ik} by:

$$u(x_i, x_k) = u(x_i) \cdot u(x_k) \cdot r_{ik} \qquad (25)$$

The *expanded uncertainty* U is obtained by multiplying the combined standard uncertainty $u_c(y)$ by a *coverage factor k:*

$$U = k\, u_c(y) \qquad (26)$$

The result of a measurement is then conveniently expressed as $Y = y \pm U$, which means that the best estimate of the value attributable to the measurand Y is y and that $y - U$ to $y + U$ is an interval that may be expected to encompass a large fraction of the distribution of values that could be reasonably attributed to Y. This interval can also be expressed as $y - U \leq Y \leq y + U$.

The coverage factor k is a numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty. The

value of coverage factor is chosen on the basis of the level of confidence required of the interval $y - U$ to $y + U$. In general, k will be in the range 2 to 3. In practice, one can assume that taking $k = 1$ produces an interval having a level of confidence of approximately 68%, k values 2 and 3 result in the level of confidence of 95% and 99% respectively.

1.4.2. Uncertainty sources in chemical measurements

Numerous uncertainty sources are operational in chemical and physicochemical measurements.[3] Below is a short commented list of them.

1. Solution preparation
   - calibration of glassware: the uncertainty in the certified internal volume of the flask, variation in filling the flask to the mark, the flask and solution temperatures differing from the temperature at which the volume of the flask was calibrated
   - weighing of the substance: repeatability of weighing, readability of the balance scale, drift of the balance, interference from electrostatic disturbances
   - Purity of the substance. For example, acids can contain some impurity acids with different dissociation constants.
2. Calibration of instruments
   - reference materials: uncertainty arising from the limited accuracy of the values assigned to the standards. The commercially available standards have their value and uncertainty in which limits the true value of the standard have to be in given confidence level. Standard values can depend on temperature and also on other environmental variables.
   - uncertainty from assuming linear response of the instrument when the response in reality is nonlinear.
3. Analysis
   - repeatability of the instrument. To a greater or lesser extent all instruments are sensitive to extraneous disturbances. These are manifested by less than ideal repeatability – differences between results of successive measurements made under identical conditions.
   - drift of the measurement system. If the time between calibration and unknown solution measurement is too long, the drift of the measurement system can take a place.
   - resolution of the instruments display
4. data treatment
   - averaging
   - how well the used calculation model corresponds to real situation

1.4.3. Uncertainty estimation according to the ISO GUM method

The uncertainty estimation procedures described in this thesis are based on mathematical models of pH and pK_a measurement and involve identification and quantification of individual uncertainty sources according to the ISO GUM/EURACHEM approach[2,3]. This approach of uncertainty estimation consists of the following steps:

1) Specifying the measurand and defining of the mathematical model. The relationship between the measurand and the input quantities (e.g. measured quantities, constants, calibration standard values etc.) upon which it depends are written down.
2) Identification of the sources of uncertainty. The possible uncertainty sources of input parameters are listed.
3) Modification of the mathematical model (if necessary).
4) Quantification of the uncertainty components. The size of the uncertainty component associated with each potential source is measured or estimated.
5) Calculating the combined uncertainty. The quantified contributions have to be expressed as standard uncertainties. In order to calculate combined uncertainty, the relative weights of input quantities are summarized according to equation 23 or 24.

Before the combined uncertainty calculation all uncertainty components are converted to the level of standard uncertainty. In this work we assume the B-type uncertainties for which no information on distribution function is available and which are expressed with the "±" sign have rectangular (uniform) distribution.[3] In order to convert them to standard uncertainties they are divided by $\sqrt{3}$.[2,3]

The uncertainty calculations in this work have been carried out using two software packages: general purpose spreadsheet calculation package MS Excel and dedicated uncertainty estimation software GUM Workbench[32,33].

The Kragten method[34] for calculation of uncertainty has been used in the MS Excel software. According to EURACHEM/CITAC guide[3] all the partial derivatives in equations 23 and 24 are approximated as follows:

$$\frac{\partial y}{\partial x_i} \approx \frac{y(x_i + \Delta x_i) - y(x_i)}{\Delta x_i} \tag{27}$$

where $y = f(x_1,x_2,\ldots,x_n)$ is the output quantity, x_i is the i-th input quantity and Δx_i is a small increment of x_i. In the EURACHEM/CITAC guide it is proposed to take $\Delta x_i = u(x_i)$, but in this work $\Delta x_i = u(x_i)/10$ or $\Delta x_i = u(x_i)/2$ have been used. This is safer with respect to the possible nonlinearities of the function $f(x_1, x_2,.. x_n)$. For further details of this method see ref 3.

The uncertainty contribution of an input quantity (termed x_j) in the uncertainty budgets (uncertainty index) is found according to eq 28 (see ref 2 for background info) where the sum is taken over all input quantities:

$$index(x_j) = \frac{\left(\frac{\partial y}{\partial x_j} u(x_j)\right)^2}{\sum_i \left(\frac{\partial y}{\partial x_i} u(x_i)\right)^2} \cdot 100\% \qquad (28)$$

The effective number of degrees of freedom (df) of the results was found using the GUM Workbench software that utilizes the modified Welch-Satterthwaite approach.[2,33]

2. UNCERTAINTY ESTIMATION PROCEDURE FOR ROUTINE pH MEASUREMENT

2.1. Introduction

In this section uncertainty estimation procedure for the mainstream routine pH measurement equipment is presented. The procedure is applied to a real-life measurement and the obtained uncertainty budget is used for discussing the contributions of various uncertainty sources.

The mainstream pH measurement equipment is an electrode system consisting of a glass electrode and reference electrode (or a combined electrode) with liquid junction, connected to a digital pH-meter with two-point calibration (bracketing calibration). The system may or may not have temperature sensor for automatic temperature compensation. This procedure is valid for measurements in solutions that are neither too acidic nor too basic ($2 < \mathrm{pH} < 12$) and do not have too high ionic strength.

2.2. Mathematical model

The mathematical model is derived based on equations 6–8 and can be expressed in the following form:

$$\mathrm{pH_x} = \frac{(E_{\mathrm{is}} - E_{\mathrm{x}}) \cdot (\mathrm{pH_1} - \mathrm{pH_2})}{(E_2 - E_1) \cdot (1 + \alpha \cdot \Delta t)} + \frac{E_1 - E_{\mathrm{is}}}{E_2 - E_1}(\mathrm{pH_1} - \mathrm{pH_2}) + \mathrm{pH_1} + \delta\mathrm{pH_{xm}} \qquad (29)$$

The additional term $\delta\mathrm{pH_{xm}}$ is introduced to allow to take into account the existence of the following implicit sources of uncertainty: readability of the pH meter display, repeatability of pH measurement and drift of the pH meter characteristics between calibration and measurement (see publication I for details). It is defined in such a way that $\delta\mathrm{pH_{xm}} = 0$. Therefore its introduction does not influence the value of $\mathrm{pH_x}$, but its uncertainty $u(\delta\mathrm{pH_{xm}})$ does influence the standard uncertainty $u_{\mathrm{c}}(\mathrm{pH_x})$.

Detailed description of definition of the mathematical model, description of the setup, identification and quantification of the uncertainty sources and calculation of combined uncertainty is given in publication I and in ESM of publication I available from http://dx.doi.org/10.1007/s00769-002-0470-2

2.3. Application example

The derived uncertainty estimation procedure is applied to a routine pH measurement example. Below the measurement conditions are described.

General. Both calibration and measurement were carried out on the same day at 25 ± 3°C. In this example the temperature sensor was not connected and the temperature of the pH meter was set to 25°C. The system was calibrated using the pH 4.00 and 10.00 standard solutions. The emf values were 180 and –168 mV, respectively. pH value was measured in a solution (a 0.05 M phosphate buffer solution), for which the emf of the electrode system was –24 mV and the pH value was 7.52. The reading was considered stable if during 30 s (for measurement) or 60 s (for calibration) there was no change. Both measurement and calibration were done without stirring (the solution was stirred just enough to mix it and then the stirring was stopped).

pH meter. Metrohm 744 pH meter was used in this study. The meter has digital display with resolution of 0.01 units in the pH measurement mode. The meter can be calibrated using two-point calibration with one out of 5 buffer series stored in the memory of the meter. The pH values of the buffer series are stored at various temperatures. If the temperature sensor is connected then the meter automatically uses the correct pH corresponding to the temperature of calibration. If no temperature sensor is connected then the user can input the temperature (default is 25°C). If the temperature sensor is connected and the measurement temperature is different from the calibration temperature then correction is automatically applied to the slope. The theoretical value 0.00335 K^{-1} (at 25°C) for the temperature coefficient α is used.[6] For the E_{is} the pH meter uses value of 0 mV. This value cannot be adjusted with this type of pH meter. However, this is a reasonable average value for Metrohm combined pH electrodes (see below the description of the electrode system). The error limits of the meter are ± 1 mV in the mV mode and ± 0.01 pH units in the pH mode. The error limits in temperature measurement are ± 1°C. No data on the drift is given in the manual.

Electrode system. Combined glass electrode Metrohm 6.0228.000 was used. The inner reference electrode is Ag/AgCl electrode in 3 M KCl solution with porous liquid junction. The electrode has a built-in Pt1000 temperature sensor. This electrode has sodium error starting from pH values around 12. The E_{is} for this electrode is 0 ± 15 mV.

Calibration. Fisher buffer solutions with pH 4.00 ± 0.02, 7.00 ± 0.02 and 10.00 ± 0.02 were used (pH values are given at 25°C) as calibration standards. At 25°C the pH of these standard solutions have the temperature dependence 0.001, 0.002 and 0.01 pH units per centigrade, respectively. The calibration of the system was carried out daily.

2.4. Results and Discussion

The uncertainty budgets are presented in Table 1 (calibration with pH 4.00 and pH 10.00) and Table 2 (calibration with 4.00 and 7.00).

The overall expanded uncertainty $U(pH_x)$ = 0.054 (uncertainties with 3 decimal places has been used in order to detect small differences in uncertainty introduced by modifications of the experimental procedure) is primarily determined by the uncertainty contributions originating from the operation of pH measurement of the unknown solution δpH_{xm} (mainly the drift component), the residual junction potential and the large temperature effect of the pH 10.00 standard solution (Table 1, second row). Indeed, when taking into account only these contributions the uncertainty would be $U(pH_x)$ = 0.047.

Now the influence of modifying various parameters of the measurement procedure on the uncertainty with the aid of the model (eq 29) is explored. At first the focus is on the calibration standards set – pH 4.00 and 10.00. The 4.00 and 7.00 set will be considered afterwards. Calculation worksheets of all the uncertainty budgets discussed here are available in ESM publication I (files 4_and_10.xls and 4_and_7.xls, in MS Excel 97 format).

The effect of the temperature compensation. The pH meter used has the possibility to connect temperature sensor and to automatically take into account the measurement temperature and its difference from the calibration temperature (temperature compensation). This temperature compensation works twofold: 1) It ensures that during the calibration the pH values of the buffer solutions are used that exactly correspond to the actual temperature of the solution; 2) During the measurement of the unknown solution the slope of the electrode system is corrected to correspond to the temperature of the solution.
Taking into account the uncertainty of the temperature measurement ± 0.1°C, with temperature compensation $U(pH_x)$ = 0.049 (Table 1, column 3). This improvement is small but the pH 7.52 is well in the middle of the calibration line and near the isopotential point (according to the data: pH_{is} = 7.10). It is reasonable to expect that the uncertainties due to the temperature will be the higher the more the pH_x is removed from the isopotential point. This is indeed so. The trend is visualized in Fig 1. It is clearly seen that the further away is the pH from pH_{is} the more advantageous it is to use temperature compensation.

Table 1. The uncertainty budgets of pH measurement under various conditions. Standard solutions with pH 4.00 and 10.00 were used for calibration.

	Conditions[a]										
pH_x	**7.52**	**7.52**	**10.55**	**10.55**	**3.48**	**3.48**	**7.52**	**10.55**	**7.52**	**10.55**	**3.48**
Δt	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**3**	**35**	**35**	**35**
TS	**no**	**yes**	**no**	**yes**	**no**	**yes**	**yes**	**yes**	**yes**	**yes**	**yes**
x_i[b]	**Uncertainty budgets (contributions of various input parameters x_i: $(\partial pH_x/\partial x_i)\cdot u(x_i)$[b])**										
pH_1	0.005	0.005	–0.001	–0.001	0.013	0.013	0.005	–0.001	0.005	–0.001	0.013
pH_2	0.012	0.007	0.023	0.013	–0.002	–0.001	0.007	0.013	0.007	0.013	–0.001
E_1	0.011	0.011	–0.003	–0.003	0.030	0.030	0.011	–0.003	0.011	–0.003	0.030
E_2	0.016	0.016	0.030	0.030	–0.002	–0.002	0.016	0.030	0.016	0.030	–0.002
δpH_{xm}	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012
E_{is}	0.000	0.000	0.000	0.000	0.000	0.000	–0.001	–0.001	–0.016	–0.016	–0.016
α	0.000	0.000	0.000	0.000	0.000	0.000	–0.001	–0.005	–0.007	–0.057	0.060
Δt	–0.003	0.000	–0.028	–0.001	0.030	0.001	0.000	–0.001	0.000	–0.001	0.001
	Expanded uncertainties (k = 2) of pH_x										
$U(pH_x)$	0.054	0.049	0.098	0.070	0.092	0.070	0.049	0.071	0.060	0.138	0.142

[a] The calibration temperature is 25°C, Δt is the temperature difference between the measurement and calibration temperatures. TS = yes means that temperature sensor is connected and automatic temperature compensation used, TS = no means that automatic temperature compensation is not used and the pH meter assumes 25°C for both calibration and measurement. [b] x_i is the i-th input quantity. See equation 29 in this work and eq 13 in publication I.

Table 2. The uncertainty budgets of pH measurement under various conditions. Standard solutions with pH 4.00 and 7.00 were used for calibration.

	Conditions[a]										
pH_x	**7.52**	**7.52**	**10.55**	**10.55**	**3.48**	**3.48**	**7.52**	**10.55**	**7.52**	**10.55**	**3.48**
Δt	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**3**	**35**	**35**	**35**
TS	**no**	**yes**	**no**	**yes**	**no**	**yes**	**yes**	**yes**	**yes**	**yes**	**yes**
x_i[b]	**Uncertainty budgets (contributions of various input parameters x_i: $(\partial pH_x/\partial x_i)\cdot u(x_i)^b$)**										
pH_1	–0.002	–0.002	–0.014	–0.014	0.014	0.014	–0.002	–0.014	–0.002	–0.014	0.0136
pH_2	0.014	0.014	0.026	0.025	–0.002	–0.002	0.014	0.025	0.014	0.025	–0.0020
E_1	–0.005	–0.005	–0.033	–0.033	0.032	0.032	–0.005	–0.033	–0.005	–0.033	0.0325
E_2	0.033	0.033	0.061	0.061	–0.005	–0.005	0.033	0.061	0.033	0.061	–0.0048
δpH_{xm}	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.0119
E_{is}	0.000	0.000	0.000	0.000	0.000	0.000	–0.001	–0.001	–0.016	–0.016	–0.0157
α	0.000	0.000	0.000	0.000	0.000	0.000	–0.001	–0.005	–0.007	–0.057	0.0601
Δt	–0.003	0.000	–0.028	–0.001	0.030	0.001	0.000	–0.001	0.000	–0.001	0.0009
	Expanded uncertainties (k = 2) of pH_x										
$U(pH_x)$	0.076	0.075	0.162	0.151	0.096	0.075	0.075	0.152	0.083	0.192	0.145

[a] The calibration temperature is 25°C, Δt is the temperature difference between the measurement and calibration temperatures. TS = yes means that temperature sensor is connected and automatic temperature compensation used, TS = no means that automatic temperature compensation is not used and the pH meter assumes 25°C for both calibration and measurement. [b] x_i is the i-th input quantity. See equation 29 in this work and eq 13 in publication I.

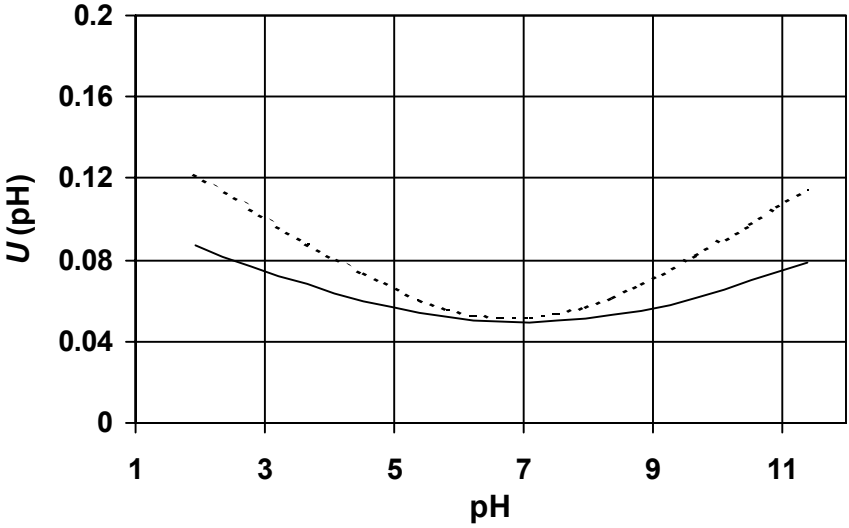

Figure 1. Dependence of the U(pH) on pH with (solid line) and without (dotted line) automatic temperature compensation. Standard solutions pH 4.00 and pH 10.00 were used for calibration.

With automatic temperature compensation the uncertainties at pH 10.55 and pH 3.48 are practically equal (Table 1, columns 5 and 7), because these pH values are about equally removed from the isopotential point. Without temperature compensation the uncertainty at 3.48 is slightly lower due to the ten times higher temperature dependence of the pH value of the pH 10.00 standard compared to the pH 4.00 standard. The main contributors to the uncertainty in the case of pH 10.55 and pH 3.48 are the $u(E_2)$ and $u(E_1)$ respectively, and $u(\Delta t)$ if no temperature compensation is used. It is also interesting to note, that although the uncertainties of α and E_{is} are large, their contribution to the overall uncertainty is negligible at $\Delta t = 0$.

As can be seen from Table 1, small differences in measurement and calibration temperature do not introduce almost any additional uncertainty if the temperature compensation is used: if calibration is carried out at 25°C and measurement at 28°C (that is, Δt = 3°C) then the increase in expanded uncertainty is not more than 0.001 (Table 1, columns 8 and 9). Things are completely different, however, if Δt is higher, and especially, if at the same time pH_x is far from pH_{is} (Table 1, last columns). Thus, if calibration is carried out at 25°C and measurement at 60°C (Δt = 35°C) then at pH 10.55 and pH 3.48 the expanded uncertainty is 0.138 and 0.142, respectively. In this case the combined uncertainty is heavily dominated by the uncertainty of α. Neglecting all other uncertainty components, $U(pH_x)$ = 0.114 and 0.120, respectively. The slightly higher uncertainty at pH 3.48 is because this pH value is slightly more distant from the pH_{is}.

The effect of the standard solution set. Other combinations of standard solutions than pH 4.00 and pH 10.00 can be used for pH meter calibration. In Table 2 and Fig 2 the changes that take place when switching to the set of pH 4.00 and pH 7.00 are explored.

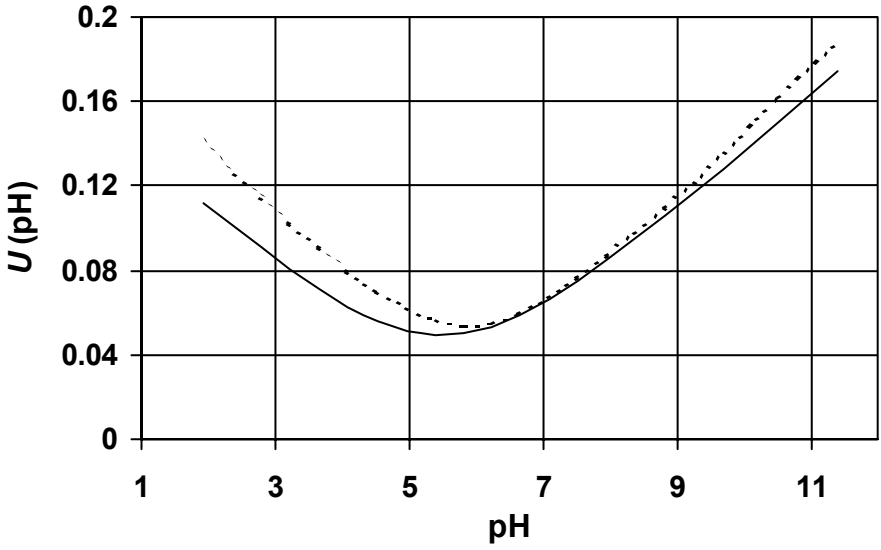

Figure 2. Dependence of the U(pH) on pH with (solid line) and without (dotted line) automatic temperature compensation. Standard solutions pH 4.00 and pH 7.00 were used for calibration.

It can be seen from Table 2 and Fig 2 that practically in all the cases (except a narrow region between pH = 5 .. 6) this leads to higher uncertainties. The effect is particularly disastrous at high pH values. Thus, at pH 10.55 if using temperature compensation the $U(\mathrm{pH_x})$ is more than twice as high as with the 4.00 and 10.00 standard set (column 5, Tables 1 and 2)

This effect is not unexpected. The calibration line is now fixed by two points that are closer to each other and therefore the line becomes less determined. In addition, at high pH values the determination of pH involves significant extrapolation. The lines for the temperature-compensated and non-compensated measurements on Fig 2 are closer in this case. This is because the temperature effect on the slope has remained the same, while the overall uncertainty is higher. Therefore the relative contribution of $u(\Delta t)$ is smaller now. This effect is especially dramatic at higher pH values where the overall uncertainty is high. The fact that the pH of the standard 7.00 is 5 times less sensitive to temperature is also a contributor.

Accuracy of the standard solutions. From Tables 1 and 2 it is apparent that with this experimental setup the uncertainty cannot be significantly reduced using more accurate standard solutions than ± 0.02 pH units. Even if the

uncertainties of the pH values of the standards were 0, the improvement in the overall uncertainty would be small. For example at pH = 10.55 the expanded uncertainties would be 0.065 instead of 0.070 and 0.094 instead of 0.098 with and without temperature compensation, respectively (Table 1, columns 5 and 4, respectively).

Limitations of the procedure. There are several additional sources of uncertainty, mostly related to the correctness of measurement, that have not been taken into account: 1) use of aged calibration buffers. The storage life of standard buffer solutions is often only a few days;[6] 2) too infrequent calibration of the system; 3) sample carryover; 4) the reading is not allowed to stabilize either during the calibration or the measurement; 5) improper handling or storage of the electrodes. Several of these (e.g. the sample carryover, which depends on the previous sample) are practically impossible to quantify with any rigor. It is therefore necessary to assure that due care is taken when measuring pH so that the above described procedure would give an adequate estimate of uncertainty of pH.

It is well known and widely recognized that the properties of the sample are very important in measurement of pH.[5] This procedure is intended for measurements with samples that are aqueous solutions with ionic strength not greater than around 0.2. Only for such solutions a quantitative meaning in terms of activity of the hydrogen ion can be ascribed to pH.[5]

Application of the Procedure to Routine Work. The presented procedure of uncertainty estimation may seem too complex for routine use. However, this is not the case. Although the procedure involves 9 input parameters and 14 components of uncertainty, it is not necessary to quantify these each time when a pH measurement is carried out, because most of them (e.g. those referring to the particular pH meter, particular electrode, etc.) will remain the same from one measurement to another.

The spreadsheet method can be proposed (like the ones in the ESM of publication I), or the GUM Workbench package for routine implementation of the procedure. This way the equipment-specific and procedure-specific components need to be quantified only once – during the method validation. Calibration data need to be input only when a new calibration is carried out. Only the E_x needs to be input separately for each measurement and when this is done, the pH and its uncertainty will be automatically calculated by the software.

2.5. Conclusions

No single uncertainty estimate can be ascribed to a pH measurement procedure. The uncertainty of pH strongly depends on changes in experimental details (standard solution set, temperature compensation, etc.) and on the pH value itself. The uncertainty is the lowest near the isopotential point (usually around pH 7) and in the center of the calibration line and can increase by a factor of 2 (depending on the details of the measurement procedure) when moving from around pH 7 to around pH 2 or 11. Therefore it is necessary to estimate the uncertainty separately for each measurement (or at least for each pH region).

At room temperature the expanded uncertainties (at $k = 2$ level) of pH values at pH 7.52 are around $U(\text{pH}) = 0.05$ either with or without automatic temperature compensation (calibrated with standards pH 4.00 and pH 10.00). At a pH value more distant from the isopotential pH the automatic temperature compensation becomes clearly advantageous: $U(\text{pH}) = 0.07$ and 0.1 with and without temperature compensation, respectively, at pH 10.55.

For routine pH measurement with an experimental setup similar to that described here the uncertainty cannot be significantly reduced by using more accurate standard solutions than ± 0.02 pH units – the uncertainty improvement is small.

A major problem in estimating the uncertainty of pH is the residual junction potential, which is almost impossible to take rigorously into account in the framework of a routine pH measurement.

3. UNCERTAINTY OF pK_a MEASUREMENT

3.1. Introduction

The uncertainty estimation procedure described in this section is intended for the mainstream routine pK_a measurement equipment: an electrode system consisting of a glass electrode and reference electrode (or a combined electrode) with liquid junction, connected to a digital pH-meter with multi-point calibration. This procedure is valid for measurements of acids that are neither too strong nor too weak. The pK_a measurement of benzoic acid was used as application example.

3.2. Mathematical model

The equations given in Table 3 form the mathematical model for pK_a measurement of the acid HA corresponding to one point of the titration curve. The main equations are 11 and 20. Table 3 contains also the full detailed list of quantities of pK_a measurement. Detailed description of derivation of the model equation and finding the uncertainty sources is given in ESM of publication II, available at http://dx.doi.org/10.1007/s00216-004-2586-1

Table 3. The uncertainty calculation of pK_a value of the acid HA corresponding to one point of the titration curve.

Estimation of Uncertainty of pKa values Determined by Potentiometric Titration

The method of pK_a determination consists in potentiometric titration (glass electrode and reference electrode with liquid junction) of a given amount V_{a0} [ml] of a solution of an acid HA of known concentration C_{a0} [mol l-1] with a solution of strong base MOH with known concentration C_{t0} [mol l-1].Below we present the procedure for calculation of the pK_{ax} value for a given point of the titration curve and estimation of the uncertainty of the pK_{ax} value.

This calculation file calculates the pK_a value and its uncertainty of the acid HA corresponding to one point of the titration curve (V_t = 0.8 ml, ie the volume of titrant added is 0.8 ml). This titration point is termed "titration point under consideration" below (point "x" in the text).

Model Equation:

{ The main equation }

$pK_{ax}=-\log((10^{(-pH_x)})*An*f_1/HA);$

$HA=V_{a0}*C_{a0}/(V_{a0}+V_t)-An;$

$An=(10^{(-pH_x)})/f_1+V_t*C_{t0}/(V_{a0}+V_t)-K_w/(10^{(-pH_x)})/f_1-C_{HCO3}-2*C_{CO3}-A_1-A_2-A_3;$

$K_w=1.008*10^{(-14+0.033*(t_{meas}-const(t_{meas})))};$

{ Concentration of the acid solution }

$C_{a0}=1000*m_a*P/V_s/M;$

$M=n_1*C+n_2*H+n_3*O;$

$m_a=m_arep+m_aread;$

$V_s=V_scal+V_stemp+V_sfill;$

$V_stemp=V_scal*\gamma*\Delta t_{Vs\_temp};$

{ Contents of impurities in the acid solution

It is assumed that the titrated acid contains some impurities. As a model approach, it is assumed that there are three

different impurities with different acidities.}

$C_{A1H\_0}=1000*m_a*P_{A1H}/V_s/M;$

$C_{A2H\_0}=1000*m_a*P_{A2H}/V_s/M;$

$C_{A3H\_0}=1000*m_a*P_{A3H}/V_s/M;$

$K_{A1H} = 10 ^ (-pK_{A1H});$

$K_{A2H} = 10 ^ (-pK_{A2H});$

$K_{A3H} = 10 ^ (-pK_{A3H});$

$A_1=C_{A1H\_0}*V_{a0}/(V_{a0}+V_t)/(1+10^{(-pH_x)}*f_1*f_1/K_{A1H});$

$A_2=C_{A2H\_0}*V_{a0}/(V_{a0}+V_t)/(1+10^{(-pH_x)}*f_1*f_1/K_{A2H});$

$A_3=C_{A3H\_0}*V_{a0}/(V_{a0}+V_t)/(1+10^{(-pH_x)}*f_1*f_1/K_{A3H})$;

{ Volume of the investigated acid solution taken for titration

The real volume at the titration point under consideration is higher due to the added titrant }

$V_{a0}=V_{a0}cal+V_{a0}temp+V_{a0}rep$;

$V_{a0}cal=V_{a0}calrep+V_{a0}caltemp$;

$V_{a0}caltemp=V_{a0}calrep*\gamma*\Delta t_{Va0\_caltemp}$;

$V_{a0}temp=V_{a0}calrep*\gamma*\Delta t_{Va0\_temp}$;

{ Volume of the titrant added to the titration cell up to the titration point under consideration }

$V_t=V_tcal+V_ttemp+V_trep$;

$V_tcal=err/vol*V_trep$;

$V_ttemp=V_trep*\gamma*\Delta t_{Vt\_temp}$;

{ Concentration of the titrant

This concentration was determined by titrating V_{at} ml of solution of potassium hydrogen phthalate}

$C_{t0}=1000*m_{at}*V_{at}*P_t*R/(V_{st}*M_t*V_{tt})$;

$M_t=n_{1t}*C+n_{2t}*H+n_{3t}*O+n_{4t}*K$;

$m_{at}=m_{at}rep+m_{at}read$;

$V_{st}=V_{st}cal+V_{st}temp+V_{st}fill$;

$V_{st}temp=V_{st}cal*\gamma*\Delta t_{Vst\_temp}$;

$V_{tt}=V_{tt}temp+V_{tt}cal+const(V_{tt}ep)$;

$V_{tt}cal=err_t/vol_t*const(V_{tt}ep)$;

$V_{tt}temp=V_{tt}ep*\gamma*\Delta t_{Vtt\_temp}$;

$R=V_{tt}ep/const(V_{tt}ep)$;

$V_{at}=V_{at}cal+V_{at}temp$;

$V_{at}cal=V_{at}calrep+V_{at}caltemp$;

$V_{at}caltemp=V_{at}calrep*\gamma*\Delta t_{Vat\_caltemp}$;

$V_{at}temp=V_{at}calrep*\gamma*\Delta t_{Vat\_temp}$;

{ Content of carbonate in the titrant }

$C_{HCO3}=C_{c0}*V_t/(V_{a0}+V_t)*K_{H2C}/f_1/10^{(-pH_x)}/(1+K_{H2C}/f_1/10^{(-pH_x)}+$

$K_{H2C}*K_{HC}/(f_1*f_2*10^{(-pH_x)}*10^{(-pH_x)}))$;

$C_{CO3}=C_{c0}*V_t/(V_{a0}+V_t)*K_{HC}*K_{H2C}/(f_1*f_2*10^{(-pH_x)}*10^{(-pH_x)})/$

$(1+K_{H2C}/f_1/10^{(-pH_x)}+K_{H2C}*K_{HC}/(f_1*f_2*10^{(-pH_x)}*10^{(-pH_x)}));$

{ Calculation of activity constant of the solution
This calculation is done via iterative approach, using three iterations.}

$I_0=C_{t0}*V_t/(V_{a0}+V_t)+10^{(-pH_x)}/f_0;$

$f_{11}=10^{(-A*sqrt(I_0)/(1+B*a*sqrt(I_0)));}$

$I_{11}=C_{t0}*V_t/(V_{a0}+V_t)+10^{(-pH_x)}/f_{11};$

$f_{12}=10^{(-A*sqrt(I_{11})/(1+B*a*sqrt(I_{11})));}$

$I_{12}=C_{t0}*V_t/(V_{a0}+V_t)+10^{(-pH_x)}/f_{12};$

$f_1=10^{(-A*sqrt(I_{12})/(1+B*a*sqrt(I_{12})));}$

$f_{21}=10^{(-A*2*sqrt(I_0)/(1+B*a*sqrt(I_0)));}$

$I_{21}=C_{t0}*V_t/(V_{a0}+V_t)+10^{(-pH_x)}/f_{21};$

$f_{22}=10^{(-A*2*sqrt(I_{21})/(1+B*a*sqrt(I_{21})));}$

$I_{22}=C_{t0}*V_t/(V_{a0}+V_t)+10^{(-pH_x)}/f_{22};$

$f_2=10^{(-A*2*sqrt(I_{22})/(1+B*a*sqrt(I_{22})));}$

{ pH of the measured solution }

$pH_x=(E_x-E_{is})/s/(1+\alpha*(t_{meas}-t_{cal}))+pH_{is};$

$E_x=E_x read+E_x drift+E_x JP+E_x rep;$

{ Calibration of pH-meter }

$\Sigma pH_iE_i=pH_1*E_1+pH_2*E_2+pH_3*E_3+pH_4*E_4+pH_5*E_5;$

$avgpH_i=(pH_1+pH_2+pH_3+pH_4+pH_5)/5;$

$avgE_i=(E_1+E_2+E_3+E_4+E_5)/5;$

$\Sigma pH_ipH_i=pH_1*pH_1+pH_2*pH_2+pH_3*pH_3+pH_4*pH_4+pH_5*pH_5;$

$s=(\Sigma pH_iE_i-5*avgpH_i*avgE_i)/(\Sigma pH_ipH_i-5*avgpH_i*avgpH_i);$

$pH_{is}=(E_{is}-(avgE_i-s*avgpH_i))/s;$

$E_1=E_1 rep+E_1 JP+E_1 read;$

$E_2=E_2 rep+E_2 JP+E_2 read;$

$E_3=E_3 rep+E_3 JP+E_3 read;$

$E_4=E_4 rep+E_4 JP+E_4 read;$

$E_5=E_5 rep+E_5 JP+E_5 read;$

$pH_1=pH_1 acc+pH_1 temp;$

$pH_2=pH_2 acc+pH_2 temp;$

$pH_3=pH_3acc+pH_3temp;$

$pH_4=pH_4acc+pH_4temp;$

$pH_5=pH_5acc+pH_5temp;$

$pH_1temp=\theta_1*(t_{cal}-const(t_{cal}));$

$pH_2temp=\theta_2*(t_{cal}-const(t_{cal}));$

$pH_3temp=\theta_3*(t_{cal}-const(t_{cal}));$

$pH_4temp=\theta_4*(t_{cal}-const(t_{cal}));$

$pH_5temp=\theta_5*(t_{cal}-const(t_{cal}));$

List of Quantities:

Quantity	Unit	Definition
pK_{ax}		acidity constant of the acid under investigation
HA	mol/l	concentration of undissociated acid molecules
An	mol/l	concentration of anion
K_w	mol/l	autoprotolysis constant of water
C_{a0}	mol/l	concentration of the acid solution
P	unitless	purity of the the acid
M	g/mol	molecular mass of the acid
n_1	unitless	number of carbon atoms in the acid molecule
C	g/mol	atomic weight of carbon
n_2	unitless	number of hydrogen atoms in the acid molecule
H	g/mol	atomic weight of hydrogen
n_3	unitless	number of oxygen atoms in the acid molecule
O	g/mol	atomic weight of oxygen
m_a	g	mass of the acid
$m_a$rep	g	value and repeatability uncertainty of the mass of the acid
$m_a$read	g	the readability (digital resolution) uncertainty of the balance scale
V_s	ml	the volume of the acid solution
$V_s$cal	ml	the value and calibration uncertainty of the volume of the acid solution
$V_s$temp	ml	the temparature uncertainty component of the volume of the acid solution
Δt_{Vs_temp}	C	the difference of calibration and usage temperatures of the flask
$V_s$fill	ml	the filling uncertainty component of the volume of the acid solution
γ	1/C	the coefficient of volume expansion of water
V_{a0}	ml	the volume of the acid solution that was initially taken for titration

Quantity	Unit	Definition
V_{a0}cal	ml	the value and calibration uncertainty component of the volume of the acid solution that was initially taken for titration
V_{a0}temp	ml	the uncertainty of volume of syringe caused by the operational temperature differing from the calibration temperature of the solution
Δt_{Va0_temp}	C	the difference of calibration and usage temperatures of the syringe
V_{a0}rep	ml	the repeatability of the volume delivered by the syringe
V_{a0}calrep	ml	the repeatability of the delivered volume during calibration of the syringe
V_{a0}caltemp	ml	uncertainty of volume of syringe coused by the solution temperature differing from the calibration temperature of the device during calibration
$\Delta t_{Va0\_caltemp}$	C	the solution temperature differing from the calibration temperature of the device during calibration
V_t	ml	volume of the titrant added to the titration cell using the piston burette
$V_t$cal	ml	calibration of the piston burette
$V_t$temp	ml	the uncertainty of volume of the titrant added to the titration cell coused by temperature difference between the operational and calibration temperature of the burette
Δt_{Vt_temp}	C	temperature difference between the operational and calibration temperature of the burette
$V_t$rep	ml	repeatability of the burette
err	ml	"absolute error" of the burette
vol	ml	full volume of the burette
C_{t0}	mol/l	concentration of titrant: sum of molar concentrations of KOH and K2CO3 in the titrant solution
m_{at}	g	mass of the standard acid
m_{at}rep	g	repeatability of weighting of the standard acid
m_{at}read	g	the readability (digital resolution) of the balance scale
V_{at}	ml	volume of the standard acid solution delivered from the syringe
V_{at}calrep	ml	the repeatability of the delivered volume during calibration
V_{at}cal	ml	the uncertainty in the stated volume (the uncertainty of calibration) of the volumetric device
V_{at}caltemp	ml	uncertainty of volume of syringe coused by the solution temperature differing from the calibration temperature of the device during calibration
$\Delta t_{Vat\_caltemp}$	C	the solution temperature differing from the calibration temperature of the device during calibration

Quantity	Unit	Definition
V_{at}temp	ml	the uncertainty of volume of syringe coused by the operational temperature differing from the calibration temperature of the solution
Δt_{Vat_temp}	C	operational temperature differing from the calibration temperature of the solution
P_t	unitless	purity of the standard acid
V_{st}	ml	volume of the standard acid solution
V_{st}cal	ml	the uncertainty in the certified internal volume of the flask
V_{st}temp	ml	the uncertainty of volume of flask coused by the flask and solution temperatures differing from the temp. at which the volume of the flask was calibrated
Δt_{Vst_temp}	C	the flask and solution temperatures differing from the temp. at which the volume of the flask was calibrated
V_{st}fill	ml	variation in filling the flask to the mark
M_t	g/mol	molecular mass of the standard acid - potassium hydrogen phtalate
n_{1t}	unitless	number of carbon atoms in potassium hydrogen phtalate molecule
n_{2t}	unitless	number of hydrogen atoms in potassium hydrogen phtalate molecule
n_{3t}	unitless	number of oxygen atoms in potassium hydrogen phtalate molecule
K	g/mol	atomic weight of the potassium
n_{4t}	unitless	number of potassium atoms in potassium hydrogen phtalate molecule
V_{tt}	ml	the titrant volume necessary to reach the equivalence point
R	unitless	overall repeatability of titration
err_t	ml	"absolute error" of the burette at the titration of standard acid
vol_t	ml	full volume of the burette
V_{tt}temp	ml	uncertainty of titration endpoint volume coused by temperature difference between the operational and calibration temperature of the burette
Δt_{Vtt_temp}	C	temperature difference between the operational and calibration temperature of the burette
V_{tt}cal	ml	calibration of the piston burette
A		constant from Debye-Hückel theory
B		constant from Debye-Hückel theory
a	ongström	ion size parameter
I_0	mol/l	interim quantity for iterative calculation of f_1
f_0	unitless	interim quantity for iterative calculation of f_1
f_{11}	unitless	interim quantity for iterative calculation of f_1
I_{11}	mol/l	interim quantity for iterative calculation of f_1

Quantity	Unit	Definition
f_{12}	unitless	interim quantity for iterative calculation of f_1
f_1	unitless	activity coefficient for univalent ions
pH_x	pH units	pH value of the solution of investigation
E_{is}	mV	emf of the isopotential point
α	1/C	temperature coefficient of the slope
E_x	mV	emf of the electrode system of the investigated solutution
$E_x$read	mV	uncertainty originating from the finite readability of the pH-meter scale of solution of investigation
$E_x$drift	mV	systematic deviations (bias) of the measured emf value from the actual value
E_xJP	mV	uncertainty caused by the residual junction potential of solution of investigation
$E_x$rep	mV	repeatability of emf measurement of solution under investigation
s	mV/pH	slope of the calibration line
ΣpH_iE_i		used for regression analysis
avgpH_i		used for regression analysis
avgE_i		used for regression analysis
ΣpH_ipH_i		used for regression analysis
pH_{is}	pH units	isopotential point pH
E_1	mV	emf of the calibration buffer solution (pH=1.679)
E_1rep	mV	repeatability of emf measurement of standard (pH=1.679)
E_1JP	mV	uncertainty caused by the residual junction potential (pH=1.679)
E_1read	mV	readability of the pH-meter scale of emf measurement of standard (pH=1.679)
E_2	mV	emf of the calibration buffer solution (pH=3.557)
E_2rep	mV	repeatability of emf measurement of standard (pH=3.557)
E_2JP	mV	uncertainty caused by the residual junction potential (pH=3.557)
E_2read	mV	readability of the pH-meter scale of emf measurement of standard (pH=3.557)
E_3	mV	emf of the calibration buffer solution (pH=4.008)
E_3rep	mV	repeatability of emf measurement of standard (pH=4.008)
E_3JP	mV	uncertainty caused by the residual junction potential (pH=4.008)
E_3read	mV	readability of the pH-meter scale of emf measurement of standard (pH=4.008)
E_4	mV	emf of the calibration buffer solution (pH=6.865)
E_4rep	mV	repeatability of emf measurement of standard (pH=6.865)
E_4JP	mV	uncertainty caused by the residual junction potential (pH=6.865)

Quantity	Unit	Definition
E_4read	mV	readability of the pH-meter scale of emf measurement of standard (pH=6.865)
E_5	mV	emf of the calibration buffer solution (pH=9.180)
E_5rep	mV	repeatability of emf measurement of standard (pH=9.180)
E_5JP	mV	uncertainty caused by the residual junction potential (pH=9.180)
E_5read	mV	readability of the pH-meter scale of emf measurement of standard (pH=9.180)
pH_1	pH units	pH of the calibration buffer solution (pH=1.679)
pH_1acc	pH units	uncertainty arising from the limited accuracy of the pH values of the standard buffer solution pH=1.679
pH_1temp	pH units	uncertainty caused by the dependence of the pH value of the standard on temperature (pH=1.679)
pH_2	pH units	pH of the calibration buffer solution (pH=3.557)
pH_2acc	pH units	uncertainty arising from the limited accuracy of the pH values of the standard buffer solution pH=3.557
pH_2temp	pH units	uncertainty caused by the dependence of the pH value of the standard on temperature (pH=3.557)
pH_3	pH units	pH of the calibration buffer solution (pH=4.008)
pH_3acc	pH units	uncertainty arising from the limited accuracy of the pH values of the standard buffer solution pH=4.008
pH_3temp	pH units	uncertainty caused by the dependence of the pH value of the standard on temperature (pH=4.008)
pH_4	pH units	pH of the calibration buffer solution (pH=6.865)
pH_4acc	pH units	uncertainty arising from the limited accuracy of the pH values of the standard buffer solution pH=6.865
pH_4temp	pH units	uncertainty caused by the dependence of the pH value of the standard on temperature (pH=6.865)
pH_5	pH units	pH of the calibration buffer solution (pH=9.180)
pH_5acc	pH units	uncertainty arising from the limited accuracy of the pH values of the standard buffer solution pH=9.180
pH_5temp	pH units	uncertainty caused by the dependence of the pH value of the standard on temperature (pH=9.180)
θ_1		temperaturecoeficient of the standard buffer solution pH=1.679
t_{cal}		calibration temperature
θ_2		temperaturecoeficient of the standard buffer solution pH=3.557
θ_3		temperaturecoeficient of the standard buffer solution pH=4.008
θ_4		temperaturecoeficient of the standard buffer solution pH=6.865
θ_5		temperaturecoeficient of the standard buffer solution pH=9.180
I_{12}	mol/l	interim quantity for iterative calculation of f_2

Quantity	Unit	Definition
$f_{2,1}$	unitless	interim quantity for iterative calculation of f_2
$I_{2,1}$	mol/l	interim quantity for iterative calculation of f_2
$f_{2,2}$	unitless	interim quantity for iterative calculation of f_2
$I_{2,2}$	mol/l	interim quantity for iterative calculation of f_2
f_2	unitless	activity coefficient for doubly charged ions
C_{HCO3}	mol/l	concentration of hydrogencarbonate ions
C_{CO3}	mol/l	concentration of carbonate ions
A_1	mol/l	concentration of dissociated contaminant A1H in the titration cell
A_2	mol/l	concentration of dissociated contaminant A2H in the titration cell
A_3	mol/l	concentration of dissociated contaminant A3H in the titration cell
C_{t0}	mol/l	overall concentration of carbonate ($C_{HCO3} + C_{CO3}$)
K_{H2C}	mol/l	the first dissocation constant of carbonic acid
K_{HC}	mol/l	the second dissocation constant of carbonic acid
C_{A1H_0}	mol/l	concentration of contaminant A1H
K_{A1H}	mol/l	dissocation constant of contaminant A1H
C_{A2H_0}	mol/l	concentration of contaminant A2H
K_{A2H}	mol/l	dissocation constant of contaminant A2H
C_{A3H_0}	mol/l	concentration of contaminant A3H
K_{A3H}	mol/l	dissocation constant of contaminant A3H
P_{A1H}	unitless	content of the contaminant A1H
P_{A2H}	unitless	content of the contaminant A2H
P_{A3H}	unitless	content of the contaminant A3H
t_{meas}	C	measurement temperature
V_{ep}	ml	volume of titrant that correspond to titration endpoint of standard solution
pK_{A1H}		pKa of the contaminant A1H
pK_{A2H}		pKa of the contaminant A2H
pK_{A3H}		pKa of the contaminant A3H

3.3. Application example

Below the measurement conditions are described.

pH meter. The meter has digital display with resolution of 0.1 units in the emf measurement mode. The theoretical value 0.00335 K^{-1} (at 25°C) for the temperature coefficient α is used[6] for calculation the temperature correction. According to the manual of the meter, the "error limits" of the meter are ± 0.2 mV in the mV mode. The "error limits" in temperature measurement are ± 0.1°C. No data on the drift is given in the manual.

Electrode system. Combined glass electrode was used. The inner reference electrode is Ag/AgCl electrode in 3 M KCl solution with porous liquid junction. This electrode has sodium error starting from pH values around 12. The E_{is} for the electrode is 0 ± 15 mV.

Calibration standards. NIST buffer solutions 1.679, 3.557, 4.008, 6.865 and 9.180 were used (pH values are given at 25°C). At 25°C the pH of these standard solutions have temperature dependence 0.0008, 0.001, 0.0014, –0.0032 and –0.009 pH units per centigrade, respectively.[5]

Weighing and volumetric apparatus and solutions.

The benzoic acid (Carlo Erba, >99.5%) was weighed on digital analytical balances (resolution 0.1 mg). Repeatability of the balance has been experimentally determined at our laboratory as a standard deviation 0.00017 g. The solution was prepared in 50.00 ± 0.03 ml (at 20°C) volumetric flask. The solution was transferred to the measurement cell using a calibrated (20 ± 2°C) syringe with stopper. The volume of delivery of the syringe was 12.5281 ml, the standard uncertainty of calibration was 0.0033 ml, repeatability 0.0033 ml.

Titration was carried out in a cell thermostated to 25.0 ± 0.1°C, maintaining an atmosphere of nitrogen over the solution and using a magnetic stirrer for stirring the solution.

A 5 ml (burette capacity) piston burette was used for titration. In the manual of the burette the following data are given: "absolute error" ± 0.015 ml per 5 ml, "repeating error" ± 0.005 ml.

Solution of KOH was used as the titrant. The concentration of the titrant was determined by titrating the potassium hydrogen phthalate standard solution. A tube filled with ascarite was mounted on the titrant bottle to avoid its contamination by atmospheric CO_2. Titrant concentration was determined by three separate titrations of potassium hydrogen phthalate standard solution.

The piston burette and the pH meter were both controlled by in-house software (running in MS Excel environment).

3.4. Results

Uncertainty calculation has been carried out for seven different titration points corresponding to 6, 12, 30, 50, 70, 90 and 95% of the overall titrant volume required to arrive at the equivalence point (V_t =0.1, 0.2, 0.4, 0.8, 1.15, 1.45 and 1.55 ml, respectively) .

The detailed uncertainty budget at one single titrant volume (V_t = 0.8 ml) is presented in Table 3 and Figure 4. It is also available as GUM Workbench file pKa_u.smu in the ESM of publication II. The uncertainty budgets of the pH values at the different V_t values are presented in Table 4. The uncertainty budgets, the resulting pK_{ax} values and the resulting combined standard uncertainties u_c(pK_{ax}) and expanded uncertainties U(pK_{ax}) are presented in Table 5. Figure 3 illustrates the variation of uncertainty of pK_a values obtained from different points of the titration curve.

3.5. Discussion

The main sources of uncertainty in pK_a determination. The uncertainty budgets of the pK_{ax} values found from different points of the titration curve are presented in Table 5. As is expected, the uncertainty is the lowest in the middle of the titration curve. The relationship is roughly symmetrical with respect to the half-neutralization point (see Figure 3). From this follows that different sources of uncertainty dominate in the beginning of the curve and in the end.

pH is clearly the key player in the uncertainty budgets corresponding to the majority of the titration curve points. In turn, the uncertainty of pH is in all titration points almost entirely determined by the uncertainty of the EMF measurement in the measured solution $u(E_x)$: leaving out all other uncertainty sources changes the $u_c(pH_x)$ by only around 0.001 pH units. The $u(E_x)$, which consists of 4 components (Table 5, 4 rows next to the E_x row), is in turn determined mainly by the residual liquid junction potential uncertainty.

It is interesting to note the different contributions of uncertainty of pH_x to the u(pK_{ax}) in different parts of the titration curve, while the uncertainty of all the pH measurements is practically identical (see Table 4): the influence of $u(pH_x)$ is stronger in the beginning and in the middle of the titration curve where it is clearly the dominating uncertainty source. At the end of the curve the dominating factors are the uncertainties of the concentrations C_{a0} and C_{t0} and the titrant volume V_t. This behaviour can be easily rationalised: in the region of equivalence point of the curve the relatively low concentration of the neutral [HA] is calculated as a difference of two relatively high concentrations C_a and [A^-], which in turn are dependent on the three parameters: C_{a0}, C_{t0} and V_t. In the beginning and in the middle of the curve where the [HA] is high this effect is not pronounced. On the contrary, in the beginning of the titration curve there is

a pronounce self-dissociation of the acid HA. Thus, in addition to determining the $a(H^+)_x$ in eq 16 pH_x also influences $[A^-]$.

The purity P of the acid under investigation is in this treatment not related just to inert compounds but involves also contaminants with acidic properties (see the Mathematical model section in ESM of publication II file pKa_u_ESM.pdf for more detailed explanations). In the application example it has been assumed that the acid contains in addition to inert impurities also three different kinds of acidic impurities with different acidities (pK_a values around 2.5, 7 and 10). Concentrations and acidities of all those acidic impurities enter the measurement equations and are thus taken into account. As can been seen from Table 5, impurities with different pK_a values have different influence on the final result. The impurity with the lowest pK_a value has the highest influence. The total uncertainty contribution of the four impurities is different in different parts of the titration curve, ranging from 8.1% (in the middle of the curve) to 31% at V_t = 1.55 ml. The input quantities related to the purity of the acid are the biggest source of uncertainty of the initial acid concentration C_{a0}.

The uncertainty of V_t is mainly determined by the accuracy of the mechanical burette. The uncertainty of the concentration of the titrant depends on several sources of similar magnitude, the most important of them are again the weighing uncertainty, the purity of standard substance and the accuracy of the burette. The effect of contamination of the titrant with carbonate becomes (at the level of carbonate, assumed in the example) visible only in the last portion of the titration curve because the pK_a value of H_2CO_3 is ca 6.3, which is well above the pH_x values.

Possibilities for optimizing the pK_a measurement procedure. The uncertainty budget is a powerful tool for optimizing the measurement procedure. From the Table 4 and Table 5 it can be concluded that the used glassware and the burette are in general appropriate for this work. The stability of temperature in the laboratory is adequate. There is no need to involve more calibration standards into the calibration of the pH meter (it is also the recommendation of IUPAC to use up to 5 buffers for multi-point calibration of pH meters[9]). The target uncertainty of pH measurement using multi-point calibration is estimated as 0.01–0.03 pH units (expanded uncertainty, k = 2), in agreement with our results. The changes that could be introduced: instead of a 50 ml flask a 250 ml flask could be used, so that a larger amount of the acid could be weighed; a smaller piston could be used for the piston burette (that can, in fact, be difficult, because at least with this manufacturer 5 ml is the smallest size). However these changes do not reduce the uncertainty significantly. The most significant decrease of the overall uncertainty of pK_a would be achieved if the residual liquid junction potential could be estimated or eliminated. That is difficult, however, without introducing significant changes to the experimental setup[8,11,35].

Finding the overall pK_a value and its uncertainty. The presented procedure is intended for finding the uncertainty of the pK_{ax} determined from a single point of the titration curve. Obviously the best estimate of the pK_a value

is the mean of the pK_{ax} values that are in the region of the lowest uncertainty, see Table 6 and Figure 3.

The overall uncertainty of pK_a should consider all the uncertainty sources in the method, including the variability between the pK_{ax} values found from different points of the titration curve. However, since the sources of variability (the various repeatabilities) are already included in the uncertainty estimates of the individual pK_{ax} values, it is unnecessary to add any repeatability contribution anymore. Based on this the average value of U(pK_{ax}) was taken as the estimate of U(pK_a). It is unreasonable to divide the uncertainty U(pK_{ax}) by square root of n (the number of pK_{ax} values used for calculating the overall pK_a value), because the pK_{ax} values are not statistically independent.

Based on this reasoning, the following results have been got (using the pK_{ax}) values corresponding to V_t 0.2, 0.4, 0.8 and 1.15 ml): pK_a = 4.219, u_c(pK_a)= 0.017, U(pK_a) = 0.034 (k = 2)

Interpretation of the mismatch of the pK_{ax} values obtained from different parts of the titration curve. From Table 5 it is seen that the pK_a values increase slightly with the increase of V_t. This is caused by various effects of systematic nature. Some of them influence the front part of the curve, some influence the rear part. For example, some mismatch always exists between the four parameters C_t, V_t, C_{a0} and V_{a0}. That leads to an increasingly erroneous concentration of the undissociated acid [HA] as the V_t gets higher ([HA] is calculated [HA] = C_a – [A^-] (eq 8 in ESM of publication II) and in the rear part of the curve the [HA] is found as the small difference of two relatively large quantities of similar magnitude) causing also the pK_{ax} values to "drift" along the titration curve. Because the presented uncertainty estimation procedure takes into account all the uncertainty sources causing the drift (including the uncertainties of the four parameters of this example), this drift is also automatically taken into account by the uncertainty estimate. Therefore, some drift of the pK_{ax} values is normal.

The question remains, however, how much of such drift is acceptable. The following criterion was proposed: the drift of a pK_{ax} value from the overall pK_a value is acceptable as long as the overall pK_a value lies within the limits of expanded uncertainty pK_{ax} – U(pK_{ax}) ... pK_{ax} + U(pK_{ax}). According to this approach the drift in Table 5 is acceptable.

Comparison of the obtained uncertainty of the pK_a value to the literature data.

The main problem with the literature is that very often no uncertainty estimate is given with the results. For example, there are 174 pK_a values for pK_a of benzoic acid measured under different conditions given in Palm tables[15]. Only for 24 of those values were uncertainty estimates reported! The results of this work can be used to obtain rough estimates of the uncertainty for such literature values if experimental details are available from original publications.

The second aspect is the validity of the reported uncertainty values. As can be seen from the results of this work, "normal" expanded uncertainties

(at k = 2 level) for pK_a values in the region of 3 .. 5 pK_a units obtained from potentiometric titration with electrode system containing liquid junction, are in the range on ± 0.03 .. 0.05 pK_a units. It is doubtful, whether with similar experimental setup it would be possible to obtain expanded uncertainty (k =2) below 0.02 pK_a units. It is outside the scope of this work to carry out extensive review of literature data but it should be noted that for carboxylic acids, for example, uncertainty claims in the range of 0.005 to 0.02 pK_a units are more frequently found in ref 15 than uncertainty claims in the range of 0.03 to 0.05 pK_a units. Quite frequently encountered situation is that values from different authors do not agree within the combined uncertainty limits. This clearly indicates underestimated uncertainties.

The acidic dissociation of the compound under study in this work – benzoic acid – has been extensively studied (using all major methods for pK_a measurement) and many different values have been found. The values given in ref 15 (at 25°C) vary from 4.16 to 4.24. The values of higher quality (estimated by the limited information available on reliability of the values) are around 4.20 to 4.21. In the compilation of Kortüm et al[13] the values estimated by the compilers as the most reliable are pK_a = 4.20. Our result 4.219 ± 0.034 agrees with the literature data well within the uncertainty limits.

Table 3. Detailed uncertainty budget of pK_a value of the acid HA corresponding to one point of the titration curve (added titrant volume: 0.8 ml).

Estimation of Uncertainty of pKa values Determined by Potentiometric Titration

Uncertainty Budget:

Quantity	Value	Standard Uncertainty	Distribution	Sensitivity Coefficient	Uncertainty Contribution	Index
P	0.99500 unitless	$2.89 \cdot 10^{-3}$ unitless	rectangular	0.88	$2.5 \cdot 10^{-3}$	2.8 %
n_1	7.0 unitless					
C	12.010700 g/mol	$400 \cdot 10^{-6}$ g/mol	normal	-0.017	$-6.8 \cdot 10^{-6}$	0.0 %
n_2	6.0 unitless					
H	1.0079400 g/mol	$35.0 \cdot 10^{-6}$ g/mol	normal	-0.022	$-780 \cdot 10^{-9}$	0.0 %
n_3	2.0 unitless					
O	15.999400 g/mol	$150 \cdot 10^{-6}$ g/mol	normal	$2.3 \cdot 10^{-3}$	$340 \cdot 10^{-9}$	0.0 %
$m_a$rep	0.049100 g	$170 \cdot 10^{-6}$ g	normal	18	$3.0 \cdot 10^{-3}$	4.1 %
$m_a$read	0.0 g	$28.9 \cdot 10^{-6}$ g	rectangular	18	$520 \cdot 10^{-6}$	0.1 %
$V_s$cal	50.0000 ml	0.0173 ml	rectangular	-0.018	$-300 \cdot 10^{-6}$	0.0 %
Δt_{Vs_temp}	0.0 C	2.31 C	rectangular	$-180 \cdot 10^{-6}$	$-430 \cdot 10^{-6}$	0.0 %
$V_s$fill	0.0 ml	0.0173 ml	rectangular	-0.018	$-300 \cdot 10^{-6}$	0.0 %
γ	$210.0 \cdot 10^{-6}$ 1/C					
Δt_{Va0_temp}	0.0 C	2.31 C	rectangular	$180 \cdot 10^{-6}$	$410 \cdot 10^{-6}$	0.0 %
V_{a0}rep	0.0 ml	$3.26 \cdot 10^{-3}$ ml	normal	0.068	$220 \cdot 10^{-6}$	0.0 %
V_{a0}calrep	12.52813 ml	$1.23 \cdot 10^{-3}$ ml	normal	0.068	$84 \cdot 10^{-6}$	0.0 %
$\Delta t_{Va0\_caltemp}$	0.0 C	1.15 C	rectangular	$180 \cdot 10^{-6}$	$210 \cdot 10^{-6}$	0.0 %
Δt_{Vt_temp}	0.0 C	2.31 C	rectangular	$-180 \cdot 10^{-6}$	$-410 \cdot 10^{-6}$	0.0 %
$V_t$rep	0.80000 ml	$2.89 \cdot 10^{-3}$ ml	rectangular	-1.1	$-3.1 \cdot 10^{-3}$	4.1 %
err	0.0 ml	$8.66 \cdot 10^{-3}$ ml	rectangular	-0.17	$-1.5 \cdot 10^{-3}$	1.0 %
vol	5.0 ml					
m_{at}rep	0.158000 g	$170 \cdot 10^{-6}$ g	normal	-5.4	$-910 \cdot 10^{-6}$	0.4 %
m_{at}read	0.0 g	$28.9 \cdot 10^{-6}$ g	rectangular	-5.4	$-160 \cdot 10^{-6}$	0.0 %
V_{at}calrep	12.52813 ml	$1.23 \cdot 10^{-3}$ ml	normal	-0.068	$-84 \cdot 10^{-6}$	0.0 %
$\Delta t_{Vat\_caltemp}$	0.0 C	1.15 C	rectangular	$-180 \cdot 10^{-6}$	$-210 \cdot 10^{-6}$	0.0 %
Δt_{Vat_temp}	0.0 C	2.31 C	rectangular	$-180 \cdot 10^{-6}$	$-410 \cdot 10^{-6}$	0.0 %
P_t	0.99800 unitless	$1.15 \cdot 10^{-3}$ unitless	rectangular	-0.85	$-980 \cdot 10^{-6}$	0.4 %
V_{st}cal	50.0000 ml	0.0173 ml	rectangular	0.017	$290 \cdot 10^{-6}$	0.0 %
Δt_{Vst_temp}	0.0 C	2.31 C	rectangular	$180 \cdot 10^{-6}$	$410 \cdot 10^{-6}$	0.0 %
V_{st}fill	0.0 ml	0.0173 ml	rectangular	0.017	$290 \cdot 10^{-6}$	0.0 %

Quantity	Value	Standard Uncertainty	Distribution	Sensitivity Coefficient	Uncertainty Contribution	Index
n_{1t}	8.0 unitless					
n_{2t}	5.0 unitless					
n_{3t}	4.0 unitless					
K	39.0983000 g/mol	$50.0 \cdot 10^{-6}$ g/mol	normal	$4.2 \cdot 10^{-3}$	$210 \cdot 10^{-9}$	0.0 %
n_{4t}	1.0 unitless					
err_t	0.0 ml	$8.66 \cdot 10^{-3}$ ml	rectangular	0.17	$1.5 \cdot 10^{-3}$	1.0 %
vol_t	5.0 ml					
Δt_{Vtt_temp}	0.0 C	2.31 C	rectangular	$180 \cdot 10^{-6}$	$410 \cdot 10^{-6}$	0.0 %
A	0.5115000	$57.7 \cdot 10^{-6}$	rectangular	0.054	$3.1 \cdot 10^{-6}$	0.0 %
B	0.3291000	$11.5 \cdot 10^{-6}$	rectangular	$-7.7 \cdot 10^{-3}$	$-89 \cdot 10^{-9}$	0.0 %
a	5.000 ongström	0.289 ongström	rectangular	$-510 \cdot 10^{-6}$	$-150 \cdot 10^{-6}$	0.0 %
f_0	1.0 unitless					
E_{is}	0.0 mV	8.66 mV	rectangular	0.0	$-220 \cdot 10^{-21}$	0.0 %
α	$3.354 \cdot 10^{-3}$ 1/C	$530 \cdot 10^{-6}$ 1/C	normal	0.0	0.0	0.0 %
$E_x$read	0.0 mV	0.0289 mV	rectangular	-0.018	$-510 \cdot 10^{-6}$	0.1 %
$E_x$drift	0.0 mV	0.353 mV	normal	-0.018	$-6.2 \cdot 10^{-3}$	16.9 %
E_xJP	0.0 mV	0.577 mV	rectangular	-0.018	-0.010	45.2 %
$E_x$rep	163.100 mV	0.115 mV	rectangular	-0.018	$-2.0 \cdot 10^{-3}$	1.8 %
E_1rep	311.700 mV	0.115 mV	rectangular	$5.0 \cdot 10^{-3}$	$570 \cdot 10^{-6}$	0.1 %
E_1JP	0.0 mV	0.205 mV	rectangular	$5.0 \cdot 10^{-3}$	$1.0 \cdot 10^{-3}$	0.5 %
E_1read	0.0 mV	0.0289 mV	rectangular	$5.0 \cdot 10^{-3}$	$140 \cdot 10^{-6}$	0.0 %
E_2rep	200.000 mV	0.115 mV	rectangular	$4.2 \cdot 10^{-3}$	$480 \cdot 10^{-6}$	0.1 %
E_2JP	0.0 mV	0.205 mV	rectangular	$4.2 \cdot 10^{-3}$	$850 \cdot 10^{-6}$	0.3 %
E_2read	0.0 mV	0.0289 mV	rectangular	$4.2 \cdot 10^{-3}$	$120 \cdot 10^{-6}$	0.0 %
E_3rep	174.600 mV	0.115 mV	rectangular	$4.0 \cdot 10^{-3}$	$460 \cdot 10^{-6}$	0.0 %
E_3JP	0.0 mV	0.205 mV	rectangular	$4.0 \cdot 10^{-3}$	$810 \cdot 10^{-6}$	0.3 %
E_3read	0.0 mV	0.0289 mV	rectangular	$4.0 \cdot 10^{-3}$	$110 \cdot 10^{-6}$	0.0 %
E_4rep	5.300 mV	0.115 mV	rectangular	$2.7 \cdot 10^{-3}$	$320 \cdot 10^{-6}$	0.0 %
E_4JP	0.0 mV	0.205 mV	rectangular	$2.7 \cdot 10^{-3}$	$560 \cdot 10^{-6}$	0.1 %
E_4read	0.0 mV	0.0289 mV	rectangular	$2.7 \cdot 10^{-3}$	$79 \cdot 10^{-6}$	0.0 %
E_5rep	-130.700 mV	0.115 mV	rectangular	$1.7 \cdot 10^{-3}$	$200 \cdot 10^{-6}$	0.0 %
E_5JP	0.0 mV	0.205 mV	rectangular	$1.7 \cdot 10^{-3}$	$350 \cdot 10^{-6}$	0.0 %
E_5read	0.0 mV	0.0289 mV	rectangular	$1.7 \cdot 10^{-3}$	$50 \cdot 10^{-6}$	0.0 %

Quantity	Value	Standard Uncertainty	Distribution	Sensitivity Coefficient	Uncertainty Contribution	Index
pH_1acc	1.6790 pH units	0.0115 pH units	rectangular	0.29	$3.4 \cdot 10^{-3}$	5.0 %
pH_2acc	3.5570 pH units	0.0115 pH units	rectangular	0.25	$2.8 \cdot 10^{-3}$	3.5 %
pH_3acc	4.0080 pH units	0.0115 pH units	rectangular	0.23	$2.7 \cdot 10^{-3}$	3.2 %
pH_4acc	6.8650 pH units	0.0115 pH units	rectangular	0.16	$1.9 \cdot 10^{-3}$	1.5 %
pH_5acc	9.1800 pH units	0.0115 pH units	rectangular	0.10	$1.2 \cdot 10^{-3}$	0.6 %
θ_1	$800.0 \cdot 10^{-6}$					
t_{cal}	25.0000	0.0577	rectangular	-0.010	$-590 \cdot 10^{-6}$	0.2 %
θ_2	$1.0 \cdot 10^{-3}$					
θ_3	$1.4 \cdot 10^{-3}$					
θ_4	$-3.2 \cdot 10^{-3}$					
θ_5	$-9.0 \cdot 10^{-3}$					
C_{c0}	$150.0 \cdot 10^{-6}$ mol/l	$86.6 \cdot 10^{-6}$ mol/l	rectangular	0.10	$9.0 \cdot 10^{-6}$	0.0 %
K_{H2C}	$450.0 \cdot 10^{-9}$ mol/l					
K_{HC}	$48.0 \cdot 10^{-12}$ mol/l					
P_{A1H}	$3.50 \cdot 10^{-3}$ unitless	$2.02 \cdot 10^{-3}$ unitless	rectangular	1.7	$3.5 \cdot 10^{-3}$	5.3 %
P_{A2H}	$3.50 \cdot 10^{-3}$ unitless	$2.02 \cdot 10^{-3}$ unitless	rectangular	$3.1 \cdot 10^{-3}$	$6.3 \cdot 10^{-6}$	0.0 %
P_{A3H}	$3.50 \cdot 10^{-3}$ unitless	$2.02 \cdot 10^{-3}$ unitless	rectangular	$3.1 \cdot 10^{-6}$	$6.3 \cdot 10^{-9}$	0.0 %
t_{meas}	25.0000 C	0.0577 C	rectangular	$9.6 \cdot 10^{-3}$	$550 \cdot 10^{-6}$	0.1 %
$V_{tt}ep$	3.11174 ml	$1.19 \cdot 10^{-3}$ ml	normal	-0.27	$-320 \cdot 10^{-6}$	0.0 %
pK_{A1H}	2.50	1.15	rectangular	not valid!	$-610 \cdot 10^{-6}$	0.2 %
pK_{A2H}	7.00	1.15	rectangular	not valid!	$-76 \cdot 10^{-6}$	0.0 %
pK_{A3H}	10.00	1.15	rectangular	not valid!	$-77 \cdot 10^{-9}$	0.0 %
pK_{ax}	4.2199	0.0151				

Result: Quantity: pK_{ax}
Value: 4.220
Expanded Uncertainty: ±0.030
Coverage Factor: 2.00
Coverage: manual

Table 4. Uncertainty budgets and combined uncertainties of pH_x corresponding to the different points of the titration curve.

	Titrant volume and pH						
V_t=	0.1	0.2	0.4	0.8	1.15	1.45	1.55
pH_x=	3.335	3.491	3.757	4.194	4.589	5.152	5.631
	Uncertainty contributions of input quantities (%)[a]						
pH_1	7.8	7.3	6.7	5.1	3.0	0.6	0.1
pH_2	4.4	4.3	4.2	3.5	2.4	0.6	0.1
pH_3	3.7	3.6	3.6	3.2	2.3	0.6	0.1
pH_4	0.7	0.8	1.1	1.5	1.5	0.6	0.2
pH_5	0.0	0.0	0.1	0.6	1.0	0.7	0.2
E_1	0.9	0.9	0.8	0.6	0.4	0.1	0.0
E_2	0.5	0.5	0.5	0.4	0.3	0.1	0.0
E_3	0.4	0.4	0.4	0.4	0.3	0.1	0.0
E_4	0.1	0.1	0.1	0.2	0.2	0.1	0.0
E_5	0.0	0.0	0.0	0.1	0.1	0.1	0.0
E_x[b]	*59.1*	*60.6*	*64.7*	*64.2*	*50.6*	*15.7*	*3.2*
$E_{x,rep}$	1.7	1.7	1.8	1.8	1.4	0.4	0.1
$E_{x,read}$	0.1	0.1	0.1	0.1	0.1	0.0	0.0
$E_{x,drift}$	15.6	16.0	17.1	16.9	13.4	4.1	0.9
$E_{x,JP}$	41.7	42.8	45.7	45.3	35.7	11.1	2.3
E_{is}	0.0	0.0	0.0	0.0	0.0	0.0	0.0
α	0.0	0.0	0.0	0.0	0.0	0.0	0.0
t_{cal}	0.2	0.2	0.2	0.2	0.1	0.0	0.0
t_{meas}	0.2	0.2	0.2	0.1	0.1	0.0	0.0
	Standard uncertainties of pH values						
$u_C(pH_x)$=	0.013	0.013	0.013	0.013	0.013	0.013	0.013

[a] The uncertainty contributions percentages are given for the uncertainty of the respective pK_{ax} value (i.e. the percentages (excluding the row "E_x"[b]) sum up to give the uncertainty contribution of the pH_x value in Table). The uncertainty contributions have been found according to eq 28. The full uncertainty budgets can be found in ESM of publication II (files pKa_u.smu and pKa_u.xls). [b] The separate uncertainty contributions of components of E_x – the most important input quantity – are given in the next 4 rows.

Table 5. Uncertainty budgets and combined uncertainties of pK_{ax} values calculated for different added titrant volumes V_t.

	Titrant volume and pH						
V_t=	0.1	0.2	0.4	0.8	1.15	1.45	1.55
pH_x=	3.335	3.491	3.757	4.194	4.589	5.152	5.631
	Uncertainty contributions of input quantities (%)[a]						
pH_x	78.0	79.0	82.8	80.1	62.3	19.2	4.0
m_a	0.6	0.9	1.7	4.2	10.3	24.6	29.8
V_s	0.0	0.0	0.1	0.2	0.4	1.0	1.2
P	0.4	0.6	1.1	2.8	6.9	16.7	20.2
P_{A1H}	9.1	9.0	7.0	5.3	6.6	10.1	10.8
pK_{A1H}	2.8	1.8	0.4	0.0	0.0	0.0	0.0
pK_{A2H}	0.0	0.0	0.0	0.0	0.0	0.0	0.4
C_{t0}	0.1	0.3	0.7	2.1	5.4	13.3	16.3
V_{a0}	0.0	0.0	0.0	0.1	0.3	0.7	0.9
V_t	9.1	8.4	6.2	5.2	7.7	14.3	16.3
C_{c0}	0.0	0.0	0.0	0.0	0.0	0.0	0.1
	pK_a values and their uncertainties (standard and expanded)						
pK_{ax}=	4.229	4.217	4.214	4.220	4.226	4.250	4.313
u_c(pK_{ax})=	0.024	0.020	0.016	0.015	0.017	0.030	0.066
U(pK_{ax})=	0.048	0.039	0.032	0.030	0.033	0.060	0.132

[a] The uncertainty contributions have been found according to eq 28. Those input quantities that contribute negligibly to the overall uncertainty of pK_{ax} have been omitted. The full uncertainty budgets can be found in ESM of publication II (files pKa_u.smu and pKa_u.xls).

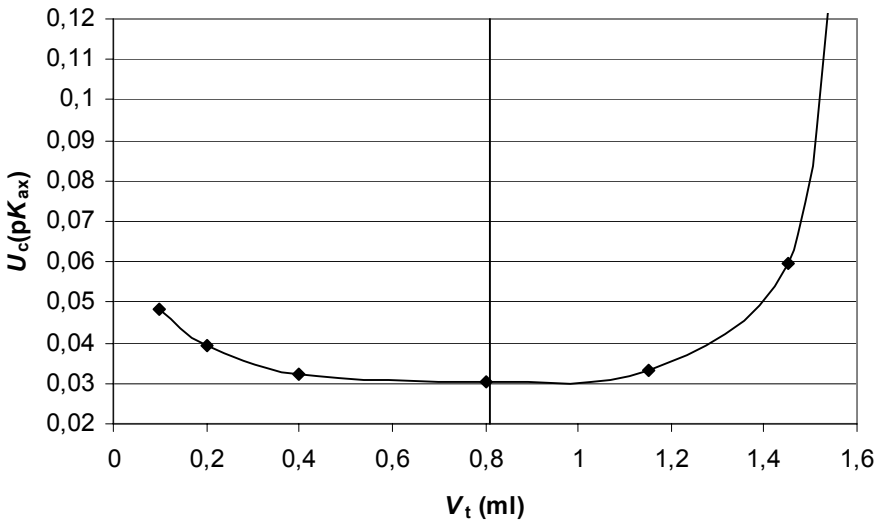

Figure 3. Uncertainty (k=2) of pK_{ax} in different points of the titration curve.

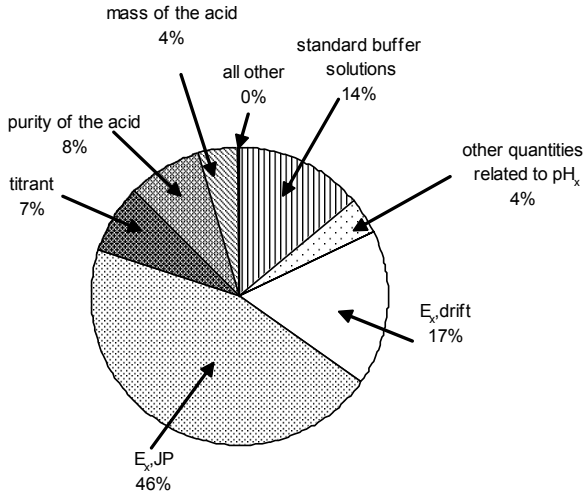

Figure 4. Uncertainty contributions of the most important input quantities of pK_{ax} in the titration point $V_t = 0.8$.

4. UNCERTAINTY CALCULATION FOR pK_a(I=0) AND pK_a(I=0.1M KCl)

4.1. Introduction

In this section the procedures for estimation of measurement uncertainty for acidity constant K_a (or pK_a value) in different media I=0 and I=0.1M KCl determined by potentiometric titration are presented. The uncertainty budgets (the relative contributions of different input quantities on the uncertainty of the result) of the pK_a(I=0) and pK_a(I=0.1M KCl) values are compared. Unlike the values (see the Introduction), their uncertainties and uncertainty budgets are comparable.

In order to be able to compare the uncertainty budgets of the pK_a(I=0) and pK_a(I=0.1M KCl) values, the calculation approaches have to be the same. In very broad terms there are two approaches for calculation of pK_a values from potentiometric titration curves. One (approach 1 below) is the approach described in the book of Albert and Serjeant[17,18] whereby a pK_a value is found for every point of the titration curve and the result is calculated as average of these values. This approach was also used in previous section (section 3) of this work. The more modern one (approach 2 below) is to find a single pK_a value from the titration curve by modeling the curve and then minimizing the sums of squares (often weighed) of the differences of the modeled curve from the experimental curve by adjusting the pK_a value (and possibly other parameters, such as concentrations). This approach has been used in several computer programs, which have been described in refs 19–23.

In this work the approach 1 was chosen and the uncertainty calculation for a single point (denoted below as x) on the titration curve was presented. The point x was chosen near the half-neutralization point. The advantages of approach 1 are simplicity and transparency for better following of the uncertainty propagation. It has been shown previously (section 3 of this work) that the uncertainty of a pK_a value determined as an average of several values obtained from different titration points is not significantly lower than a single-point pK_a value due to the strong correlation between the pK_a values from different points of the curve (similar influence of the systematic effects). Also, the principal conclusions drawn from uncertainty analysis of pK_a values obtained by approach 1 are applicable to pK_a values obtained from approach 2, because in the end the pK_a values are calculated from the same data with the same uncertainty sources.

4.2. Experimental

Below the measurement conditions are described.

Weighing and volumetric apparatus.

Two benzoic acid solutions were prepared: one for pK_a(I=0) measurement and the second for the pK_a(I=0.1M KCl) measurement. First solution was prepared using only deionized CO_2-free water. The second one was prepared in 0.1M KCl medium.

The benzoic acid (Carlo Erba, >99.5%) was weighed on digital analytical balances (resolution 0.1 mg). Repeatability of the balance has been experimentally determined at our laboratory as a standard deviation 0.00017 g. The solution was prepared in 250.00 ± 0.15 ml (at 20°C) volumetric flask. The solution was transferred to the measurement cell by a 50 ml pipette, calibrated in our laboratory.

Titration was carried out in a cell thermostated to 25.0 ± 0.1°C, maintaining an atmosphere of nitrogen over the solution and using a magnetic stirrer for stirring the solution.

A 5 ml (burette capacity) piston burette was used for titration. In the manual of the burette the following data are given: "absolute error" ± 0.015 ml per 5 ml, "repeating error" ± 0.005 ml.

Solution of 0.1000M KOH (Merck) was used as the titrant. Its uncertainty as provided by the supplier was ±0.1%. A tube filled with ascarite was mounted on the titrant bottle to avoid its contamination by atmospheric CO_2. Titrant concentration was determined by three separate titrations of potassium hydrogen phthalate standard solution.

pH meter. The meter has digital display with resolution of 0.1 units in the emf measurement mode. The theoretical value 0.00335 K^{-1} (at 25°C) for the temperature coefficient α is used[6] for calculation the temperature correction. According to the manual of the meter, the "error limits" of the meter are ± 0.2 mV in the mV mode. The "error limits" in temperature measurement are ± 0.1°C. No data on the drift is given in the manual.

Electrode system. Combined glass electrode was used. The inner reference electrode is Ag/AgCl electrode in 3 M KCl solution with porous liquid junction. This electrode has sodium error starting from pH values around 12.

Buffer solutions for pK_a(I=0) measurement (calibration in hydrogen activity scale). Aldrich buffer solutions 4.00, 6.99 and 9.95 were used. The accuracy ± 0.02 pH units was given for all buffer solutions. The temperature dependences of the pH at 25°C of these standard solutions were 0.000, 0.002 and 0.010 pH units per centigrade, respectively.

Calibration in terms of hydrogen ion concentration for pK_a(I=0.1M KCl) measurement. For the pK_a(I=0.1M KCl) value, the calibration procedure was carried out in terms of hydrogen ion concentration by means of a strong acid – strong base titration in 0.1M KCl medium and the program GLEE[29] was used for calculation of the calibration parameters E_0 and s.

4.3. Mathematical model

The mathematical models are based on main equations 12, 16 and 17 for pK_a(I=0) measurement and on 14, 18 and 19 for pK_a(I=0.1M KCl) measurement. The full models for calculation the pK_a(I=0) and pK_a(I=0.1M KCl) value and their uncertainties are presented in Table 7 and Table 8, respectively. The explanations and definitions of all the quantities are presented in Table 9. The factors that are taken into account include all uncertainty sources related to weighing and volumetric operations, purities of the measured acid, carbonate content of the titrant, dilution effect from added titrant, and pH-related uncertainty sources, such as uncertainties of calibration parameters and uncertainty of pH measurement.

Table 6. Mathematical model of calculation of pK_a(I=0) value and its uncertainty. (printout of GUM Wb file)

Model Equation:

{ The main equation }

$pK_a=-\log(a_H*An*f_1/HA)$;

$a_H=10^{(-pH_x)}$;

$HA=V_{a0}*C_{a0}/(V_{a0}+V_t)-An$;

$An=a_H/f_1+V_t*C_{t0}/(V_{a0}+V_t)-K_w/a_H/f_1-C_{HCO3}-2*C_{CO3}-A_1-A_2-A_3$;

$K_w=1.008*10^{(-14+0.033*(t_{meas}-\text{const}(t_{meas})))}$;

{ Initial concentration of the acid solution taken for pKa measurement }

$C_{a0}=1000*m_a*P/V_s/M$;

$M=n_1*C+n_2*H+n_3*O$;

$m_a=m_a\text{rep}+m_a\text{read}+m_a\text{drift}+m_a\text{electrost}$;

$V_s=V_s\text{cal}+V_s\text{temp}+V_s\text{fill}$;

$V_s\text{temp}=V_s\text{cal}*\gamma*\Delta t_{Vs\_temp}$;

{ Contents of impurities in the acid solution

It is assumed that the titrated acid contains some impurities. As a model approach, it is assumed that there are three

different impurities with different acidities.}

$C_{A1H\_0}=1000*m_a*P_{A1H}/V_s/M$;

$C_{A2H\_0}=1000*m_a*P_{A2H}/V_s/M$;

$C_{A3H\_0}=1000*m_a*P_{A3H}/V_s/M$;

$K_{A1H} = 10 ^ (-pK_{A1H})$;

$K_{A2H} = 10 ^ (-pK_{A2H})$;

$K_{A3H} = 10 ^ (-pK_{A3H})$;

$A_1=C_{A1H\_0}*V_{a0}/(V_{a0}+V_t)/(1+a_H*f_1*f_1/K_{A1H})$;

$A_2=C_{A2H\_0}*V_{a0}/(V_{a0}+V_t)/(1+a_H*f_1*f_1/K_{A2H})$;

$A_3=C_{A3H\_0}*V_{a0}/(V_{a0}+V_t)/(1+a_H*f_1*f_1/K_{A3H})$;

{ Volume of the investigated acid solution taken for titration

The real volume at the titration point under consideration is higher due to the added titrant }

$V_{a0}=V_{a0}cal+V_{a0}temp+V_{a0}rep;$

$V_{a0}cal=V_{a0}calrep+V_{a0}caltemp;$

$V_{a0}caltemp=V_{a0}calrep*\gamma*\Delta t_{Va0\_caltemp};$

$V_{a0}temp=V_{a0}calrep*\gamma*\Delta t_{Va0\_temp};$

{ Volume of the titrant added to the titration cell up to the titration point under consideration }

$V_t=V_tcal+V_ttemp+V_trep;$

$V_tcal=err/vol*V_trep;$

$V_ttemp=V_trep*\gamma*\Delta t_{Vt\_temp};$

{ Content of carbonate in the titrant }

$C_{HCO3}=C_{c0}*V_t/(V_{a0}+V_t)*K_{H2C}/f_1/a_H/(1+K_{H2C}/f_1/a_H+$

$K_{H2C}*K_{HC}/(f_1*f_2*a_H*a_H));$

$C_{CO3}=C_{c0}*V_t/(V_{a0}+V_t)*K_{HC}*K_{H2C}/(f_1*f_2*a_H*a_H)/$

$(1+K_{H2C}/f_1/a_H+K_{H2C}*K_{HC}/(f_1*f_2*a_H*a_H));$

{ Calculation of activity constant of the solution

This calculation is done via iterative approach, using three iterations.}

$I_{00}=C_{t0}*V_t/(V_{a0}+V_t)+a_H/f_{000};$

$f_{00}=10\text{^}(-A*sqrt(I_{00})/(1+B*å*sqrt(I_{00})));$

$I_0=C_{t0}*V_t/(V_{a0}+V_t)+a_H/f_{00};$

$f_0=10\text{^}(-A*sqrt(I_0)/(1+B*å*sqrt(I_0)));$

$I=C_{t0}*V_t/(V_{a0}+V_t)+a_H/f_0;$

$logf_1=-A*sqrt(I)/(1+B*å*sqrt(I))+\Delta logf_{1SIT}+\Delta logf_{1DH};$

$f_1=10\text{^}(logf_1);$

$logf_2=-A*2*sqrt(I)/(1+B*a*sqrt(I))+\Delta logf_{2SIT}+\Delta logf_{2DH};$

$f_2=10\text{^}(logf_2);$

{ pH of the measured solution }

$pH_x=(E_x-E_0)/s/(1+\alpha*(t_{meas}-t_{cal}));$

$E_x=E_xread+E_xdrift+E_xJP+E_xrep;$

{ Calibration of pH-meter }

$\Sigma pH_iE_i=pH_1*E_1+pH_2*E_2+pH_3*E_3;$

$avgpH_i=(pH_1+pH_2+pH_3)/3;$

$avgE_i=(E_1+E_2+E_3)/3;$

$\Sigma pH_ipH_i=pH_1*pH_1+pH_2*pH_2+pH_3*pH_3;$

$s=(\Sigma pH_iE_i-3*avgpH_i*avgE_i)/(\Sigma pH_ipH_i-3*avgpH_i*avgpH_i);$

E_0=avgE_i-s*avgpH_i;

E_1=E_1rep+E_1JP+E_1read;
E_2=E_2rep+E_2JP+E_2read;
E_3=E_3rep+E_3JP+E_3read;

pH_1=pH_1acc+pH_1temp;
pH_2=pH_2acc+pH_2temp;
pH_3=pH_3acc+pH_3temp;

pH_1temp=θ_1*(t_{cal}-const(t_{cal}));
pH_2temp=θ_2*(t_{cal}-const(t_{cal}));
pH_3temp=θ_3*(t_{cal}-const(t_{cal}));

Table 7. Mathematical model of calculation of pK_a(I=0.1M KCl) value and its uncertainty. (printout of GUM Wb file)

Model Equation:

{ The main equation }

pK_a=pH_c-log(An/HA);

HA=V_{a0}*C_{a0}/(V_{a0}+V_t)-An;

An=10^(-pH_c)+V_t*C_{t0}/(V_{a0}+V_t)-K_w/(10^(-pH_c))-C_{HCO3}-2*C_{CO3}-A_1-A_2-A_3;

K_w=10^(-13.78+0.034*(t_{meas}-const(t_{meas})));

{ Initial concentration of the acid solution taken for pKa measurement }

C_{a0}=1000*m_a*P/V_s/M;

M=n_1*C+n_2*H+n_3*O;

m_a=$m_a$rep+$m_a$read+$m_a$drift+$m_a$electrost;

V_s=$V_s$cal+$V_s$temp+$V_s$fill;

$V_s$temp=$V_s$cal*γ*Δt_{Vs_temp};

{ Contents of impurities in the acid solution

It is assumed that the titrated acid contains some impurities. As a model approach, it is assumed that there are three

different impurities with different acidities.}

C_{A1H_0}=1000*m_a*P_{A1H}/V_s/M;

C_{A2H_0}=1000*m_a*P_{A2H}/V_s/M;

C_{A3H_0}=1000*m_a*P_{A3H}/V_s/M;

K_{A1H} = 10 ^ (-pK_{A1H});

K_{A2H} = 10 ^ (-pK_{A2H});

K_{A3H} = 10 ^ (-pK_{A3H});

A_1=C_{A1H_0}*V_{a0}/(V_{a0}+V_t)/(1+(10^(-pH_c))/K_{A1H});

A_2=C_{A2H_0}*V_{a0}/(V_{a0}+V_t)/(1+(10^(-pH_c))/K_{A2H});

A_3=C_{A3H_0}*V_{a0}/(V_{a0}+V_t)/(1+(10^(-pH_c))/K_{A3H});

{ Volume of the investigated acid solution taken for titration

The real volume at the titration point under consideration is higher due to the added titrant }

$V_{a0}=V_{a0}cal+V_{a0}temp+V_{a0}rep;$

$V_{a0}cal=V_{a0}calrep+V_{a0}caltemp;$

$V_{a0}caltemp=V_{a0}calrep*\gamma*\Delta t_{Va0\_caltemp};$

$V_{a0}temp=V_{a0}calrep*\gamma*\Delta t_{Va0\_temp};$

{ Volume of the titrant added to the titration cell up to the titration point under consideration }

$V_t=V_tcal+V_ttemp+V_trep;$

$V_tcal=err/vol*V_trep;$

$V_ttemp=V_trep*\gamma*\Delta t_{Vt\_temp};$

{ Content of carbonate in the titrant }

$C_{HCO3}=C_{c0}*V_t/(V_{a0}+V_t)*K_{H2C}/(10^{(-pH_c)})/(1+K_{H2C}/(10^{(-pH_c)})+$

$K_{H2C}*K_{HC}/(10^{(-pH_c)}*10^{(-pH_c)}));$

$C_{CO3}=C_{c0}*V_t/(V_{a0}+V_t)*K_{HC}*K_{H2C}/(10^{(-pH_c)}*10^{(-pH_c)})/$

$(1+K_{H2C}/(10^{(-pH_c)})+K_{H2C}*K_{HC}/(10^{(-pH_c)}*10^{(-pH_c)}));$

{ pH of the measured solution }

$pH_c=(E_x-E_0)/s/(1+\alpha*(t_{meas}-t_{cal}));$

$E_x=E_xread+E_xdrift+E_xJP+E_xrep;$

Table 8. List of Quantities in the Mathematical Models of calculation of pK_a(I=0) and pK_a(I=0.1M KCl).

Quantity	Unit	Definition
pK_a		acidity constant of the acid under investigation
HA	mol/l	concentration of the undissociated acid molecules
An	mol/l	concentration of the anion
K_w	mol/l	autoprotolysis constant of water
C_{a0}	mol/l	concentration of the acid solution
P	unitless	purity of the the acid
M	g/mol	molecular mass of the acid
n_1	unitless	number of carbon atoms in the acid molecule
C	g/mol	atomic weight of carbon
n_2	unitless	number of hydrogen atoms in the acid molecule
H	g/mol	atomic weight of hydrogen
n_3	unitless	number of oxygen atoms in the acid molecule
O	g/mol	atomic weight of oxygen
m_a	g	mass of the acid taken for titration
$m_a$rep	g	value and repeatability uncertainty of the mass of the acid
$m_a$read	g	the readability (digital resolution) uncertainty of the balance scale
V_s	ml	the volume of the acid solution
$V_s$cal	ml	the value and calibration uncertainty of the volume of the acid solution
$V_s$temp	ml	the temparature uncertainty component of the volume of the acid solution
Δt_{Vs_temp}	C	the difference of calibration and usage temperatures of the flask
$V_s$fill	ml	the filling uncertainty component of the volume of the acid solution
γ	1/C	the coefficient of volume expansion of water
V_{a0}	ml	the volume of the acid solution that was initially taken for titration
pH_c		-log([H+]) value of the acid solution at the titration point at which the pKa value is found

Quantity	Unit	Definition
V_{a0}cal	ml	the value and calibration uncertainty component of the volume of the acid solution that was initially taken for titration
V_{a0}temp	ml	the uncertainty of volume of pipette caused by the operational temperature differing from the calibration temperature of the solution
Δt_{Va0_temp}	C	the difference of calibration and usage temperatures of the pipette
V_{a0}rep	ml	the repeatability of the volume delivered by the pipette
V_{a0}calrep	ml	the repeatability of the delivered volume during calibration of the pipette
V_{a0}caltemp	ml	uncertainty of volume of pipette coused by the solution temperature differing from the calibration temperature of the device during calibration
$\Delta t_{Va0\_caltemp}$	C	the solution temperature differing from the calibration temperature of the device during calibration
V_t	ml	volume of the titrant added to the titration cell using the piston burette
$V_t$cal	ml	calibration uncertainty of the piston burette
$V_t$temp	ml	the uncertainty of volume of the titrant added to the titration cell coused by temperature difference between the operational and calibration temperature of the burette
Δt_{Vt_temp}	C	temperature difference between the operational and calibration temperature of the burette
$V_t$rep	ml	repeatability uncertainty of the burette
err	ml	"absolute error" of the burette
vol	ml	full volume of the burette
C_{t0}	mol/l	concentration of titrant: sum of molar concentrations of KOH and K2CO3 in the titrant solution
A	(l/mol)½	constant from the Debye-Hückel theory
B	(l/mol)½/Å	constant from the Debye-Hückel theory
å	Å	ion size parameter from the Debye-Hückel theory
I_{00}	mol/l	interim quantity for iterative calculation of f_1
f_{000}	unitless	interim quantity for iterative calculation of f_1
f_{00}	unitless	interim quantity for iterative calculation of f_1
I_0	mol/l	interim quantity for iterative calculation of f_1
f_0	unitless	interim quantity for iterative calculation of f_1
f_1	unitless	activity coefficient for univalent ions
pH_x		pH value of the acid solution at the titration point at which the pKa value is found
α	1/C	temperature coefficient of the slope
E_x	mV	emf of the electrode system at the titration point at which the pKa value is found
$E_x$read	mV	uncertainty originating from the finite readability of the pH-meter scale of solution of investigation
$E_x$drift	mV	systematic deviations (bias) of the measured emf value from the actual value

Quantity	Unit	Definition
E_xJP	mV	uncertainty caused by the residual junction potential of solution of investigation
$E_x$rep	mV	repeatability of emf measurement of solution under investigation
s	mV	slope of the calibration line
ΣpH_iE_i		used for regression analysis
avgpH_i		used for regression analysis
avgE_i		used for regression analysis
ΣpH_ipH_i		used for regression analysis
E_1	mV	emf of the calibration buffer solution 1
E_1rep	mV	repeatability of emf measurement of buffer solution 1
E_1JP	mV	uncertainty caused by the residual junction potential in buffer solution 1
E_1read	mV	readability of the pH-meter scale of emf measurement of buffer solution 1
E_2	mV	emf of the calibration buffer solution 2
E_2rep	mV	repeatability of emf measurement of buffer solution 2
E_2JP	mV	uncertainty caused by the residual junction potential in buffer solution 2
E_2read	mV	readability of the pH-meter scale of emf measurement of buffer solution 2
E_3	mV	emf of the calibration buffer solution 3
E_3rep	mV	repeatability of emf measurement of buffer solution 3
E_3JP	mV	uncertainty caused by the residual junction potential in buffer solution 3
E_3read	mV	readability of the pH-meter scale of emf measurement of buffer solution 3
pH_1		pH of the calibration buffer solution 1
pH_1acc		uncertainty arising from the limited accuracy of the pH values of the buffer solution 1
pH_1temp		uncertainty caused by the dependence of the pH value of the standard on temperature in buffer solution 1
pH_2		pH of the calibration buffer solution 2
pH_2acc		uncertainty arising from the limited accuracy of the pH values of the buffer solution 2
pH_2temp		uncertainty caused by the dependence of the pH value of the standard on temperature in buffer solution 2
pH_3		pH of the calibration buffer solution 3
pH_3acc		uncertainty arising from the limited accuracy of the pH values of the buffer solution 3
pH_3temp		uncertainty caused by the dependence of the pH value of the standard on temperature in buffer solution 3
θ_1		temperature coefficient of the standard buffer solution 1
t_{cal}	C	temperature at which the electrode system was calibrated
θ_2		temperature coefficient of the standard buffer solution 2
θ_3		temperature coefficient of the standard buffer solution 3
I	mol/l	interim quantity for iterative calculation of f_2

Quantity	Unit	Definition
f_2	unitless	activity coefficient for doubly charged ions
C_{HCO3}	mol/l	concentration of hydrogencarbonate ions
C_{CO3}	mol/l	concentration of carbonate ions
A_1	mol/l	concentration of dissociated contaminant A1H in the titration cell
A_2	mol/l	concentration of dissociated contaminant A2H in the titration cell
A_3	mol/l	concentration of dissociated contaminant A3H in the titration cell
C_{c0}	mol/l	overall concentration of carbonate ($C_{HCO3} + C_{CO3}$)
K_{H2C}	mol/l	the first dissocation constant of carbonic acid
K_{HC}	mol/l	the second dissocation constant of carbonic acid
C_{A1H_0}	mol/l	concentration of contaminant A1H
K_{A1H}	mol/l	dissocation constant of contaminant A1H
C_{A2H_0}	mol/l	concentration of contaminant A2H
K_{A2H}	mol/l	dissocation constant of contaminant A2H
C_{A3H_0}	mol/l	concentration of contaminant A3H
K_{A3H}	mol/l	dissocation constant of contaminant A3H
P_{A1H}	unitless	content of the contaminant A1H
P_{A2H}	unitless	content of the contaminant A2H
P_{A3H}	unitless	content of the contaminant A3H
t_{meas}	C	measurement temperature
pK_{A1H}		pKa of the contaminant A1H
pK_{A2H}		pKa of the contaminant A2H
pK_{A3H}		pKa of the contaminant A3H
a_H	mol/l	activity of the hydrogen ions in the measured solution
E_0	mV	standard potentcial of electrode system
$m_a$drift	g	drift of the balance
$m_a$electrost	g	interference from electrostatic disturbances
$logf_1$		logarithm of activity coefficient for singly charged ions
$\Delta logf_{1SIT}$		uncertainty given by SIT program
$logf_2$		logarithm of activity coefficient for doubly charged ions
$\Delta logf_{2SIT}$		uncertainty given by SIT program
$\Delta logf_{1DH}$		difference between $logf_1$ calculated by Debye-Hückel theory and SIT program
$\Delta logf_{2DH}$		difference between $logf_2$ calculated by Debye-Hückel theory and SIT program

4.4. Results

The uncertainty budgets for the pK_a(I=0) and pK_a(I=0.1M KCl) values are presented in Table 9. Table 9 omits some of the input parameters that have marginal influence and some input parameters have been grouped together to provide a better overview. Detailed information will be available in the calculation files provided in the ESM of publication III when it will be published on the web.

Table 9. Uncertainty budgets and combined uncertainties for the pK_a(I=0) and pK_a(I=0.1M KCl) values.

	pK_a (I=0.1M KCl)	pK_a(I=0)	pK_a(I=0.1M KCl) without the unc of V_{HCl}	pK_a(I=0) without the unc E_xJP
	Uncertainty contributions of input quantities (%)[a]			
pH$_1$		31.2		47.8
pH$_2$		5.3		8.1
pH$_3$		0.9		1.3
E_1		3.7		5.7
E_2		0.7		0.9
E_3		0.0		0.1
E_0 + s[b]	*38.0*		*12.1*	
E_0 + s // V_{HCl}	21.5		0.0	
E_0 + s // V_{KCl}	1.4		0.9	
E_0 + s // C_{HCl}	1.5		1.4	
E_0 + s // C_{t0}	0.9		0.8	
E_0 + s // pK_w	2.0		1.1	
E_0 + s // range	6.8		4.4	
E_0 + s // V_t	4.0		3.5	
E_x[c]	*23.9*	*47.0*	*33.8*	*19.0*
$E_x$rep	6.2	3.0	8.8	4.6
$E_x$read	0.4	0.0	0.5	0.1
$E_x$drift	17.3	9.4	24.5	14.3
E_xJP	0.0	34.6	0.0	0.0
t_{cal}	0.9	0.3	1.3	0.4
t_{meas}	0.9	0.2	1.3	0.4
m_a	3.3	0.9	4.8	1.5
V_s	0.3	0.0	0.5	0.0
P	7.8	2.1	11.1	3.2
P_{A1H}	15.8	3.9	22.4	6.0
P_{A2H}	0.0	0.0	0.0	0.0
P_{A3H}	0.0	0.0	0.0	0.0
pK_{A1H}	1.3	0.1	1.8	0.2
pK_{A2H}	0.0	0.0	0.0	0.0
pK_{A3H}	0.0	0.0	0.0	0.0
C_{t0}	0.6	0.2	0.8	0.2
V_{a0}	1.6	0.3	2.3	0.5
V_t	5.5	1.7	7.7	2.6
C_{c0}	0.0	0.0	0.0	0.0
logf_1		0.8		1.2
logf_2		0.0		0.0

	pK_a (I=0.1M KCl)	pK_a(I=0)	pK_a(I=0.1M KCl) without the unc of V_{HCl}	pK_a(I=0) without the unc E_xJP
	pK_a values and their uncertainties			
pK_a	4.019	4.217	4.019	4.217
***u*$_c$(pK_a)**	0.0086	0.017	0.0072	0.014
***Eff. df*[d]**	33	2100	120	880
k (95.45% CI)	2.09	2.00	2.00	2.00
***U*(pK_a)**	0.018	0.035	0.014	0.028

[a] The uncertainty contributions in percentages (found according to eq 28) are given for the uncertainty of the respective pK_a value. [b] The contribution "E_0 + s" is applicable only to pK_a(I=0.1M KCl) and is the sum of the 7 subsequent contributions. [c] The contribution of E_x is the sum of the subsequent 4 contributions. [d] The significantly larger number of degrees of freedom in the case of the pK_a (I=0) is due to the fact that the most significantly contributing input quantities –the residual liquid junction potential and the accuracy of the pH 4.00 buffer solution – have been estimated as rectangularly distributed, meaning that they have infinite number of degrees of freedom by definition.

4.5. Discussion

It is first of all important to stress that the purpose was not to compare the pK_a(I=0) and pK_a(I=0.1M KCl) values. These pK_a values refer to different standard states and are thus not directly comparable[20,21]. Instead the uncertainties and the uncertainty budgets (the relative contributions of different input quantities on the uncertainty of the result) were compared.

Also, it is important to keep in mind that the numerical data given in Table 9 and discussed below apply strictly speaking only to the particular example (benzoic acid of the purity that was used) using the particular experimental setup (glassware, chemicals, titrator) that is described in the Experimental section. However, the main trends and the logic of the calculations remain the same and can be used for other examples. Also, with the aid of the calculation files that are provided in the ESM interested user can explore changes in uncertainty and in the uncertainty budgets that take place when changes to the experimental parameters are introduced.

The main contributors to the uncertainty budget are in both cases the uncertainty components due to the hydrogen ion concentration/activity measurement. These contributions are: 89.3% and 63.7% for the pK_a(I=0) and pK_a(I=0.1M KCl), respectively. The different size of these contributions reflects the different accuracy of measuring the activity and concentration of the hydrogen ion. The structure of these contributions is different, as can be expected keeping in mind the different measurand – log[H^+] and pH – in these two cases and the different approach to calibrate the pH meter.

The main uncertainty contribution to the pH measurement arises from the residual liquid junction potential (34.6%) and the uncertainty of the pH values of the calibration buffer solutions (37.4%). The contributions of the calibration buffer solutions are very different being 31.2%, 5.3% and 0.9% for the buffer solutions 4.00, 6.99 and 9.95, respectively. The heavily dominating contribution of the first buffer solution is due to the vicinity of the determined pK_a value to pH 4.00. Varying the determined pK_a value between 4 and 9 (keeping all other influencing factors the same) leads to a large change of the relative contributions of the different calibration buffer solutions and also to some change of their overall contribution. This is due to the fact that the combined uncertainty of the pK_a value varies in the following range 0.018 (pK_a=4), 0.014 (pK_a=7), 0.021 (pK_a=10). Using two-point calibrations with buffer solutions 4.00 and 6.99, 4.00 and 9.95, the expanded uncertainty increases only by 0.001 pK_a units in both cases. If, however, higher quality buffer solutions with stated uncertainties of ± 0.005 pH units would be used then some decrease of the uncertainty would be obtained: the expanded uncertainty would decrease from 0.035 to 0.028 pK_a units.

The main uncertainty contribution to the log[H^+] is the combined contribution of the calibration parameters – slope s and the intercept E_0 – of the pH electrode system (38.0%). It is impossible to separate this contribution into the sub-contributions of s and E_0, because strong correlation exists between these two quantities (R = –0.87, see publication III for details about its estimation). However, these parameters are obtained in turn from a titration of HCl solution with KOH and it is possible to quantify the contributions of the different uncertainty sources of that titration. There are altogether 7 such uncertainty sources and the information about their contributions is presented in Table 9. The main contributor is the uncertainty of the volume of HCl that is transferred to the titration cell (21.5%) followed by the uncertainty introduced by the ill-defined range of titration points taken for the calibration using the GLEE software (6.8%). The rest are of minor significance.

The pH electrode in our hands worked in a more stable manner in 0.1M KCl solution than in the low ionic strength benzoic acid solution. For this reason the repeatability and drift uncertainty components of E_x are different for pK_a(I=0) and pK_a(I=0.1M KCl). The following uncertainty estimates values are used for pK_a(I=0): E_x rep = 0.17 mV and E_x drift = 0.3 mV. The following uncertainty estimates are used for pK_a(I=0.1M KCl): E_x rep = 0.12 mV and E_x drift = 0.2 mV. According to the literature data [5,6] and our own experience the reason for this is twofold: (1) The conductivity of the solution with 0.1 M KCl background electrolyte is higher and (2) the liquid junction potential is stable and constant.

The remaining contribution in the uncertainty of the pK_a(I=0.1M KCl) comes for the most part from the purity of the acid P (around 24.9%), volume measurement of the titrant V_t (5.5%) and uncertainty of mass measurement of benzoic acid m_a (3.3%). The same uncertainty sources are also the remaining

main contributors to the uncertainty of pK_a(I=0) with relative contributions 6.1%, 1.7% and 0.9%. These strongly different relative contributions of the same effects that in absolute terms have almost identical uncertainties is due to the strongly different contribution of hydrogen ion activity/concentration measurement (89.3% vs 63.7%).

The purity of the acid under investigation P is in this treatment not related just to inert compounds but involves also contaminants with acidic properties. In the application example it has been assumed that the acid contains in addition to inert impurities also three different kinds of acidic impurities with different acidities (pK_a values around 2.5, 7 and 10). Concentrations and acidities of all those acidic impurities enter the measurement equations and are thus taken into account. As is seen from Table 9, impurities with different pK_a values have different influence on the final result. The impurity with the lowest pK_a value has the highest influence since the others behave in this example more or less as inert impurities.

The uncertainty of V_t is mainly determined by the accuracy of the mechanical burette. The effect of contamination of the titrant with carbonate at the level of carbonate, that was in the titrant in the example almost insignificant.

The mass uncertainty of benzoic acid is almost entirely determined by the effect of electrostatic disturbances that occur when weighing very dry substances. All other uncertainty sources can in principle be neglected.

The uncertainty of pK_a(I=0) is almost two times higher than the uncertainty of pK_a(I=0.1M KCl). The following effects mainly contribute to this: (1) the large uncertainty contributions to the pK_a(I=0) that arises from the ill-defined residual liquid junction potential E_xJP and (2) the higher repeatability and drift contributions in the case of the solutions with low ionic strength as compared to the solutions with 0.1M KCl background electrolyte.

If the uncertainty due to the residual liquid junction potential E_xJP were absent then the expanded uncertainty of pK_a(I=0) would decrease from 0.035 to 0.028 pK_a units and would still be significantly higher than that of the pK_a(I=0.1M KCl). The uncertainty of the pK_a(I=0.1M KCl) in turn is heavily dependent on the uncertainty of the HCl volume taken for electrode calibration. If weighing could be used instead then the uncertainty would decrease from 0.018 to 0.014 pK_a units.

Interestingly, the two totally different calibration approaches – with standard buffer solutions and with acid-base titration – lead to rather similar relative uncertainty contributions: 42.3% and 39.8% in the case of pK_a(I=0) and pK_a(I=0.1M KCl), respectively.

4.6. Conclusions

From this work a generalization can be made that the uncertainty of the pK_a(I=0.1M KCl) values tends to be lower than that of the pK_a(I=0). The main reasons are: (1) the uncertainty of the residual liquid junction potential is virtually absent in the case of pK_a(I=0.1M KCl) due to the similar high concentration of background electrolyte in the calibration solutions and measured solution and (2) The electrode system is more stable in solutions containing the 0.1 M KCl background electrolyte and more stable readings can be obtained.

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6. SUMMARY

As a result of this work uncertainty estimation procedures based on mathematical models of pH and pK_a potentiometric measurements were developed. The procedures involve identification and quantification of individual uncertainty sources according to the ISO GUM approach.

In the first part (section 2) of this work the sources of uncertainty in pH measurement using the most widespread potentiometric equipment (glass electrode and reference electrode with liquid junction) were studied and a generally applicable procedure for calculating the uncertainty of such pH measurement was developed. The procedure was applied to a real-life measurement and the obtained uncertainty budget was used for discussing the contributions of various uncertainty sources and their dependence on experimental conditions.

In the second part (section 3) of this work the uncertainty sources in measurement of pK_a value having I=0 as the standard state were systematically studied. A generally applicable procedure for calculating the uncertainty of such pK_a measurement was developed. The procedure gives uncertainty separately for each point of the titration curve. A novel approach was used for taking into account the purity of the acid: the impurities are not treated as inert compounds only: their possible acidic dissociation is also taken into account.

In the third part (section 4) of this work the uncertainty estimation procedures for pK_a values having I=0 and I=0.1 M KCl as the standard states were elaborated and the uncertainty budgets of those pK_a values based on different standard states were compared.

No single uncertainty estimate can be ascribed to a pH measurement procedure: the uncertainty of pH strongly depends on changes in experimental details and on the pH value itself. The uncertainty is the lowest near the isopotential point and in the center of the calibration line and can increase by a factor of 2 (depending on the details of the measurement procedure) when moving from around pH 7 to around pH 2 or 11. Therefore it is necessary to estimate the uncertainty separately for each measurement. For routine pH measurement the uncertainty cannot be significantly reduced by using more accurate standard solutions than ± 0.02 pH units – the uncertainty improvement is small. A major problem in estimating the uncertainty of pH is the residual junction potential, which is almost impossible to take rigorously into account.

The uncertainty of pK_a measurement is dependent on the amount of titrant added, being the lowest in the central part of the titration curve. The main contributors to the uncertainty budget of pK_a(I=0) and pK_a(I=0.1M KCl) are the measurements of hydrogen ion activity and concentration, respectively. However, due to the different nature of hydrogen ion activity and concentration measurements and fully different calibration approach the structure of their uncertainty budgets is different.

The remaining uncertainty contributions arise mostly from the limited purity of the acid. From this work a generalization can be made that the uncertainty of the pK_a(I=0.1M KCl) values tends to be lower than that of the pK_a(I=0). The main reasons are: (1) the uncertainty due to the residual liquid junction potential is absent in the case of pK_a(I=0.1M KCl) because of the similar high concentration of background electrolyte in the calibration solutions and measured solution and (2) the electrode system is more stable in solutions containing the 0.1 M KCl background electrolyte and more stable readings can be obtained.

The applicability of the ISO GUM to these uncertainty estimations was confirmed.

7. KOKKUVÕTE

Potentsiomeetrilisel meetodil mõõdetud pH ja pK_a mõõtemääramatuse hindamine

Praegu peetakse väga oluliseks, et iga mõõdetud tulemus tuleb anda koos määramatuse hinnanguga – mõõtetulemusele omistatavate väärtuste hajuvust iseloomustava parameetriga. Käesoleva töö tulemusena loodi matemaatilistel mudelitel põhinevad määramatuse hindamise protseduurid pH ja pK_a väärtuste potentsiomeetriliseks mõõtmiseks. Protseduurid baseeruvad ISO GUM meetodil ning sisaldavad määramatuse allikate identifitseerimist ja kvantiseerimist.

Töö esimeses osas (section 2) identifitseeriti ja analüüsiti määramatuse allikaid tavataseme potentsiomeetrilise pH mõõtmise juures (kasutades klaaselektroodi ja poorse ühendusega võrdluselektroodi) ning töötati välja üldkasutatav protseduur sellise pH mõõtmise määramatuse hindamiseks. Seda protseduuri rakendati reaalsetele mõõtmistele ja saadud määramatuse allikate panuste struktuuri analüüsiti mõõtmistingimuste seisukohast.

Töö teises osas (section 3) uuriti süstemaatiliselt määramatuse allikaid pK_a ioontugevusel I=0 mõõtmiste juures. Töötati välja üldine protseduur pK_a mõõtemääramatuse arvutamiseks. Protseduuri abil on võimalik leida mõõtemääramatus eraldi iga tiitrimiskõvera punkti jaoks. Tiitritava happe puhtus võeti arvesse uudsel moel: lisandeid ei käsitletud ainult inertsete lisanditena vaid võeti arvesse ka nende võimalik happeline dissotsieerumine.

Töö kolmandas osas (section 4) töötati välja mõõtemääramatuse protseduurid erinevatel ioontugevustel I=0 ja I=0.1 M KCl mõõdetud pK_a väärtustele ja võrreldi saadud määramatuste allikate panuste struktuure.

pH mõõtmise protseduurile ei ole võimalik esitada ühtset määramatuse hinnangut: pH mõõtemääramatus sõltub oluliselt mõõtmise läbiviimise tingimistest ja pH väärtusest endast. Määramatus on madalaim isopotentsiaalipunkti läheduses ja kalibratsioonisirge keskel ja võib kasvada 2 korda (sõltuvalt mõõtmise tingimutest) pH 2 ja 11 juures. Niisiis on vajalik hinnata mõõtemääramatus eraldi igale mõõtmisele. Rutiinse pH mõõtmise juures ei ole määramatust võimalik märkimisväärselt alandada kui kasutada täpsema pH väärtusega puhvreid kui ± 0.02 pH ühikut. Kõige keerulisem probleem pH mõõtemäärmatuse hindamise juures on jääkdifusioonipotentsiaali mittekonstantsusest tingitud määramatuse arvessevõtmine.

pK_a mõõtemääramatus sõltub lisatud titrandi ruumalast, olles madalaim tiitrimiskõvera keskpaigas. Kõige olulisema panuse määramatusse pK_a(I=0) ja pK_a(I=0.1M KCl) puhul annab vastavalt vesinikiooni aktiivsuse ja kontsentratsiooni mõõtmine. Kuna need mõõtmised viiakse läbi erinevates standardtingimustes ja kalibratsiooniprotseduur on erinev, siis on vastavate määramatuse

panuste struktuur täiesti erinev. Olulisuselt järgneb tiitritava happe ebapuhtusest tingitud määramatus.

Tehtud tööst saab järeldada, et pK_a(I=0.1M KCl) määramatus on väiksem kui pK_a(I=0) määramatus. Peamised põhjused on järgmised: (1) jääkdifusioonipotentsiaalist tingitud määramatuse puudumine pK_a(I=0.1M KCl) mõõtmise juures kuna kalibratsioonilahuste ja mõõdetava lahuse ioontugevused on sama väärtusega ja mõõtmised viiakse läbi kõrgel ioontugevasul lisades foonelektrolüüti; (2) elektroodisüsteem on stabiilsem lahustes, mis sisaldavad 0.1 M KCl foonelektrolüüti ja on võimalik saada stabiilsemad mõõteriista näidud.

Töös leidis kinnitust ISO GUM meetodi sobivus pH ja pK_a väärtuste määramatuse hindamise jaoks.

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PUBLICATIONS

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Acidity Constant in different media I=0 and I=0.1M KCl:
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Acidity Constant in different media I=0 and I=0.1M KCl: from the Uncertainty Perspective

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1. ABSTRACT

Procedures for estimation of measurement uncertainty for acidity constant K_a (or pK_a value) in different media I=0 and I=0.1 mol L^{-1} KCl determined by potentiometric titration are presented. The uncertainty budgets (the relative contributions of different input quantities on the uncertainty of the result) of the pK_a(I=0) and pK_a(I=0.1 mol L^{-1} KCl) values are compared. Unlike the values, their uncertainties and uncertainty budgets are comparable.

The uncertainty estimation procedures are based on mathematical models of pK_a measurement and involve identification and quantification of individual uncertainty sources according to the ISO GUM approach. The mathematical model involves 52 and 48 input parameters for pK_a(I=0) and pK_a(I=0.1 mol L^{-1} KCl), respectively. The relative importance of different uncertainty sources is discussed. The main contributors to the uncertainty budget are in both cases the uncertainty components due to the hydrogen ion concentration/activity measurement, 63.7% and 89.3%, respectively. The remaining uncertainty contributions arise mostly from the limited purity of the acid. From this work a generalization can be made that the uncertainty of the pK_a(I=0.1 mol L^{-1} KCl) values tends to be lower than that of the pK_a(I=0). The main reasons are: (1) the uncertainty due to the residual liquid junction potential is nominally absent in the case of pK_a(I=0.1 mol L^{-1} KCl) because of the similar high concentration of background electrolyte in the calibration solutions and measured solution and (2) the electrode system is more stable in solutions containing the 0.1 mol L^{-1} KCl background electrolyte and more stable readings can be obtained.

Keywords: dissociation constant, pK_a value, uncertainty, ISO GUM, potentiometric titration.

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2. INTRODUCTION

In recent years the quality of results of chemical measurements – Metrology in Chemistry (traceability of results, measurement uncertainty, etc) – has become an increasingly important topic. It is reflected by the growing number of publications, conferences, etc [1–3]. One of the main points that is now widely recognized is that every measurement result should be accompanied by an estimate of uncertainty – a parameter of the result that characterizes the dispersion of the values that could reasonably be attributed to the measurand [2,4].

The dissociation constant K_a or its negative decadic logarithm pK_a value is one of the most important physicochemical characteristics of a compound which has acidic (or basic) properties. Reliable pK_a data are indispensable in analytical chemistry, biochemistry, chemical technology, etc. A huge amount of pK_a data has been reported in the literature and collected into several compilations [5–9]. The literature data in these compilations was published prior to the GUM [2] and that GUM-compliant uncertainty data are not available in these compilations.

There is one additional aspect that is stressed less often than it should be: in broad terms the pK_a data that are available are divided into two categories:

1) pK_a values having infinite dilution as the standard state. Sometimes these are also called thermodynamic pK_a values. Their measurement involves either extrapolation of the values found at finite ionic strengths to zero ionic strength or (more often) calculation of the activity coefficients using some model approach, such as the Debye-Hückel theory, and expressing the pK_a via activities. In this work we denote them as pK_a (I=0)
2) pK_a values having 0.1 mol L^{-1} KCl (or 0.1 mol·kg^{-1} or other specified concentration) as the standard state. These are determined in solutions containing 0.1 mol L^{-1} KCl background electrolyte that generates constant and sufficiently high ionic strength to the solution. Sometimes these are called concentrational pK_a values as opposed to thermodynamic ones. In reality they are also thermodynamic, but with a standard state that is not infinite dilution but 0.1 mol L^{-1} KCl. In this work we denote them as pK_a (I=0.1M[b] KCl)

Both types of pK_a values have their advantages and ample data on both is available in the literature. Most importantly – these values cannot be directly compared to each other. It is unfortunate that the same symbol pK_a is used for both values. Hence it is sometimes difficult to distinguish which of the two has been used.

[b] The symbol M is used as a shortcut instead of mol L^{-1} in the much-used prefix pKa(I=0.1 mol L^{-1})

Potentiometric titration methods of pK_a determination using the glass electrode are the most widely used for both pK_a(I=0) and pK_a(I=0.1M KCl) values and the art of such pK_a measurement can be considered mature. Numerous methodologies have been described, starting from those described in the classic books [10–12] and finishing with the modern computational approaches (for example MINIPOT [13], PHCONST [13], HYPERQUAD [14], SUPERQUAD [15], PKPOT [16], MINIGLASS [17] etc) for calculation and refinement of pK_a values from potentiometric data.

Efforts have also been devoted to investigating the sources of uncertainty of both types of pK_a values. The various computer programs mentioned above are very useful in this respect. They can be used in the search of systematic errors, because many parameters are adjustable. Standard errors of the parameters are obtained by weighted or unweighted non-linear regression and curve-fitting [13–20]. The influence of various uncertainty sources in pH and titrant volume measurements on the accuracy of acid-base titration has been studied using logarithmic approximation functions by Kropotov [21]. The uncertainty of titration equivalence point (predict values and detect systematic errors) was investigated by a graphical method using spreadsheets by Schwartz [22]. Gran plots can also be used to determine titration equivalence point [23] and they are useful to assess the extent of carbonate contamination in the alkaline titrant. The various sources of uncertainty have thus been investigated quite extensively. Also, recently [24] a procedure taking into account all uncertainty sources of a pK_a(I=0) value and propagating them (using the corresponding mathematical model) to give the combined uncertainty of the pK_a(I=0) value was published. No similar procedure is available for pK_a(I=0.1M KCl) values. Furthermore, to the best of our knowledge there is no comprehensive study available in the literature on comparison of the uncertainty budgets of these two types of pK_a values.

In this paper we present procedures of estimation of uncertainty of both pK_a(I=0) and pK_a(I=0.1M KCl) values determined by potentiometric titration and we compare the uncertainty budgets of the two types of pK_a values. We provide the realization of the uncertainty calculation in two different software packages – MS Excel and GUM Workbench – that are available in the Electronic Supplementary Material (ESM).

The uncertainty estimation procedures are based on mathematical models of pK_a measurement and involve identification and quantification of individual uncertainty sources according to the ISO GUM/EURACHEM approach [2,4]. This approach of uncertainty estimation consists of the following steps: 1) Specifying the measurand and definition of the mathematical model; 2) Identification of the sources of uncertainty; 3) Quantification of the uncertainty components; 4) Converting the uncertainty components to standard uncertainties; 5) Calculating the combined uncertainty. In this paper the section "Derivation of the Uncertainty Estimation Procedures" includes step 1, the section "Experimental" includes steps 2–4. The mathematical details of

calculating the combined uncertainty (step 5) are given in section "Calculating the combined uncertainty".

3. DERIVATION OF THE UNCERTAINTY ESTIMATION PROCEDURES

In order to be able to compare the uncertainty budgets of the pK_a(I=0) and pK_a(I=0.1M KCl) values, the calculation approaches have to be the same. In very broad terms there are two approaches for calculation of pK_a values from potentiometric titration curves. One (approach 1 below) is the approach described in the book of Albert and Serjeant [10,11] whereby a pK_a value is found for every point of the titration curve and the result is calculated as average of these values. This approach was also used in our previous work on the subject [24]. The more modern one (approach 2 below) is to find a single pK_a value from the titration curve by modeling the curve and then minimizing the sums of squares (often weighed) of the differences of the modeled curve from the experimental curve by adjusting the pK_a value (and possibly other parameters, such as concentrations). This approach has been used in several computer programs, which have been described in refs [13–17].

In this work we opt for approach 1 and present the uncertainty calculation for a single point (denoted below as x) on the titration curve, near the half-neutralization point. The advantages are simplicity and transparency for better following of the uncertainty propagation. It has been shown previously [24] that the uncertainty of a pK_a value determined as an average of several values obtained from different titration points is not significantly lower than a single-point pK_a value due to the strong correlation between the pK_a values from different points of the curve (similar influence of the systematic effects). Also, the principal conclusions drawn from uncertainty analysis of pK_a values obtained by approach 1 are applicable to pK_a values obtained from approach 2, because in the end the pK_a values are calculated from the same data with the same uncertainty sources.

The dissociation of a Brønsted acid HA refers to the simplified equation:

$$\mathrm{HA} \rightleftarrows \mathrm{H^+} + \mathrm{A^-} \tag{1}$$

The pK_a(I=0) is given by (quantities in eq (2) through (10) are defined after eq(10)):

$$K_\mathrm{a}(I=0) = \frac{a(\mathrm{H^+}) \cdot a(\mathrm{A^-})}{a(\mathrm{HA})}, \tag{2}$$

$$\mathrm{p}K_\mathrm{a}(I=0) = -\log K_\mathrm{a}(I=0) \tag{3}$$

The pH value in the titration point x is given by

$$\mathrm{pH_x} = \frac{E_\mathrm{x} - E_0}{s(1 + \alpha(t_\mathrm{meas} - t_\mathrm{cal}))} \tag{4}$$

and the pK_a(I=0) for that titration point is calculated as follows:

$$\mathrm{p}K_\mathrm{a}(I = 0) = \mathrm{pH_x} - \log\frac{[\mathrm{A^-}] \cdot f_1}{C_\mathrm{a} - [\mathrm{A^-}]} \tag{5}$$

where

$$\log f_1 = \frac{-A \cdot \sqrt{I}}{1 + B \cdot \mathring{a} \cdot \sqrt{I}} \tag{6}$$

In the case of pK_a(I=0.1M KCl) the respective equations are:

$$K_\mathrm{a}(I = 0.1\mathrm{M\ KCl}) = \frac{[\mathrm{H^+}] \cdot [\mathrm{A^-}]}{[\mathrm{HA}]} \tag{7}$$

$$\mathrm{p}K_\mathrm{a}(I = 0.1\mathrm{M\ KCl}) = -\log K_\mathrm{a}(I = 0.1\mathrm{M\ KCl}) \tag{8}$$

$$\mathrm{pH_c} = \frac{E_\mathrm{x} - E_0}{s(1 + \alpha(t_\mathrm{meas} - t_\mathrm{cal}))} \tag{9}$$

$$\mathrm{p}K_\mathrm{a}(I = 0.1\mathrm{M\ KCl}) = \mathrm{pH_c} - \log\frac{[\mathrm{A^-}]}{C_\mathrm{a} - [\mathrm{A^-}]} \tag{10}$$

where $a(\mathrm{H^+})$ and $[\mathrm{H^+}]$, $a(\mathrm{A^-})$ and $[\mathrm{A^-}]$, a(HA) and [HA] are the activities and concentrations of the hydrogen ion, the anion and the undissociated acid molecules, respectively. E_x is the electromotive force (emf) of the electrode system at point x of the titration curve, $\mathrm{pH_x}$ and $\mathrm{pH_c}$ are the measured pH and $-\log[\mathrm{H^+}]$ at that point in I=0 and I=0.1M KCl solutions, respectively. C_a is the total concentration of the benzoic acid in the titration cell. The activity coefficient of a singly charged ion f_1 in eq 6 is estimated from Debye-Hückel theory [25], where A and B are constants, $\mathring{a}$ is the mean distance of closest approach of the ions (ion size parameter) and I is the ionic strength of the solution. The activity coefficient of the neutral molecule HA is assumed to be 1, hence a(HA) is equal to [HA]. E_0 is the standard potential of the electrode system, s is the slope of the calibration line, α is the temperature coefficient of the slope [26], t_meas and t_cal are the measurement temperature and the calibration temperature. For the pK_a(I=0) value, the calibration parameters s and E_0 are found by calibrating the system using standard buffer solutions of known pH values $\mathrm{pH_i}$ having emf values $\mathrm{E_i}$. For the pK_a(I=0.1M KCl) value, the calibration was carried out in terms of hydrogen ion concentration by means of a strong acid – strong base titration in 0.1M KCl medium. The program GLEE

[27] was used for calculation of the calibration parameters. From the pH measurements and the amounts and concentrations of the solutions the $a(H^+)$ or $[H^+]$, the ratios $a(A^-)/[HA]$ or $[A^-]/[HA]$ can be calculated and a pK_a value can be calculated for every point of the titration curve. We deliberately use here the same quantity names E_x, s and E_0 for both $pK_a(I=0)$ and $pK_a(I=0.1M\ KCl)$, because these are by their essence the same quantities, only found under different conditions in these two cases. Since the calculation formulae are distinctly different this does not create any confusion.

The equations above present the core of the mathematical models used in this work. The full models for calculation the $pK_a(I=0)$ and $pK_a(I=0.1M\ KCl)$ value and their uncertainties are presented in Table 1 and Table 2, respectively. The explanations and definitions of all the quantities are presented in Table 3. The factors that are taken into account include all uncertainty sources related to weighing and volumetric operations, purities of the measured acid, carbonate content of the titrant, dilution effect from added titrant, and pH-related uncertainty sources, such as uncertainties of calibration parameters and uncertainty of pH measurement. Effects from the liquid-junction (diffusion) potential are not explicitly included in the equations 2 to 10 as separate quantities but are taken account as an uncertainty component.

4. EXPERIMENTAL

The overall uncertainty of the result arises from many sources, including measurement conditions, uncertainties of masses and volumetric equipment, reference values, etc. In this section we describe the experimental setup and the titration procedures and identify the uncertainty sources and quantify most of the uncertainty components. Some of the less influent uncertainty components have been left out of thorough consideration here and information about them can be found in the Electronic Supplementary Material (ESM) files. All the uncertainty contributions below are marked with the same symbols as used in Tables 1, 2, 3 and in the ESM.

Before the combined uncertainty calculation all uncertainty components are converted to the level of standard uncertainty. In this work we assume that the B-type uncertainties for which no information on distribution function is available and which are expressed with the "±" sign have rectangular (uniform) distribution [4]. In order to convert them to standard uncertainties they are divided by $\sqrt{3}$ [2,4].

It is important to stress that the uncertainty calculations presented here are meaningful only if the pH measurement and titration systems are in good working order (no leaks in the piston burette, no bad connections or electrical interference problems in pH measurement, etc.)

4.1. pK_a *determination procedure*

The pK_a values were determined using potentiometric titration procedure that was essentially the same for I=0 and I=0.1M KCl.

Calibration of pH meter for pK_a(I=0) measurement was carried out using 3 standard buffer solutions and the calibration parameters were calculated by three-point linear regression.

For the pK_a(I=0.1M KCl) value, the calibration procedure was carried out in terms of hydrogen ion concentration by means of a strong acid – strong base titration in 0.1M KCl medium and the program GLEE [27] was used for calculation of the calibration parameters.

The laboratory temperature was 25 ± 4°C during all the experimental work (preparing the solutions, calibrations, titrations). The solution was transferred to the thermostated measurement cell by pipette. Titration was carried out in a cell thermostated to 25.0 ± 0.1°C (t_{cal}, t_{meas}), maintaining an atmosphere of nitrogen over the solution and using a magnetic stirrer for stirring the solution. The titrant was generally added in 0.05 mL increments. The solution was stirred at constant speed (the same stirring speed was used for calibration and measurement). The piston burette and the pH meter were both controlled by in-house software (running in MS Excel environment). The software monitors the emf after each titrant addition and when the reading remains stable a new aliquot of titrant is added. The following stability criterion is used: the change in the mV reading is less than 0.1 mV during 4 seconds (the adequacy of this criterion was checked separately by waiting longer time). On an average 25 s was necessary for full stabilization of the reading of a titration point. This way the titration was completely automatic, eliminating the human interference. Each titration experiment lasted for around 0.5 hours.

4.2. Solutions and Volumetric Ware

Concentration of the benzoic acid solution. Two benzoic acid solutions were prepared: one for pK_a(I=0) measurement and the second for the pK_a(I=0.1M KCl) measurement. First solution was prepared using only deionized CO_2-free water. The second one was prepared in 0.1M KCl medium.

Purity of the benzoic acid P. The purity of the acid is handled the same way as described in ref 24. Carlo Erba, >99.5% benzoic acid was used. We interpret the purity P > 99.5% in a conservative way: we assume that the purity is rectangularly distributed between 99 and 100%, i.e. 99.5 ± 0.5%. In addition we attempt to take the acidic behaviour of the possible acidic impurities into account (impurities in acids are very often acidic). We use the following general approach to take the influence of the impurities into account:

1. We assume that there are 4 different types of impurities in HA: inert impurities and acidic impurities A_1H, A_2H, A_3H. The different acidic impurities are assumed to have different acidities. We assume that the benzoic acid

contains three different impurities A_1H, A_2H and A_3H with the following pK_a values: pK_{A1H}= 2.5 ± 2.0, pK_{A2H}= 7.0 ± 2.0 and pK_{A3H}= 10.0 ± 2.0. Their relative contents in benzoic acid (P_{A1H}, P_{A2H}, P_{A3H}) are 0.0035 ± 0.0035 (i.e 0.35% ± 0.35%). The very wide uncertainty ranges are used here because in reality we do not know what the impurities exactly are. It is assumed that what is left from the three acidic impurities are the inert impurities.

2. The acidic impurities generate concentrations C_{A1H_0}, C_{A2H_0} and C_{A3H_0} to the solution of the acid AH.
3. All these impurities taken together form the overall purity P (unitless, denotes the mass fraction of the substance that corresponds to HA) of HA.

Mass of the acid m_a. The benzoic acid was weighed on Sartorius BP 210 S digital analytical balances, resolution 0.1 mg. Four uncertainty sources were considered.

The uncertainty caused by rounding of the digital reading $m_a$read is half of the last digit: ± 0.00005 g.

Repeatability of the balance reading $m_a$rep has been experimentally determined at our laboratory as a standard deviation 0.00017 g. This value has been determined with taring included.

Interference from electrostatic disturbances $m_a$electrost. When weighing the standard substance, interference from electrostatics (instability of the reading) was quite serious and is thus taken into account as an uncertainty component. This uncertainty component is estimated as maximum 0.3% of the mass of the substance (assuming rectangular distribution). For the pK_a(I=0) the mass of benzoic acid was 0.1716 g making this uncertainty contribution ± 0.00053 g. For pK_a(I=0.1M KCl) the mass was 0.2239 g, making this uncertainty component ± 0.00067 g.

Drift of the balance $m_a$drift. This uncertainty contribution refers to the effect that mass of the same body is different in different times of the day due to changes in the temperature of the laboratory, etc. Our balance did not show detectable drift under our conditions.

Molar mass of the benzoic acid M. The uncertainty in the molar mass of the compound can be determined by combining the uncertainties of the atomic weights of its constituent elements. A table of atomic weights including uncertainty estimates has been published [36]. Atomic masses of C, H and O are 12.0107, 1.00794 and 15.9994 g mol L^{-1}, respectively and standard uncertainties of them are 0.0004, 0.000035 and 0.00015 g mol^{-1} (converted to standard uncertainties from the k=2 uncertainties given in ref 36.

Volume of the benzoic acid solution V_s.

Calibration uncertainty $V_s$cal. The solution was prepared in 250.00 ± 0.15 mL (at 20°C) volumetric flask (Brand).

Filling uncertainty $V_s$fill. If the flask is filled drop by drop, according to our experience, the uncertainty of filling is ± 2 drops. We take 0.03 mL as an average volume of a drop.

Temperature effect to the volume of solution $V_s$temp. Our laboratory is not thermostated and we estimate its temperature as 20 ± 4°C. The estimation bases on long-term experience from the laboratory.

Volume of the investigated acid solution taken for titration V_{a0}. The solution was transferred to the measurement cell by a 50 mL pipette (Brand).

The uncertainty in the stated volume of the pipette V_{a0}cal. It has two major sources: the standard deviation of the mean volume obtained from repetitive calibrations V_{a0}calrep and the uncertainty due to temperature effect V_{a0}caltemp. The standard deviation of average of the volume is used as V_{a0}calrep (0.019 mL). The pipette was calibrated at 20 ± 2°C. The temperature uncertainty is estimated based on long-term experience from the laboratory. Temperature is more closely controlled during calibration than during routine measurement.

The variability (or repeatability) of the delivered volume V_{a0}rep. Repeatability determined in-house using 10 parallel measurements and the standard deviation of the volume obtained from calibration data is used as the estimate of this repeatability.

The temperature component of the uncertainty V_{a0}temp. The solution temperature differing from the calibration temperature of the device. The temperature in the laboratory was 20 ± 4°C during the preparation and transferring of solutions. The estimation bases on long-term experience from the laboratory. The uncertainty of this component was calculated as described in ref 4.

The titrant volume V_t. A 5 mL (burette capacity) piston burette "Liquid Handling Station LHS 300/600" (Brand GmbH) was used for titration. The titrant was delivered to the cell by means of a PTFE tubing equipped with a PTFE nozzle. The nozzle is immersed into the solution throughout the titration. We have found that diffusion of the titrant solution from the tubing to the solution is negligible, because the hole in the nozzle is very fine. In mechanical burettes like ours air bubbles develop sometimes. This was carefully checked and made sure that no air bubbles were present during the experiment.

Repeatability of the burette $V_t$rep. We take the "repeating error" ± 0.005 mL given in the manual of the piston burette as the estimate of the repeatability uncertainty.

The calibration uncertainty of the piston burette $V_t$cal. We use the "absolute error" given in the manual of the burette (quantity "err" in Tables 1 and 2) as the estimate of the calibration uncertainty of the burette. The absolute error is given for the full volume of the burette (5 mL) and is ± 0.015 mL. We assume that it is mainly due to the variability of the inner diameter of the glass cylinder of the burette. Therefore the uncertainty is proportional to the delivered volume and different for each point.

Temperature uncertainty $V_t$temp. The uncertainty caused by the difference between the operational and calibration temperature of the burette. The burette

at the factory has been calibrated at 20°C and the temperature in our laboratory during the measurements was 20±4°C (assuming rectangular distribution).

Concentration of titrant C_{t0}. Solution of 0.1000M KOH (Merck) was used as the titrant. Its uncertainty as provided by the supplier was ±0.1%. This uncertainty has been given by the manufacturer after titrant standardization but it is clear that after opening the bottle and transporting it to the measurement system, the uncertainty increases. Taking into account all those possible uncertainty sources, we evaluate this additional uncertainty as ±0.1%. With both these components we assume rectangular distributions. The overall uncertainty is thus ±0.2% and the distribution is triangular. In concentration units that is ±0.0002 mol L^{-1}. Care was taken not to contaminate the titrant with CO_2. A tube filled with ascarite was mounted on the titrant bottle for this purpose.

Content of carbonate in the titrant C_{c0}. The carbonate content of the titrant was found using the electrode calibration program GLEE. It was found to be 1.59% of titrant concentration (percentage of molar concentration of K_2CO_3 from the molar concentration of KOH). It is conservatively assumed in this work that the carbonate content of the titrant can be anywhere between 0% and 3.18%, giving C_{c0}= 0.00159 mol L^{-1}, with the uncertainty ± 0.00159 mol L^{-1}.

4.3. pH *meter and electrode system*

Readability of pH-meter scale $E_x$read, $E_i$read. Metrohm 713 pH meter was used in this study. The meter has digital display with resolution of 0.1 mV in the emf measurement mode. The error due to the finite readability of pH meter's display is at most half of the last digit, that is ± 0.05 mV.

Repeatability of emf measurement $E_x$rep, $E_i$rep. According to the manual of the meter, the "error limits" of the meter are ± 0.2 mV in the mV mode. In our hands the electrode system worked in a more stable and reproducible manner in the 0.1 M KCl solution than in benzoic acid solution where no background electrolyte was added. In the case of the 0.1M KCl background electrolyte the repeatability of the measurement is not noticeably higher than the repeatability caused by the meter only. Thus in the case of pK_a (I=0.1M KCl) we use the ±0.2 mV as the estimate of repeatability. This estimate is also valid for the calibration buffer measurements (the $E_i$rep components) in the case of pK_a (I=0) measurement. In the case of the I=0 medium the higher repeatability uncertainty contribution has been assumed. For the pK_a (I=0) determination we, based on the long-term experience from our laboratory, increased this uncertainty contribution to ±0.3 mV.

Drift of the electrode system $E_x$drift. The drift of the instrument during the time from calibration to measurement will be taken into account as an uncertainty component $E_x$drift. Similarly to the situation with repeatability the drift was also found more serious in the case of pK_a (I=0) than in the case of pK_a (I=0.1M KCl). According to our experience the standard uncertainty due to drift in I=0 solution is 0.3 mV and in I=0.1M KCl 0.2 mV.

Uncertainty caused by junction potential (JP) of the reference electrode E_iJP, E_xJP. Combined glass electrode Metrohm 6.0253.100 was used. The inner reference electrode is Ag/AgCl electrode in 3 M KCl solution with porous liquid junction. As it is known, the diffusion potential in the liquid junction of the reference electrode is not exactly the same in all solutions. The residual liquid junction potential (RLJP), although one of the most important components of uncertainty, is very difficult to quantify (*vide supra*). Based on the estimates published in the literature [25, 28, 29–31] we use the following estimate for the pH standards: ± 0.006 in pH units, it makes 0.2 mV (E_iJP) and the assumption that no extreme samples are measured we use an estimate of ± 1 mV (E_xJP) for the sample. Depending on the type of the liquid junction it is possible that the RLJP could be underestimated.

In the case of measuring log[H^+] (pH_c) in the 0.1M KCl medium and if the pH_c measurements are done in the pH_c range 2.5 to 11.3 then the JP supposed to be constant and have the same value during the calibration and measurement, hence, the uncertainty due to the RLJP does not exist.

Calibration of the pH meter.

Buffer solutions for pK_a(I=0) measurement. Calibration of pH meter for pK_a(I=0) measurement was carried out using 3 standard buffer solutions and the calibration parameters were calculated by three-point linear regression.

Stated uncertainty of the pH value of standard buffer solutions pH_iacc. Aldrich buffer solutions 4.00 (pH_1), 6.99 (pH_2) and 9.95 (pH_3) were used. pH values at 25°C are calculated from given pH values at 20°C and 30°C. The uncertainty of ± 0.02 pH units was stated by the manufacturer for all the buffer solutions.

Temperature dependences of buffer solutions pH values pH_itemp. The temperature dependences of the pH at 25°C of these standard solutions are calculated also from those given data and they are 0.000 (θ_1), 0.002 (θ_2) and 0.010 (θ_3) pH units per centigrade, respectively.

Calibration in terms of hydrogen ion concentration for pK_a(I=0.1M KCl) measurement. For the pK_a(I=0.1M KCl) value, the calibration procedure was carried out in terms of hydrogen ion concentration by means of a strong acid – strong base titration in 0.1M KCl medium and the program GLEE [27] was used for calculation of the calibration parameters E_0 and s.

50.02 mL (V_{KCl}) of 0.1M KCl solution was pipetted into thermostated measurement cell (see above for the uncertainty of pipetting). 2 mL (V_{HCl}) of (0.1000 ± 0.0001)M HCl solution (Reagecon) (C_{HCl}) was added. This acidic solution was titrated with the above-described 0.1000M KOH solution (C_t). The calibration uncertainty of 2 mL pipette was given as ± 0.015 mL (V_{HCl} cal), the repeatability was 0.0055mL (V_{HCl}rep) and temperature effect 0.001 mL (V_{HCl}temp). The uncertainty of 0.1M HCl was ±0.14% and was found the same way as for 0.1M KOH above. pK_w for 0.1M KCl is 13.78, the uncertainty of pK_w(I=0.1M KCl) is mostly caused by the uncertainty of temperature and using

the table of temperature dependencies given in help text of the program GLEE [27] we get 0.002. The uncertainty of added titrant volume (V_t) has three components: repeatability of the piston burette, calibration uncertainty of the burette and temperature uncertainty, see above. Here we can neglect the repeatability component because numerous points (59 in our case) on the curve are used for the calibration. The uncertainty is thus caused only by calibration and temperature effect that are both proportional to the added volume. In order to account for this effect it is found that for V_t =1 mL the uncertainty is 0.0018 mL (see the file pKa_I_01M.xls in the ESM) and for other volumes the effect is proportional. For uncertainty estimation of E_0 and s the influencing parameters described above (V_{KCl}, V_{HCl}, C_{HCl}, C_t, V_t, pK_w(I=0.1M KCl)) and in addition the uncertainty introduced by the ill-defined range of titration points taken for the calibration using the GLEE software (the "range" uncertainty) were taken into account. With all the parameters except the "range" the uncertainty contributions to E_0 and s were calculated by the Kragten method [32] as described in section "Calculation of the combined uncertainty" according to eq 11 (see the file pKa_I_01M.xls in the ESM). The respective Δy values were found by calculating the E_0 and s values using the GLEE program with the parameters at their assigned values ($y(x_i)$) and with one of the parameters changed slightly ($y(x_i + \Delta x_i)$).

The "range" uncertainty contribution is an interesting one. It not straightforward to express numerically, because it is not directly connected to any single numerical input parameter. Instead it was quantified by the following approach. First the best estimates of the E_0 and s values (the optimal values) were found by including the points that were on the curve in the following two pH ranges: 2.5 – 3.8, 10.7 – 11.3. These ranges are very similar to those recommended by the GLEE software manual. Then the E_0 and s values were estimated using various less than optimum but still reasonable sets of points on the curve. The standard deviations of the so obtained E_0 and s values were taken as the uncertainty estimates.

Correlation between E_0 and s. The correlation coefficient between E_0 and s was found using the simulation method, where changing the values of the input parameters (listed in previous subsection), many new E_0 and s values were obtained. From those data the correlation coefficient was calculated. Necessity for taking into account the correlation arises in the case of finding the uncertainty for the pK_a(I=0.1M KCl) value because E_0 and s are calculated from the same parameters and are thus significantly correlated and in the further calculations they are used as two distinct quantities with their own uncertainties. The correlation coefficient (R) was found to be –0.866. In the case of the uncertainty for the pK_a(I=0) value similar correlation is taken into account automatically, because E_0 and s are used as interim values (calculated from the data of the calibration buffers and the measurement system), not as input quantities [33, 34].

Temperature coefficient of the slope. The theoretical value 0.00335 K^{-1} (at 25°C) for the temperature coefficient α is used [26] for calculation the temperature correction of the slope. An uncertainty estimate of α (derived from a study involving many glass electrodes) is given in ref [26]: $u(\alpha) = 0.00053\ K^{-1}$.

Activity coefficients f_1 and f_2. The activity coefficients for $pK_a(I=0)$ measurements were calculated according to Debye-Hückel (DH) theory [25], see above eq 6. Term f_1 was used for singly charged ions and f_2 for doubly charged ions (carbonate ions). The uncertainty of the activity coefficients is composed of two main groups of contributions: (1) the uncertainties of the coefficients a, A and B as well as the ionic strength I and (2) the uncertainty due to the mismatch between the Debye-Hückel model and the real activity coefficients in the solution – the "mismatch" uncertainty.

The uncertainty of the ionic strength is built up automatically from the volumetric data in the model. The ion size parameter a is assumed 5 Å for all ions and we conservatively estimate its uncertainty as ± 4 Å (rectangular distribution). We assume that the uncertainties of the following three parameters: A = 0.5115 $mol^{-1/2}$ $litre^{1/2}$[25], B = 0.3291· 10^8 cm^{-1} $mol^{1/2}$ [25] and also $K_w(I=0) = 1.008 \cdot 10^{-14}$ mol L^{-1} are mostly caused by the uncertainty of temperature. Given that the uncertainty of temperature in the measurement cell is ± 0.1°C and using the tables of temperature dependencies given in ref [25] we get corresponding uncertainties as follows: for A $5.8 \cdot 10^{-5}$ $mol^{-1/2}$ $litre^{1/2}$, for B $1.2 \cdot 10^3$ cm^{-1} $mol^{1/2}$ and for $K_w(I=0)$ $4.4 \cdot 10^{-17}$.

For an estimate of the "mismatch" uncertainty the SIT method (via the SIT program [35]) was used as the reference activity coefficient calculation method. The program allows to carry out the calculations at the ionic strength of 0.05M, which is by an order of magnitude higher than in our case. This certainly leads to an overestimated uncertainty. At the same time the program does not provide a possibility to carry out the calculation for the benzoate ion and thus acetate – the structurally closest anion available in the program – was used instead. This probably leads to an underestimation of uncertainty. We expect that all in all the uncertainty will thus be somewhat overestimated, mainly because of the much lower ionic strength in our case. The "mismatch" uncertainty has two components: (a) the deviation between the DH values and the SIT values and (b) the uncertainties of the SIT values. For activity coefficients calculated by SIT program the uncertainties were given as $\Delta\log f_{1SIT}$ =0.002 and $\Delta\log f_{2SIT}$=0.001 for singly and doubly charged ions, respectively (rectangular distribution assumed). The deviations between the two calculation methods were correspondingly $\Delta\log f_{1DH}$=0.002 and $\Delta\log f_{2DH}$=0.006. These deviations were taken as uncertainty estimates (rectangular distribution assumed). See Table 1. for the full equations of f_1 and f_2.

Table 1. Mathematical model of calculation of pK_a(I=0) value and its uncertainty. (printout of GUM Wb file)

Model Equation:

{ The main equation }

$pK_a=-\log(a_H*An*f_1/HA)$;

$a_H=10^{(-pH_x)}$;

$HA=V_{a0}*C_{a0}/(V_{a0}+V_t)-An$;

$An=a_H/f_1+V_t*C_{t0}/(V_{a0}+V_t)-K_w/a_H/f_1-C_{HCO3}-2*C_{CO3}-A_1-A_2-A_3$;

$K_w=1.008*10^{(-14+0.033*(t_{meas}-const(t_{meas})))}$;

{ Initial concentration of the acid solution taken for pKa measurement }

$C_{a0}=1000*m_a*P/V_s/M$;

$M=n_1*C+n_2*H+n_3*O$;

$m_a=m_arep+m_aread+m_adrift+m_aelectrost$;

$V_s=V_scal+V_stemp+V_sfill$;

$V_stemp=V_scal*\gamma*\Delta t_{Vs\_temp}$;

{ Contents of impurities in the acid solution

It is assumed that the titrated acid contains some impurities. As a model approach, it is assumed that there are three

different impurities with different acidities.}

$C_{A1H\_0}=1000*m_a*P_{A1H}/V_s/M$;

$C_{A2H\_0}=1000*m_a*P_{A2H}/V_s/M$;

$C_{A3H\_0}=1000*m_a*P_{A3H}/V_s/M$;

$K_{A1H} = 10 ^ (-pK_{A1H})$;

$K_{A2H} = 10 ^ (-pK_{A2H})$;

$K_{A3H} = 10 ^ (-pK_{A3H})$;

$A_1=C_{A1H\_0}*V_{a0}/(V_{a0}+V_t)/(1+a_H*f_1*f_1/K_{A1H})$;

$A_2=C_{A2H\_0}*V_{a0}/(V_{a0}+V_t)/(1+a_H*f_1*f_1/K_{A2H})$;

$A_3=C_{A3H\_0}*V_{a0}/(V_{a0}+V_t)/(1+a_H*f_1*f_1/K_{A3H})$;

{ Volume of the investigated acid solution taken for titration

The real volume at the titration point under consideration is higher due to the added titrant }

$V_{a0}=V_{a0}cal+V_{a0}temp+V_{a0}rep;$

$V_{a0}cal=V_{a0}calrep+V_{a0}caltemp;$

$V_{a0}caltemp=V_{a0}calrep*\gamma*\Delta t_{Va0\_caltemp};$

$V_{a0}temp=V_{a0}calrep*\gamma*\Delta t_{Va0\_temp};$

{ Volume of the titrant added to the titration cell up to the titration point under consideration }

$V_t=V_tcal+V_ttemp+V_trep;$

$V_tcal=err/vol*V_trep;$

$V_ttemp=V_trep*\gamma*\Delta t_{Vt\_temp};$

{ Content of carbonate in the titrant }

$C_{HCO3}=C_{c0}*V_t/(V_{a0}+V_t)*K_{H2C}/f_1/a_H/(1+K_{H2C}/f_1/a_H+$

$K_{H2C}*K_{HC}/(f_1*f_2*a_H*a_H));$

$C_{CO3}=C_{c0}*V_t/(V_{a0}+V_t)*K_{HC}*K_{H2C}/(f_1*f_2*a_H*a_H)/$

$(1+K_{H2C}/f_1/a_H+K_{H2C}*K_{HC}/(f_1*f_2*a_H*a_H));$

{ Calculation of activity constant of the solution

This calculation is done via iterative approach, using three iterations.}

$I_{00}=C_{t0}*V_t/(V_{a0}+V_t)+a_H/f_{000};$

$f_{00}=10\text{^}(-A*sqrt(I_{00})/(1+B*å*sqrt(I_{00})));$

$I_0=C_{t0}*V_t/(V_{a0}+V_t)+a_H/f_{00};$

$f_0=10\text{^}(-A*sqrt(I_0)/(1+B*å*sqrt(I_0)));$

$I=C_{t0}*V_t/(V_{a0}+V_t)+a_H/f_0;$

$logf_1=-A*sqrt(I)/(1+B*å*sqrt(I))+\Delta logf_{1SIT}+\Delta logf_{1DH};$

$f_1=10\text{^}(logf_1);$

$logf_2=-A*2*sqrt(I)/(1+B*a*sqrt(I))+\Delta logf_{2SIT}+\Delta logf_{2DH};$

$f_2=10\text{^}(logf_2);$

{ pH of the measured solution }

$pH_x=(E_x-E_0)/s/(1+\alpha*(t_{meas}-t_{cal}));$

$E_x=E_xread+E_xdrift+E_xJP+E_xrep;$

{ Calibration of pH-meter }

$\Sigma pH_iE_i=pH_1*E_1+pH_2*E_2+pH_3*E_3;$

$avgpH_i=(pH_1+pH_2+pH_3)/3;$

$avgE_i=(E_1+E_2+E_3)/3;$

$\Sigma pH_ipH_i=pH_1*pH_1+pH_2*pH_2+pH_3*pH_3;$

$s=(\Sigma pH_iE_i-3*avgpH_i*avgE_i)/(\Sigma pH_ipH_i-3*avgpH_i*avgpH_i);$

E_0=avgE_i-s*avgpH_i;

E_1=E_1rep+E_1JP+E_1read;
E_2=E_2rep+E_2JP+E_2read;
E_3=E_3rep+E_3JP+E_3read;

pH_1=pH_1acc+pH_1temp;
pH_2=pH_2acc+pH_2temp;
pH_3=pH_3acc+pH_3temp;

pH_1temp=θ_1*(t_{cal}-const(t_{cal}));
pH_2temp=θ_2*(t_{cal}-const(t_{cal}));
pH_3temp=θ_3*(t_{cal}-const(t_{cal}));

Table 2. Mathematical model of calculation of pK_a(I=0.1M KCl) value and its uncertainty. (printout of GUM Wb file)

Model Equation:

{ The main equation }

pK_a=pH_c-log(An/HA);

HA=V_{a0}*C_{a0}/(V_{a0}+V_t)-An;

An=10^(-pH_c)+V_t*C_{t0}/(V_{a0}+V_t)-K_w/(10^(-pH_c))-C_{HCO3}-2*C_{CO3}-A_1-A_2-A_3;

K_w=10^(-13.78+0.034*(t_{meas}-const(t_{meas})));

{ Initial concentration of the acid solution taken for pKa measurement }

C_{a0}=1000*m_a*P/V_s/M;

M=n_1*C+n_2*H+n_3*O;

m_a=$m_a$rep+$m_a$read+$m_a$drift+$m_a$electrost;

V_s=$V_s$cal+$V_s$temp+$V_s$fill;

$V_s$temp=$V_s$cal*γ*Δt_{Vs_temp};

{ Contents of impurities in the acid solution

It is assumed that the titrated acid contains some impurities. As a model approach, it is assumed that there are three

different impurities with different acidities.}

C_{A1H_0}=1000*m_a*P_{A1H}/V_s/M;

C_{A2H_0}=1000*m_a*P_{A2H}/V_s/M;

C_{A3H_0}=1000*m_a*P_{A3H}/V_s/M;

K_{A1H} = 10 ^ (-pK_{A1H});

K_{A2H} = 10 ^ (-pK_{A2H});

K_{A3H} = 10 ^ (-pK_{A3H});

A_1=C_{A1H_0}*V_{a0}/(V_{a0}+V_t)/(1+(10^(-pH_c))/K_{A1H});

A_2=C_{A2H_0}*V_{a0}/(V_{a0}+V_t)/(1+(10^(-pH_c))/K_{A2H});

A_3=C_{A3H_0}*V_{a0}/(V_{a0}+V_t)/(1+(10^(-pH_c))/K_{A3H});

{ Volume of the investigated acid solution taken for titration

The real volume at the titration point under consideration is higher due to the added titrant }

$V_{a0}=V_{a0}cal+V_{a0}temp+V_{a0}rep;$

$V_{a0}cal=V_{a0}calrep+V_{a0}caltemp;$

$V_{a0}caltemp=V_{a0}calrep*\gamma*\Delta t_{Va0\_caltemp};$

$V_{a0}temp=V_{a0}calrep*\gamma*\Delta t_{Va0\_temp};$

{ Volume of the titrant added to the titration cell up to the titration point under consideration }

$V_t=V_tcal+V_ttemp+V_trep;$

$V_tcal=err/vol*V_trep;$

$V_ttemp=V_trep*\gamma*\Delta t_{Vt\_temp};$

{ Content of carbonate in the titrant }

$C_{HCO3}=C_{c0}*V_t/(V_{a0}+V_t)*K_{H2C}/(10^{(-pH_c)})/(1+K_{H2C}/(10^{(-pH_c)})+$

$K_{H2C}*K_{HC}/(10^{(-pH_c)}*10^{(-pH_c)}));$

$C_{CO3}=C_{c0}*V_t/(V_{a0}+V_t)*K_{HC}*K_{H2C}/(10^{(-pH_c)}*10^{(-pH_c)})/$

$(1+K_{H2C}/(10^{(-pH_c)})+K_{H2C}*K_{HC}/(10^{(-pH_c)}*10^{(-pH_c)}));$

{ pH of the measured solution }

$pH_c=(E_x-E_0)/s/(1+\alpha*(t_{meas}-t_{cal}));$

$E_x=E_xread+E_xdrift+E_xJP+E_xrep;$

Table 3. List of Quantities in the Mathematical Models of calculation of pK_a(I=0) and pK_a(I=0.1M KCl).

Quantity	Unit	Definition
pK_a		acidity constant of the acid under investigation
HA	mol/l	concentration of the undissociated acid molecules
An	mol/l	concentration of the anion
K_w	mol/l	autoprotolysis constant of water
C_{a0}	mol/l	concentration of the acid solution
P	unitless	purity of the the acid
M	g/mol	molecular mass of the acid
n_1	unitless	number of carbon atoms in the acid molecule
C	g/mol	atomic weight of carbon
n_2	unitless	number of hydrogen atoms in the acid molecule
H	g/mol	atomic weight of hydrogen
n_3	unitless	number of oxygen atoms in the acid molecule
O	g/mol	atomic weight of oxygen
m_a	g	mass of the acid taken for titration
$m_a$rep	g	value and repeatability uncertainty of the mass of the acid
$m_a$read	g	the readability (digital resolution) uncertainty of the balance scale
V_s	ml	the volume of the acid solution
$V_s$cal	ml	the value and calibration uncertainty of the volume of the acid solution
$V_s$temp	ml	the temparature uncertainty component of the volume of the acid solution
Δt_{Vs_temp}	C	the difference of calibration and usage temperatures of the flask
$V_s$fill	ml	the filling uncertainty component of the volume of the acid solution
γ	1/C	the coefficient of volume expansion of water
V_{a0}	ml	the volume of the acid solution that was initially taken for titration
pH_c		-log([H+]) value of the acid solution at the titration point at which the pKa value is found

Quantity	Unit	Definition
V_{a0}cal	ml	the value and calibration uncertainty component of the volume of the acid solution that was initially taken for titration
V_{a0}temp	ml	the uncertainty of volume of pipette caused by the operational temperature differing from the calibration temperature of the solution
Δt_{Va0_temp}	C	the difference of calibration and usage temperatures of the pipette
V_{a0}rep	ml	the repeatability of the volume delivered by the pipette
V_{a0}calrep	ml	the repeatability of the delivered volume during calibration of the pipette
V_{a0}caltemp	ml	uncertainty of volume of pipette coused by the solution temperature differing from the calibration temperature of the device during calibration
$\Delta t_{Va0\_caltemp}$	C	the solution temperature differing from the calibration temperature of the device during calibration
V_t	ml	volume of the titrant added to the titration cell using the piston burette
$V_t$cal	ml	calibration uncertainty of the piston burette
$V_t$temp	ml	the uncertainty of volume of the titrant added to the titration cell coused by temperature difference between the operational and calibration temperature of the burette
Δt_{Vt_temp}	C	temperature difference between the operational and calibration temperature of the burette
$V_t$rep	ml	repeatability uncertainty of the burette
err	ml	"absolute error" of the burette
vol	ml	full volume of the burette
C_{t0}	mol/l	concentration of titrant: sum of molar concentrations of KOH and K2CO3 in the titrant solution
A	(l/mol)½	constant from the Debye-Hückel theory
B	(l/mol)½/Å	constant from the Debye-Hückel theory
å	Å	ion size parameter from the Debye-Hückel theory
I_{00}	mol/l	interim quantity for iterative calculation of f_1
f_{000}	unitless	interim quantity for iterative calculation of f_1
f_{00}	unitless	interim quantity for iterative calculation of f_1
I_0	mol/l	interim quantity for iterative calculation of f_1
f_0	unitless	interim quantity for iterative calculation of f_1
f_1	unitless	activity coefficient for univalent ions
pH_x		pH value of the acid solution at the titration point at which the pKa value is found
α	1/C	temperature coefficient of the slope
E_x	mV	emf of the electrode system at the titration point at which the pKa value is found
$E_x$read	mV	uncertainty originating from the finite readability of the pH-meter scale of solution of investigation
$E_x$drift	mV	systematic deviations (bias) of the measured emf value from the actual value

Quantity	Unit	Definition
E_xJP	mV	uncertainty caused by the residual junction potential of solution of investigation
$E_x$rep	mV	repeatability of emf measurement of solution under investigation
s	mV	slope of the calibration line
ΣpH_iE_i		used for regression analysis
avgpH_i		used for regression analysis
avgE_i		used for regression analysis
ΣpH_ipH_i		used for regression analysis
E_1	mV	emf of the calibration buffer solution 1
E_1rep	mV	repeatability of emf measurement of buffer solution 1
E_1JP	mV	uncertainty caused by the residual junction potential in buffer solution 1
E_1read	mV	readability of the pH-meter scale of emf measurement of buffer solution 1
E_2	mV	emf of the calibration buffer solution 2
E_2rep	mV	repeatability of emf measurement of buffer solution 2
E_2JP	mV	uncertainty caused by the residual junction potential in buffer solution 2
E_2read	mV	readability of the pH-meter scale of emf measurement of buffer solution 2
E_3	mV	emf of the calibration buffer solution 3
E_3rep	mV	repeatability of emf measurement of buffer solution 3
E_3JP	mV	uncertainty caused by the residual junction potential in buffer solution 3
E_3read	mV	readability of the pH-meter scale of emf measurement of buffer solution 3
pH_1		pH of the calibration buffer solution 1
pH_1acc		uncertainty arising from the limited accuracy of the pH values of the buffer solution 1
pH_1temp		uncertainty caused by the dependence of the pH value of the standard on temperature in buffer solution 1
pH_2		pH of the calibration buffer solution 2
pH_2acc		uncertainty arising from the limited accuracy of the pH values of the buffer solution 2
pH_2temp		uncertainty caused by the dependence of the pH value of the standard on temperature in buffer solution 2
pH_3		pH of the calibration buffer solution 3
pH_3acc		uncertainty arising from the limited accuracy of the pH values of the buffer solution 3
pH_3temp		uncertainty caused by the dependence of the pH value of the standard on temperature in buffer solution 3
θ_1		temperature coefficient of the standard buffer solution 1
t_{cal}	C	temperature at which the electrode system was calibrated
θ_2		temperature coefficient of the standard buffer solution 2
θ_3		temperature coefficient of the standard buffer solution 3
I	mol/l	interim quantity for iterative calculation of f_2

Quantity	Unit	Definition
f_2	unitless	activity coefficient for doubly charged ions
C_{HCO3}	mol/l	concentration of hydrogencarbonate ions
C_{CO3}	mol/l	concentration of carbonate ions
A_1	mol/l	concentration of dissociated contaminant A1H in the titration cell
A_2	mol/l	concentration of dissociated contaminant A2H in the titration cell
A_3	mol/l	concentration of dissociated contaminant A3H in the titration cell
C_{c0}	mol/l	overall concentration of carbonate ($C_{HCO3} + C_{CO3}$)
K_{H2C}	mol/l	the first dissocation constant of carbonic acid
K_{HC}	mol/l	the second dissocation constant of carbonic acid
C_{A1H_0}	mol/l	concentration of contaminant A1H
K_{A1H}	mol/l	dissocation constant of contaminant A1H
C_{A2H_0}	mol/l	concentration of contaminant A2H
K_{A2H}	mol/l	dissocation constant of contaminant A2H
C_{A3H_0}	mol/l	concentration of contaminant A3H
K_{A3H}	mol/l	dissocation constant of contaminant A3H
P_{A1H}	unitless	content of the contaminant A1H
P_{A2H}	unitless	content of the contaminant A2H
P_{A3H}	unitless	content of the contaminant A3H
t_{meas}	C	measurement temperature
pK_{A1H}		pKa of the contaminant A1H
pK_{A2H}		pKa of the contaminant A2H
pK_{A3H}		pKa of the contaminant A3H
a_H	mol/l	activity of the hydrogen ions in the measured solution
E_0	mV	standard potentcial of electrode system
$m_a$drift	g	drift of the balance
$m_a$electrost	g	interference from electrostatic disturbances
$logf_1$		logarithm of activity coefficient for singly charged ions
$\Delta logf_{1SIT}$		uncertainty given by SIT program
$logf_2$		logarithm of activity coefficient for doubly charged ions
$\Delta logf_{2SIT}$		uncertainty given by SIT program
$\Delta logf_{1DH}$		difference between $logf_1$ calculated by Debye-Hückel theory and SIT program
$\Delta logf_{2DH}$		difference between $logf_2$ calculated by Debye-Hückel theory and SIT program

5. CALCULATION OF THE COMBINED UNCERTAINTY

Before the combined uncertainty calculation all uncertainty components are converted to the level of standard uncertainty. In this work we assume that the B-type uncertainties for which no information on distribution function is available and which are expressed with the "±" sign have rectangular (uniform) distribution [4]. In order to convert them to standard uncertainties they are divided by $\sqrt{3}$ [2,4]. All the input quantities of the pK_a(I=0) and pK_a(I=0.1M KCl) calculation procedures are presented in Tables 1 to 3. The uncertainties of all the input quantities can be found in the ESM (in the GUM Workbench files

or their PDF printouts). The uncertainty calculation for both pK_a(I=0) and pK_a(I=0.1M KCl) have been carried out according to the ISO GUM procedure [2,4] using two different software packages: MS Excel (Microsoft Inc) and GUM Workbench (Metrodata GmbH). The full calculation files of both software packages for both pK_a(I=0) and pK_a(I=0.1M KCl) are available in the Electronic Supplementary Material.

As explained above the uncertainty calculation has been carried out for a single point x on the titration curve. The point on the titration curve for which the calculation was done was chosen near the half-neutralization point. The total uncertainty, termed combined standard uncertainty $u_c(y)$ of a value y is related to the uncertainties of the individual parameters x_1, x_2, …x_n as follows [2]:

$$u_c(y) = \sqrt{\sum_{i=1,n} c_i^2 u(x_i)^2 + 2 \sum_{\substack{i,k=1,n \\ i \neq k}} c_i c_k \cdot u(x_i, x_k)} \tag{11}$$

where $y = f(x_1,x_2,\ldots,x_n)$ is the output quantity (pK_a(I=0) or pK_a(I=0.1M KCl) in our case), x_i is the i-th input quantity, c_i is the sensitivity coefficient evaluated as $c_i=\partial y/\partial x_i$, $u(x_i,x_k)$ is the covariance between x_i and x_k and it is related to the correlation coefficient r_{ik} by:

$$u(x_i, x_k) = u(x_i) \cdot u(x_k) \cdot r_{ik} \tag{12}$$

The Kragten method [32] for calculation of uncertainty has been used in the MS Excel software. According to EURACHEM/CITAC guide [4] all the partial derivatives are approximated as follows:

$$\frac{\partial y}{\partial x_i} \approx \frac{y(x_i + \Delta x_i) - y(x_i)}{\Delta x_i} \tag{13}$$

where $y = f(x_1,x_2,\ldots,x_n)$ is the output quantity (pK_a in our case), x_i is the i-th input quantity and Δx_i is a small increment of x_i. In the EURACHEM/CITAC guide it is proposed to take $\Delta x_i = u(x_i)$, but we have used $\Delta x_i = u(x_i)/2$. This is safer with respect to the possible nonlinearities of the function $f(x_1, x_2,.. x_n)$. For further details on this method see ref [4].

In the uncertainty budgets in Table 4 the uncertainty contribution of an input quantity (termed x_j) is found according to eq 14 (see ref 2 for background info) where the sum is taken over all input quantities:

$$\%(x_j) = \frac{\left(\frac{\partial \mathrm{p}K_a}{\partial x_j} u(x_j) \right)^2}{\sum_i \left(\frac{\partial \mathrm{p}K_a}{\partial x_i} u(x_i) \right)^2} \cdot 100\% \tag{14}$$

The effective number of degrees of freedom (df) of the results was found using the GUM Workbench software that utilizes the modified Welch-Satterthwaite approach [2,37].

6. RESULTS

The uncertainty budgets for the pK_a(I=0) and pK_a(I=0.1M KCl) values are presented in Table 4.

Table 4 omits some of the input parameters that have marginal influence and some input parameters have been grouped together to provide a better overview. Fully detailed information is available in the calculation files provided in the Electronic Supplementary Material.

Table 4. Uncertainty budgets and combined uncertainties for the pK_a(I=0) and pK_a(I=0.1M KCl) values.

	pK_a(I=0.1M KCl)	pK_a(I=0)	pK_a(I=0.1M KCl) without the unc of V_{HCl}	pK_a(I=0) without the unc E_xJP
	Uncertainty contributions of input quantities (%)[a]			
pH_1		31.2		47.8
pH_2		5.3		8.1
pH_3		0.9		1.3
E_1		3.7		5.7
E_2		0.7		0.9
E_3		0.0		0.1
E_0 + s[b]	*38.0*		*12.1*	
E_0 + s // V_{HCl}	21.5		0.0	
E_0 + s // V_{KCl}	1.4		0.9	
E_0 + s // C_{HCl}	1.5		1.4	
E_0 + s // C_{t0}	0.9		0.8	
E_0 + s // pK_w	2.0		1.1	
E_0 + s // range	6.8		4.4	
E_0 + s // V_t	4.0		3.5	
E_x[c]	*23.9*	*47.0*	*33.8*	*19.0*
$E_x$rep	6.2	3.0	8.8	4.6
$E_x$read	0.4	0.0	0.5	0.1
$E_x$drift	17.3	9.4	24.5	14.3
E_xJP	0.0	34.6	0.0	0.0
t_{cal}	0.9	0.3	1.3	0.4
t_{meas}	0.9	0.2	1.3	0.4
m_a	3.3	0.9	4.8	1.5
V_s	0.3	0.0	0.5	0.0

	pK_a(I=0.1M KCl)	pK_a(I=0)	pK_a(I=0.1M KCl) without the unc of V_{HCl}	pK_a(I=0) without the unc E_xJP
P	7.8	2.1	11.1	3.2
P_{A1H}	15.8	3.9	22.4	6.0
P_{A2H}	0.0	0.0	0.0	0.0
P_{A3H}	0.0	0.0	0.0	0.0
pK_{A1H}	1.3	0.1	1.8	0.2
pK_{A2H}	0.0	0.0	0.0	0.0
pK_{A3H}	0.0	0.0	0.0	0.0
C_{t0}	0.6	0.2	0.8	0.2
V_{a0}	1.6	0.3	2.3	0.5
V_t	5.5	1.7	7.7	2.6
C_{c0}	0.0	0.0	0.0	0.0
$logf_1$		0.8		1.2
$logf_2$		0.0		0.0
	pK_a values and their uncertainties			
pK_a	4.019	4.217	4.019	4.217
u_c(pK_a)	0.0086	0.017	0.0072	0.014
Eff. df [d]	33	2100	120	880
k (95.45% CI)	2.09	2.00	2.00	2.00
***U*(pK_a)**	0.018	0.035	0.014	0.028

[a] The uncertainty contributions percentages (found according to eq 14) are given for the uncertainty of the respective pK_a value. [b] The contribution "E_0 + s" is applicable only to pK_a(I=0.1M KCl) and is the sum of the 7 subsequent contributions. [c] The contribution of E_x is the sum of the subsequent 4 contributions. [d] The significantly larger number of degrees of freedom in the case of the pK_a (I=0) is due to the fact that the most significantly contributing input quantities –the residual liquid junction potential and the accuracy of the pH 4.00 buffer solution – have been estimated as rectangularly distributed, meaning that they have infinite number of degrees of freedom by definition.

Below we compare the uncertainties and the uncertainty budgets of the two calculation approaches.

7. DISCUSSION

It is first of all important to stress that we are not comparing the pK_a(I=0) and pK_a(I=0.1M KCl) values. These pK_a values refer to different standard states and are thus not directly comparable [14,15]. Instead we compare the uncertainties and the uncertainty budgets (the relative contributions of different input quantities on the uncertainty of the result).

Also, it is important to keep in mind that the numerical data given in Table 4 and discussed below apply strictly speaking only to the particular example (benzoic acid of the purity that was used) using the particular experimental setup (glassware, chemicals, titrator) that is described in the Experimental section. However, the main trends and the logic of the calculations remain the same and can be used for other examples. Also, with the aid of the calculation files that are provided in the ESM interested user can explore changes in uncertainty and in the uncertainty budgets that take place when changes to the experimental parameters are introduced. Below we deliberately use uncertainty contribution percentages with one decimal digit, although this is certainly an overkill in precision. This is done to facilitate the readers to follow the data.

The main contributors to the uncertainty budget are in both cases the uncertainty components due to the hydrogen ion concentration/activity measurement. These contributions are: 89.3% and 63.7% for the $pK_a(I=0)$ and $pK_a(I=0.1M\ KCl)$, respectively, including those noted under $E_0 + s$ and E_x, t_{cal} and t_{meas}. The different size of these contributions reflects the different accuracy of measuring the activity and concentration of the hydrogen ion. The structure of these contributions is different, as can be expected keeping in mind the different measurand – $\log[H^+]$ and pH – in these two cases and the different approach to calibrate the pH meter.

The main uncertainty contribution to the pH measurement arises from the residual liquid junction potential (34.6%) and the uncertainty of the pH values of the calibration buffer solutions (37.4%). The contributions of the different buffer solution are very different being 31.2%, 5.3% and 0.9% for the buffer solutions 4.00, 6.99 and 9.95, respectively. The heavily dominating contribution of the first buffer solution is due to the vicinity of the determined pK_a value to pH 4.00. Varying the determined pK_a value between 4 and 9 (keeping all other influencing factors the same) leads to a large change of the relative contributions of the different calibration buffer solutions and also to some change of their overall contribution. This is due to the fact that the combined uncertainty of the pK_a value varies in the following range 0.018 (pK_a=4), 0.014 (pK_a=7), 0.021 (pK_a=10). Using two-point calibrations with buffer solutions 4.00 and 6.99, 4.00 and 9.95, the expanded uncertainty increases only by 0.001 pK_a units in both cases. If, however, higher quality buffer solutions with stated uncertainties of ± 0.005 pH units would be used then some decrease of the uncertainty would be obtained: the expanded uncertainty would decrease from 0.035 to 0.028 pK_a units.

The main uncertainty contribution to the log[H+] is the combined contribution of the calibration parameters – slope s and the intercept E_0 – of the pH electrode system (38.0%). It is impossible to separate this contribution into the sub-contributions of s and E_0, because strong correlation exists between these two quantities (R = –0.87). However, these parameters are obtained in turn from a titration of HCl solution with KOH and it is possible to quantify the contributions of the different uncertainty sources of that titration. There are

altogether 7 such uncertainty sources and the information about their contributions is presented in Table 4. The main contributor is the uncertainty of the volume of HCl that is transferred to the titration cell (21.5%) followed by the uncertainty introduced by the ill-defined range of titration points taken for the calibration using the GLEE software (6.8%). The rest are of minor significance.

The pH electrode in our hands worked in a more stable manner in 0.1M KCl solution than in the low ionic strength benzoic acid solution. For this reason the repeatability and drift uncertainty components of E_x are different for pK_a(I=0) and pK_a(I=0.1M KCl). The following uncertainty estimates values are used for pK_a(I=0): E_x rep = 0.17 mV and E_x drift = 0.3 mV. The following uncertainty estimates are used for pK_a(I=0.1M KCl): E_x rep = 0.12 mV and E_x drift = 0.2 mV. According to the literature data [25,26] and our own experience the reason for this is twofold: (1) The conductivity of the solution with 0.1 M KCl background electrolyte is higher and (2) the liquid junction potential is stable and constant.

The remaining contribution in the uncertainty of the pK_a(I=0.1M KCl) comes for the most part from the purity of the acid P (around 24.9%), volume measurement of the titrant V_t (5.5%) and uncertainty of mass measurement of benzoic acid m_a (3.3%). The same uncertainty sources are also the remaining main contributors to the uncertainty of pK_a(I=0) with relative contributions 6.1%, 1.7% and 0.9%. These strongly different relative contributions of the same effects that in absolute terms have almost identical uncertainties is due to the strongly different contribution of hydrogen ion activity/concentration measurement (89.3% vs 63.7%).

The purity of the acid under investigation P is in this treatment not related just to inert compounds but involves also contaminants with acidic properties (see the section Solutions and Volumetric Ware). In the application example it has been assumed that the acid contains in addition to inert impurities also three different kinds of acidic impurities with different acidities (pK_a values around 2.5, 7 and 10). Concentrations and acidities of all those acidic impurities enter the measurement equations and are thus taken into account. As is seen from Table 4, impurities with different pK_a values have different influence on the final result. The impurity with the lowest pK_a value has the highest influence since the others behave in this example more or less as inert impurities.

The uncertainty of V_t is mainly determined by the accuracy of the mechanical burette. The effect of contamination of the titrant with carbonate at the level of carbonate, that was in the titrant in the example almost insignificant.

The volume-based components of uncertainty could be nominally eliminated (or substantially decreased) by performing the titration gravimetrically (i.e., with the titrant directly added by mass instead of by volume). The improvement would be particularly evident in un-thermostated laboratories as used in the present work.

The mass uncertainty of benzoic acid is almost entirely determined by the effect of electrostatic disturbances that occur when weighing very dry substances. All other uncertainty sources can in principle be neglected.

The uncertainty of pK_a(I=0) is almost two times higher than the uncertainty of pK_a(I=0.1M KCl). The following effects mainly contribute to this: (1) the large uncertainty contributions to the pK_a(I=0) that arises from the ill-defined residual liquid junction potential E_xJP and (2) the higher repeatability and drift contributions in the case of the solutions with low ionic strength as compared to the solutions with 0.1M KCl background electrolyte.

If the uncertainty due to the residual liquid junction potential E_xJP were absent then the expanded uncertainty of pK_a(I=0) would decrease from 0.035 to 0.028 pK_a units and would still be significantly higher than that of the pK_a(I=0.1M KCl). The uncertainty of the pK_a(I=0.1M KCl) in turn is heavily dependent on the uncertainty of the HCl volume taken for electrode calibration. If weighing could be used instead then the uncertainty would decrease from 0.018 to 0.014 pK_a units.

Interestingly, the two totally different calibration approaches – with standard buffer solutions and with acid-base titration – lead to rather similar relative uncertainty contributions: 42.3% and 39.8% in the case of pK_a(I=0) and pK_a(I=0.1M KCl), respectively.

8. CONCLUSIONS

From this work a generalization can be made that the uncertainty of the pK_a(I=0.1M KCl) values tends to be lower than that of the pK_a(I=0). The main reasons are: (1) the uncertainty of the residual liquid junction potential is absent in the case of pK_a(I=0.1M KCl) due to the similar high concentration of background electrolyte in the calibration solutions and measured solution and (2) The electrode system is more stable in solutions containing the 0.1 M KCl background electrolyte and more stable readings can be obtained.

9. ELECTRONIC SUPPLEMENTARY MATERIAL

The uncertainty calculations are available in GUM Workbench files pKa_I_0.smu for the pK_a (I=0) and pKa_I_01M.smu for the pK_a (I=0.1M KCl). For those users who do not have the GUM Workbench software the PDF print-outs of the files are available in files pKa_I_0.pdf and pKa_I_01M.pdf, respectively. These printouts contain all the information about the calculations (the model, definitions, values and uncertainties of the input quantities and comments). The calculation in the MS Excel format are available as pKa_I_0.xls and pKa_I_01M.xls.

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Main scientific publications

1. Leito, I., Strauss, L., Koort, E., Pihl, V. Estimation of uncertainty in routine pH measurement. *Accred Qual Assur* (2002) 7:242–249.
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