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AGNES HEERING

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Experimental realization and applications of the unified acidity scale

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“pH measurement is often deceptively easy . . . pH measurement can also be exasperatingly difficult.”
G. Mattock, 1963

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1. LIST OF ORIGINAL PUBLICATIONS[1]

I **Suu, A.**; Jalukse, L.; Liigand, J.; Kruve, A.; Himmel, D.; Krossing, I.; Rosés, M.; Leito, I. Unified pH Values of Liquid Chromatography Mobile Phases. *Anal. Chem.* **2015**, *87* (5), 2623–2630.

II Liigand, P.; **Heering (Suu), A.**; Kaupmees, K.; Leito, I.; Girod, M.; Antoine, R.; Kruve, A. The Evolution of Electrospray Generated Droplets Is Not Affected by Ionization Mode. *J. Am. Soc. Mass Spectrom.* **2017**, *28* (10), 2124–2131.

III Lõkov, M.; Tshepelevitsh, S.; **Heering, A**.; Plieger, P. G.; Vianello, R.; Leito, I. On the Basicity of Conjugated Nitrogen Heterocycles in Different Media. *European J. Org. Chem.* **2017**, *2017* (30), 4475–4489.

Author's contribution

Paper I: Performed all of the experimental work and was the main person writing the manuscript.

Paper II: Performed all of the experimental work related to unified acidity measurements and participated in writing the manuscript.

Paper III: Performed all of the experimental work related to unified acidity measurements and participated in writing the manuscript.

[1] Roman numbers are used to cite the original publications.

2. ABBREVATIONS AND SYMBOLS

a	Activity
B	Salt bridge electrolyte
c	Molarity
C_6mim^+	1-Hexyl-3-methylimidazolium ion
E	Potential
E_j	Liquid junction potential (LJP)
F	Faraday constant
g	Gas phase
HPLC	High performance liquid chromatography
IL	Ionic liquid
Ind	Indicator electrode
ISE	Ion selective electrode
IUPAC	International Union of Pure and Applied Chemistry
LJP	Liquid junction potential
m	Molality
$m°$	Standard molality, 1 kg mol^{-1}
MeCN	Acetonitrile, systematic name is ethanenitrile
MeOH	Methanol
N_{2225}^+	Triethylpentylammonium ion
NTf_2^-	Bis(trifluoromethylsulfonyl)imide ion
p_{vap}	Vapour pressure
pH_{abs}	pH expressed on unified acidity scale
$pH_{abs}^{H_2O}$	pH_{abs} value that is shifted by a constant value to obtain the aqueous pH scale
$pH_{reference}$	Aqueous buffer solution with known pH value
${}_s^spH$	pH scale relative to particular solvent (calibrated and measured in solvent s)
${}_w^spH$	pH scale relative to water (calibrated in water, measured in solvent s)
pK_a	Negative logarithm of acid dissociation constant K_a ($pK_a = -\log K_a$)
R	Molar gas constant
RMS	Root mean square
solv	Solvated
t	Ionic transport number
T	Absolute temperature
TFA	Trifluoroacetic acid
s	Consistency standard deviation of the scale
s or S	Solvent
u_c	Combined standard uncertainty (uncertainty estimation approach 2)
u_{RW}	Long-term within-laboratory reproducibilities (uncertainty estimation approach 1)
UV-Vis	Ultraviolet-visible

v/v	Volume percentage
w	Water
w/w	Weight percentage
γ	Activity coefficient
ΔG	Free energy difference
$\Delta_{solv}G°(H^+)$	Standard Gibbs solvation energy of proton
$\Delta_{vap}G°(H^+)$	Standard Gibbs vaporization energy of proton
$\mu°_{abs}(H^+)$	Absolute standard chemical potential of proton
$\mu(H^+)$	Chemical potential of proton
$\mu°(H^+)$	Standard chemical potential of proton
|	Denotes phase boundary
¦	Denotes free-diffusion junction

3. INTRODUCTION

Acidity influences many processes like catalytic cycles and chromatographic retention. Acidity of a solution is expressed via pH value that refers to activity of the solvated proton (hydrogen ion). pH is one of the most frequently measured analytical quantities. Every solvent has its own pH scale and due to unknown shifts of zero points of these scales, pH values in different solvents are incomparable.

pH measurement in water is well-defined and straightforward, but the same does not apply to non-aqueous media. Lack of suitable electrodes and calibration standards makes pH measurements in non-aqueous solvents difficult and in many situations it is currently still impossible to obtain rigorous pH values in non-aqueous media. To overcome this problem, various acidity functions are in use to estimate the acidity of a non-aqueous solution. These acidity functions are not directly comparable to each other or to pH values.

The concept of unified pH scale has been proposed to define and compare acidities of any medium, in principle in any phase. It is based on a single universal reference point – absolute chemical potential of the hydrogen ion in the gas phase – which is the same for any medium. The first experimental realization of unified pH scale was achieved in the framework of this thesis (Paper I). The first unified acidities were measured by differential potentiometry with metal-coated glass electrodes.

The overall aim of this thesis was to develop and validate a method to measure unified acidities and secondly to use the developed method to measure unified acidity values of numerous liquid chromatography mobile phases (Paper II). Various instruments, glass cells and salt bridge electrolytes were tested. Validation was done by two separate approaches. Initial validation of the experimental method was carried out using a series of aqueous buffers with known pH values and as a second step, the method was validated by comparing potentiometric measurement results with ultraviolet-visible spectrophotometry results (Paper III).

4. LITERATURE OVERVIEW

4.1. Definition of pH

The pH is defined via the relative activity of hydrogen ions in solution

$$\mathrm{pH} = -\log a_{\mathrm{H^+}} = -\log\left(m_{\mathrm{H^+}}\gamma_{\mathrm{H^+}} / m^{\circ}\right) \quad (1)$$

where $a_{\mathrm{H^+}}$ is relative activity of hydrogen ions (H^+) in molal scale, $\gamma_{\mathrm{H^+}}$ is the molal activity coefficient of the hydrogen ions at the molality $m_{\mathrm{H^+}}$ and $m°$ is the standard molality (1 mol/kg).[1] For the purposes of this thesis it is useful to use pH defined via the molar, not molal scale, and molar scale will be used throughout, although the latest IUPAC (International Union of Pure and Applied Chemistry) recommendations[1] do not explicitly address this definition. More details are provided in section 4.3. Either way, this is a notional pH definition, because a single ion activity cannot be measured without extrathermodynamic assumptions. Operational pH is defined through measurement methods with Harned cell being the primary method for the measurement of pH.[1] Recently Rockwood[2] defined single-ion activities through fugacities instead of chemical potentials as accepted by IUPAC[3], which is another possible approach to pH definition.

In non-aqueous solvents and solvent mixtures hydrogen electrode is often substituted with other H^+-sensing electrodes and different comparison methods with transference cells are used to assign pH values.[4]

This notional definition means that the zero point of pH scale is activity of 1 of solvated H^+ in the given solvent and therefore every solvent and solvent mixture has its own pH scale. The comparison of pH values in different scales is unachievable because of unknown shifts of the zero points.

4.2. Acidity functions

Acidity function is any function that measures the thermodynamic proton-donating or -accepting ability of a solvent system, or a closely related thermodynamic property, such as the tendency of the lyate ion of the solvent system to form Lewis adducts. Acidity functions are not unique properties of the solvent system alone, but depend on the solute (or family of closely related solutes) with respect to which the thermodynamic property is measured.[5]

The best known is the Hammett acidity function H_0, which has been created for uncharged bases and in ideal dilute aqueous solution becomes pH. Hammett also defined acidity functions for cationic and anionic bases and these acidity functions deviate from one another. Hammett acidity function H_0 is based on

indicator (mostly nitro-substituted primary aromatic amines[6]) ultraviolet-visible (UV-Vis) spectrophotometric measurements.[7]

There are also acidity functions based on electrochemical functions, such as works by Strehlow[8] and Janata[9], infrared based functions (e.g Stoyanov[10]) and nuclear magnetic resonance based functions by Fărcaşiu[11] for example. A review by Cox and Yates[6] in 1983 references over 400 acidity functions and a number of new acidity functions have been developed since. Acidity functions are used in different applications, for example to describe the acidity of ionic liquids.[12,13] However, because of the way acidity functions are built they cannot be considered universal thermodynamic acidity scales and are thus no substitute for the concept of pH.

4.3. The pH of liquid chromatography mobile phases

High performance liquid chromatography (HPLC) is one of the most widely used separation techniques in analytical chemistry. In spite of its mature state of development and wide range of applications, the suitable conditions for separations – including the mobile phase pH – are still frequently developed by trial and error. An alternative way of developing separations could be based on predictions rooted in theoretical models, which mainly consider the effects of temperature, the ratio of organic and aqueous components of the mobile phase and the mobile phase pH.[14]

IUPAC[15–17] and others[4,18–20] have rules and procedures for the measurement of pH in aqueous-organic solvent mixtures, but the limited availability of appropriate reference pH data in mixed solvents have limited the application of these procedures in practical HPLC work.[20]

Altough IUPAC's most recent recommendation[1] defines pH only in molal scale, in analytical chemistry molarity is commonly used in preparation of solutions because of its simplicity.[21,22] Previous recommendations gave both concentration scales.[23] Activity and pH are dimensionless quantities, but activity must be referred to a concentration scale and so must be pH. These concentration scales give different pH scales, because activity 1 is achieved with different concentration of hydrogen ions, thus leading to different zero points of the pH scale. These pH scales can be converted through density of the solution. In water concentrations in molality and molarity scale are quite similar, but greater deviation occurs in solutions where density is higher than 1 kg dm^{-3}. One zero point for pH scale within a solvent at fixed temperature can be achieved with a consensus on compositions of standard buffers and their pH values.

In the case of nonaqueous and aqueous mixtures there is also the question of calibration. It can be done in particular solvent or in water, which leads to two different pH scales: ${}^{s}_{s}\mathrm{pH}$ or ${}^{s}_{w}\mathrm{pH}$, respectively, where subscript shows calibration and superscript measurement medium (s is solvent and w is water). Calibration sets the conditions for which the activity coefficient of hydrogen ion is

considered to be equal to unity. This question is discussed in detail by the Rosés group.[21,22]

4.4. Unified acidity scale

Himmel et al. proposed a unified acidity scale[24] that is based on the absolute standard chemical potential of the proton $\mu°_{abs}(H^+)$ and where the zero point of the scale is the absolute standard chemical potential of the proton in the ideal gas phase (1 bar, 298.15 K), which is arbitrarily set to 0 kJ mol^{-1} ($\mu°_{abs}(H^+) = 0$). Importantly, this zero point of the scale is universal to all possible media, thereby enabling expressing the acidities in any given medium on one scale. In any solvent the chemical potential of the solvated proton is decreased (becomes more negative) by solvation of proton. The more negative is the proton's chemical potential the lower is its activity and consequently the acidity of the solution.

The chemical potential of the proton in the gas phase or in solution $\mu(H^+)$ can be generally described by a standard chemical potential $\mu°(H^+)$ and a concentration-depending activity term:

$$\mu\left(\mathrm{H}^+\right) = \mu°\left(\mathrm{H}^+\right) + RT \ln a_{\mathrm{H}^+} = \mu°\left(\mathrm{H}^+\right) - RT \ln 10 \times \mathrm{pH}. \quad (2)$$

Himmel et al. defined the absolute chemical potential of the proton $\mu_{abs}(H^+)$ as:

$$\mu_{\mathrm{abs}}\left(\mathrm{H}^+\right) = \mu\left(\mathrm{H}^+\right) - \mu°_{\mathrm{abs}}\left(\mathrm{H}^+\right). \quad (3)$$

When $\mu°_{abs}(H^+) = 0$ kJ mol^{-1} (set arbitrarily by definition) the standard Gibbs energy of solvation $\Delta_{solv}G°(H^+)$ can be used to construct a unified acidity (absolute pH, pH_{abs}) in a solvent as follows:

$$\mu_{\mathrm{abs}}\left(\mathrm{H}^+,\ \mathrm{solv}\right) = \Delta_{\mathrm{solv}}G°\left(\mathrm{H}^+\right) - RT \ln 10 \times \mathrm{pH} \quad (4)$$

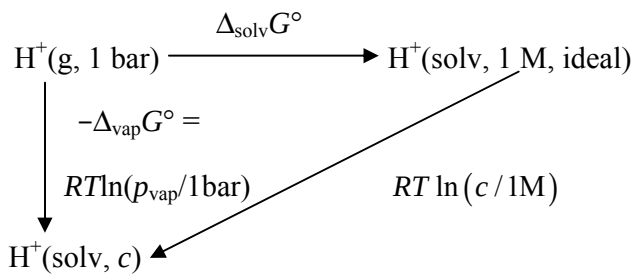
where R is the molar gas constant, and T is the absolute temperature, pH is the conventional pH and $\mu_{abs}(H^+,solv)$ is the absolute chemical potential of solvated proton. To get pH_{abs} from absolute chemical potential we have to consider how much a pH unit difference ($\Delta pH = 1$) amounts to a change of the chemical potential from Eq. 2:

$$\Delta\mu\left(\mathrm{H}^+\right) = RT \ln 10 \times \Delta\mathrm{pH} \quad (5)$$

Which gives 5.71 kJ/mol per pH unit difference (at 298.15 K) and therefore pH_{abs} is defined as

$$pH_{abs} = -\frac{\mu_{abs}\left(H^+, solv\right)}{5.71\ kJ/mol}. \quad (6)$$

The relations between different states are shown in Scheme 1.



Scheme 1. Relation between acidities in the gas phase and solution with concentration c (Adopted from Ref 24). $\Delta_{vap}G°$ is Gibbs standard vaporization energy and p_{vap} is vapour pressure and c is molar concentration.

This approach is also fully universal in the sense that it does not set any limitations to the solvation sphere of the proton. At the same time, the properties of the solvation sphere (extent of solvation) of the proton are explicitly taken into account by the decrease of its chemical potential.

As was said in IUPAC recommendations[15] already in 1985 an "inter-solvental" pH scale would be ultimately referenced to water due to the indisputable key role of water as a solvent. For the same reason it is practical to link the absolute acidity to the aqueous pH scale via the Gibbs energy of solvation of the proton in water as follows:

$$pH_{abs}^{H_2O} = pH_{abs} + \frac{\Delta_{solv}G°\left(H^+, H_2O\right)}{5.71\ kJ/mol} \quad (7)$$

The notion $pH_{abs}^{H_2O}$ means that pH is expressed on the absolute scale, but values are shifted by a constant (−193.5 pH units[25] at 25 °C) in order to make the pH_{abs} values directly comparable to the conventional aqueous pH values. This way $pH_{abs}^{H_2O}$ value 7.00 refers to the acidity of the solution where the proton's chemical potential is as high as in aqueous solution with pH 7.00. Thus the unified pH scale enables to express acidity of any medium on a unified scale in the form of familiar aqueous pH values ($^{w}_{w}pH$).

4.5. Potentiometry

Potentiometry is a large group of electrochemical methods, where information on the composition of the sample is obtained from the potential difference between two electrodes operated at or close to equilibrium conditions (i.e. only very small currents are allowed to flow). In direct potentiometry the potential is measured between an indicator electrode and a reference electrode. An ion-selective electrode (ISE) is an indicator electrode capable of selectively measuring the activity of a particular ionic species (the analyte ion).[26]

In the case of pH measurement the indicator electrode is an electrode sensitive to solvated hydrogen ions. Usually it is a glass electrode, which measures the activity of solvated hydrogen ion. It is a membrane-based device and the potential produced across the membrane corresponds to the free energy difference (ΔG) associated with the difference in activities of the solvated hydrogen ions (below termed simply hydrogen ions) on both sides of the membrane. In the case of the same solvents in the measured and internal solution ΔG can be expressed as:[26]

$$\Delta G = -RT \ln\left(\frac{a_{\mathrm{H^+,\,sample}}}{a_{\mathrm{H^+,\,internal\ solution}}}\right) \tag{8}$$

where a_i is activity of the hydrogen ion in the sample solution or in the internal solution of the ISE, respectively. The potential difference E across the membrane is expressed as follows:

$$E = -\frac{\Delta G}{zF} = \frac{RT}{1F} \ln\left(\frac{a_{\mathrm{H^+,\,sample}}}{a_{\mathrm{H^+,\,internal\ solution}}}\right) \tag{9}$$

where z is the charge of the species ($z = +1$ for H^+) and F is the Faraday constant. Since the potential of a glass electrode is usually measured relative to the potential of a reference electrode and the activity of the hydrogen ion in the inner solution is constant ($a_{\mathrm{H^+,\,internal\ solution}} = \mathrm{const}$), the measured potential difference is only dependent on the activity of H^+ in the sample. This potential difference can be related to the activity of hydrogen ion in the solution:[26]

$$E = \mathrm{const} + \frac{RT \ln 10}{F} \log a_{\mathrm{H^+,\,sample}} \tag{10}$$

where the constant is independent of the hydrogen ion activity in the solution and includes the sum of the potential differences at all the interfaces other than

the membrane/sample solution interface.[26,27] Writing the above equation for pH gives:

$$E = \text{const} - \frac{RT \ln 10}{F} \text{pH} \,. \tag{11}$$

Differential potentiometry allows direct comparison of potential difference between two indicator electrodes without a reference electrode. Very importantly, this approach offers the possibility to compare the activity of the analyte ion in different solutions. When using pH-sensitive electrodes, it is possible to compare acidities, expressed as activities of solvated proton, of solutions. The measured output is the potential difference between measured solutions, which can be converted into pH difference.[28]

In the differential potentiometry the measurement setup corresponds to the following scheme:

Indicator electrode | Solution 1 | Bridge solution | Solution 2 | Indicator electrode 2

$$\text{Ind}_1 \qquad \text{S}_1 \qquad \text{B} \qquad \text{S}_2 \qquad \text{Ind}_2 \tag{12}$$

Therefore the potential difference is:

$$\Delta E = E\left(\text{Ind}_2\right) - E\left(\text{Ind}_1\right) = -\frac{RT \ln 10}{F}\left[\text{pH}\left(\text{S}_2\right) - \text{pH}\left(\text{S}_1\right)\right] \tag{13}$$

In the cell 12 two junctions are formed – one at each end of the salt bridge – and a liquid junction potentials (LJPs, see below) $\Delta E_j(\text{B}, \text{S}_1)$ and $\Delta E_j(\text{B}, \text{S}_2)$ occur across the two junctions. Therefore a correction must be done to the measured potential difference ($\Delta E_{\text{measured}}$):

$$\Delta E = \Delta E_{\text{measured}} + \Delta E_j(\text{B}, \text{S}_1) - \Delta E_j(\text{B}, \text{S}_2) \tag{14}$$

Equation 11 shows the relationship between E and pH. In principle, both theoretical and experimental slopes can be used to convert E into pH. However, if the experimental slope of the ISE differs from the theoretical slope (which is very common), then generally the experimental slope should be used. Deviations from theoretical slopes can be due to inaccuracies in the assigned activity values of calibration standards, diffusion potential between the ISE and the reference electrode, interfering ions and unaccounted changes in the reference electrode potential.[29]

The obtained $\Delta\text{pH}_{\text{abs}}$ values can be combined into a continuous scale (so-called "ladder" approach described in previous work[30]), in which the assigned pH_{abs} values are anchored to a known pH_{abs} value. The consistency of the scale (expressing the mutual consistency of the different relative acidity measure-

ments) is evaluated with the consistency standard deviation of the scale s defined as follows[30]:

$$s = \sqrt{\frac{\sum_i \left(\Delta\mathrm{pH}_{\mathrm{exp,\,i}} - \Delta\mathrm{pH}_{\mathrm{calc,\,i}}\right)^2}{n-m}} \tag{15}$$

where $\Delta\mathrm{pH}_{\mathrm{exp,\,i}}$ are measured $\Delta\mathrm{pH}_{\mathrm{abs}}$ values and $\Delta\mathrm{pH}_{\mathrm{calc,\,i}}$ are differences in assigned pH values, n is the number of measurement results and m is the number of independent assigned pH values.

Different types of electrodes can be used for pH measurements in aqueous and nonaqueous solutions. The electrodes used in this work are called metal-connected[31], all solid-state[32–34] or metal-coated[33] glass electrodes. The glass-metal contact can be achieved by filling the glass electrodes with mercury, amalgams or melts of alloys or by coating surface of the glass with metal or metal with glass.[31,32] The essence of the design of electrodes in this work is that the glass bulb is covered with a layer of metal from the inside and the current conductor (wire) is attached directly to that metal coating. Thus, there is no internal reference solution.

4.6. Liquid junction potential

Any junction between two electrolyte solutions of different composition is called a liquid junction. Across such a junction there arises a potential difference, called the liquid junction potential (LJP).[35]

The first theories[36] of LJP are more than 100 years old, but because of its complexity the topic is still under investigation nowadays[37–40]. The most used theory for junctions with the same solvent on both sides is the one developed by Henderson[41,42], which has been described in several textbooks[43,44].

LJP is a complex phenomenon and it cannot be rigorously measured or calculated without introducing extrathermodynamic assumptions.[40] Based on the nature of the assumptions there are various theories for calculating LJP. For junctions with different solvents at the sides of the junction many theories divide LJP into two parts[45–47]: ion transport and reorientation (or transport) of solvent molecules. More advanced theories add a third component, which is supposed to take into account solvent-solvent interactions[48] or solvent mixing at the boundary[49].

It is possible to minimize the LJP and/or keep it constant in two ways: (1) add an indifferent electrolyte of the same concentrations to solutions on both side of the junction or (2) use an appropriate salt bridge between solutions.[50] The indifferent electrolyte should be added in a sufficiently high concentration so that the ion transfer in the system would be dominated by the ions of the added electrolyte.[50] The bridge solvent should not strongly interact with either

of the solvents to reduce the component from interactions. The cation and anion of the bridge electrolyte should have similar mobilities and their transfer free energies (free energy change on crossing the junction) should be approximately equal. In the case of different solvents and their mixtures, it is not straightforward to find a pair of ions with similar mobilities and similar transfer free energies.[45,50] One of the electrolytes used in salt bridges with non-aqueous solvents that is assumed to have negligible junction potential is tetraethylammonium picrate.[51] Tetraethylammonium perchlorate (Et_4NClO_4) is also used.[50]

4.6.1. Calculation of liquid junction potential

Let us consider a cell with free-diffusion junction, where on one side of the junction an electrolyte MX with molar concentration c_1 is dissolved in solvent S1 and on the other side of the junction an electrolyte NY with concentration c_2 is dissolved in solvent S2:

$$c_1 \text{ MX in solvent S1} \mid c_2 \text{ NY in solvent S2.} \tag{16}$$

Perhaps the most advanced approach to calculating LJP in this case is the theory worked out by Izutsu and his co-workers[48,52–60], which says that LJP between different solvents can be regarded as composed of three components:

$$E_j = E_j(a) + E_j(b) + E_j(c). \tag{17}$$

If $c_1 >> c_2$, we may consider replacing NY by MX[53]; the components (a), (b), and (c) can then be considered as in the case of the cell:

$$c_1 \text{ MX in solvent S1} \mid c_2 \text{ MX in solvent S2.} \tag{18}$$

The concentration of the salt bridge electrolyte is considerably higher than that of the measurement solution under study and the criterion $c_1 >> c_2$ holds for this junction.

The three components of LJP in Eq. 17 for the latter cell are[50]:
a) $E_j(a)$ is caused by the difference in electrolyte activities on the two sides of the junction and the difference between the cationic and anionic mobilities is expressed as follows:

$$E_j(a) = \left(-\frac{RT}{F}\right)\left[\left(t_{M_1} - t_{X_1}\right)\ln\frac{a_{MX_2}}{a_{MX_1}} + \left(t_{M_2} - t_{M_1} - t_{X_2} + t_{X_1}\right)\times\left(1 - \frac{a_{MX_1}}{a_{MX_2} - a_{MX_1}}\ln\frac{a_{MX_2}}{a_{MX_1}}\right)\right] \tag{19}$$

where t_i are the ionic transport numbers of ions, a_i are the activities of the ions, and the subscripts 1 and 2 refer to the left and right side of the junction.

b) E_j(b) is caused by the differences in ion solvation on the two sides of the junction and is expressed as:

$$E_j(\mathrm{b}) = slope \times \left(-\frac{1}{2F}\right)\left[\left(t_{M_1} + t_{M_2}\right)\Delta G_t^0\left(M^+\right) - \left(t_{X_1} + t_{X_2}\right)\Delta G_t^0\left(X^-\right)\right] \qquad (20)$$

where $\Delta G_t^0(M^+)$ and $\Delta G_t^0(X^-)$ are the Gibbs energies of transfer of M^+ and X^- from solvent S_1 to S_2. The slopes are obtained by plotting values calculated by Eq. 20 with slope taken as unity versus experimental results. Although the slopes are less than unity, the component (b) is proportional to the calculated E_j(b). Thus, the actual values of component (b) can be estimated by multiplying the calculated E_j(b) and the empirical *slope* (given in the literature[50]). The exact reason for slopes lower than unity is not known, but mutual diffusion of the solvents at the junction and/or the incomplete replacement of the solvated molecules on transfer of ions across the junction are the probable reasons.

c) E_j(c) is caused by the solvent-solvent interactions at the junction and is nearly electrolyte-independent. It is due to the orientation of solvent molecules by solvent-solvent interaction and the solvent-side that acts as a Lewis acid is more negative than the solvent-side that acts as a Lewis base. There is no theoretical way of estimating the value of this component, but from the experimental results and under some assumptions, a rough estimation is possible. These estimates can be found in the literature.[50]

4.7. Ionic liquid salt bridge

As previously mentioned, salt bridges are used to minimize LJP. KCl and KNO_3 are the most used electrolytes in aqueous salt bridges.[43,50] Kakiuchi and his co-workers[44,61,62] have used ionic liquids (IL) instead of aqueous KCl in cells with aqueous test solutions.

A hydrophobic IL in contact with water forms a water-IL two-phase system. The interface between two immiscible electrolyte solutions is called phase boundary.[63] The phase-boundary potential (the potential drop across the interface) that develops in this system after mutual saturation of the two phases is determined by the partitioning of the IL-constituent cation and anion (difference in the transfer Gibbs energies of the cation and anion of IL) and if the interfacial electron transfer between a redox couple takes place, then the charge transfer across the interface can also participate.[64] The theory of phase-boundary potential for IL-water interface is given by Kakiuchi.[64,65] Due to its thermodynamic (equilibrium) nature the phase-boundary potential does not depend on time and also on the shape of the interface while in case of aqueous KCl salt bridge (not at equilibrium) it does. In addition IL salt bridges are free from leakage and clogging, known problems of KCl salt bridges.[44,61,62]

IL should have cation and anion with similar mobility values both in IL itself and in water or other solvents that are used. Distribution equilibrium throughout the two phases is rarely established and differences in mobilities give rise to diffusion potential. The solubility is also an important factor because it determines the electrochemical polarizability of the interface.

As a downside the stability and reproducibility of the phase boundary potential in the IL salt bridges are not yet at the level of LJPs of carefully designed KCl salt bridges and if there are hydrophobic ions in a sample solution they can interfere with the phase-boundary potential.[44,61,62]

5. EXPERIMENTAL

5.1. Instruments and electrodes

Metrohm 713 pH meter was used for measurements in Paper I and Paper II. In case of differential potentiometry the two glass electrodes were connected to the ”pH/ISE” inputs and the auxiliary electrode was connected to one of the ”Ref” inputs. Platinum electrode (Radiometer Type 101 Pt electrode or a Pt wire) was used as auxiliary electrode. The potential was stabilized for 15 min before taking the reading.

Gamry Reference 3000 Potentiostat/Galvanostat/ZRA was used for potentiometry in Paper III. Experiments were run for 1800 s with 10 s step. Noise measurements were made for 1 s with 0.1 s or 0.0001 s step.

All the measurements were done at (25±1) °C. Used metal-coated glass electrodes (Figure 1) were in different shapes and sizes, but all were made at Laboratory of Glass Electrochemistry, St. Petersburg State University.

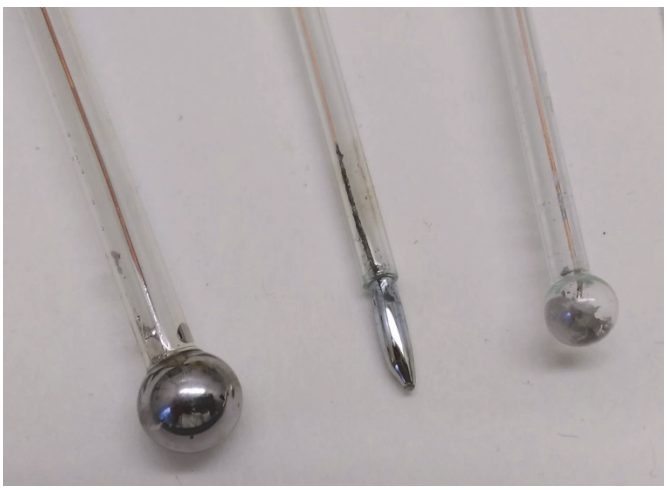


Figure 1. Metal-coated glass electrodes.

5.2. Preparation of mobile phases

Paper I: The mobile phases were mixtures of aqueous phase with acetonitrile or methanol and are given in Table 1. The aqueous buffer system used for preparing the mobile phases with different pH values was 5 mM CH_3COONH_4 (having pH approximately 6.5) titrated with HCOOH or 25% ammonium hydroxide solution for pH values below or above 6.5, respectively. In addition 0.1% (v/v) trifluoroacetic acid (TFA), 0.1% (v/v) HCOOH (pH 2.68) and 1 mM $NH_3 \cdot H_2O$ (pH 9.75) were used as aqueous phases. Origin and purity of used chemicals are described in Paper I.

Table 1. Mobile phases in Paper I.

Organic phase	Aqueous phase	Volume percentage (Organic/Aqueous)
MeCN	1 mM $NH_3 \cdot H_2O$	80/20; 50/50
MeCN	pH 5[a]	80/20; 50/50
MeCN	0.1% HCOOH	80/20; 50/50; 20/80
MeCN	pH X[a]	80/20
MeCN	0.1% TFA[b]	80/20
MeOH	1 mM $NH_3 \cdot H_2O$	80/20; 50/50
MeOH	pH 5[a]	80/20; 50/50
MeOH	0.1% HCOOH	80/20; 50/50

[a] Buffers ($HCOOH/NH_3 + CH_3COONH_4$) with pH values 5 and X = 9.01, 7.82, 7.03, 6.50, 6.05, 5.48, 5.03, 4.66, 4.02, 3.77, 3.07.
[b] TFA is trifluoroacetic acid.

Paper II**:** The mobile phases were mixtures of aqueous phase with acetonitrile. Water phase solutions were prepared by first making 0.1% (v/v) formic acid aqueous solution and then adjusting pH by adding the 25% (v/v) ammonium hydroxide aqueous solution to the desired $^{w}_{w}\mathrm{pH}$. The buffer systems are given in Table 2. Origin and purity of used chemicals are described in Paper II.

Table 2. Mobile phases in Paper II.

Organic phase	Aqueous phase[a]	Volume percentage (Organic/Aqueous)
MeCN	pH 5.50	80/20; 72/25; 70/30; 50/50; 45/55; 40/60
MeCN	pH 5.00	80/20; 72/25; 70/30; 50/50; 45/55; 40/60
MeCN	pH 4.50	80/20; 72/25; 70/30; 50/50; 45/55; 40/60
MeCN	pH 4.00	80/20; 72/25; 70/30
MeCN	pH 3.50	80/20; 72/25; 70/30

[a] Buffers $HCOOH + NH_3$.

5.3. Salt bridges

Both “classical” salt bridge – a salt dissolved in a solvent – as well as IL salt bridge were used in this work.

Tetraethylammonium (Et_4N^+) and perchlorate (ClO_4^-) ions have similar limiting ionic conductivities in acetonitrile and methanol[27]. In addition little information can be found about picrate ion in different solvents. Therefore 0.05 M Et_4NClO_4 in acetonitrile was chosen as salt bridge for mobile phase pH experiments.

ILs were chosen so that the cation and anion diffusion constants would be similar.[66] Two ILs were tested as salt bridge electrolytes – triethylpentyl-ammonium bis(trifluoromethylsulfonyl)imide ($N_{2225}NTf_2$) and 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ($C_6mimNTf_2$, Iolitec, 99%, Germany). $N_{2225}NTf_2$ was obtained from prof. Krossing group in Freiburg and was synthesized as described elsewhere.[66] These ILs are immiscible with water, but soluble in acetonitrile.

5.4. Method development

Different cells were used throughout the work. The first cells were made from glass Pasteur pipettes, a glass T-piece and silicon tubes (Figure 2).

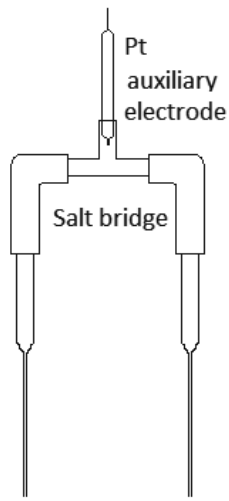
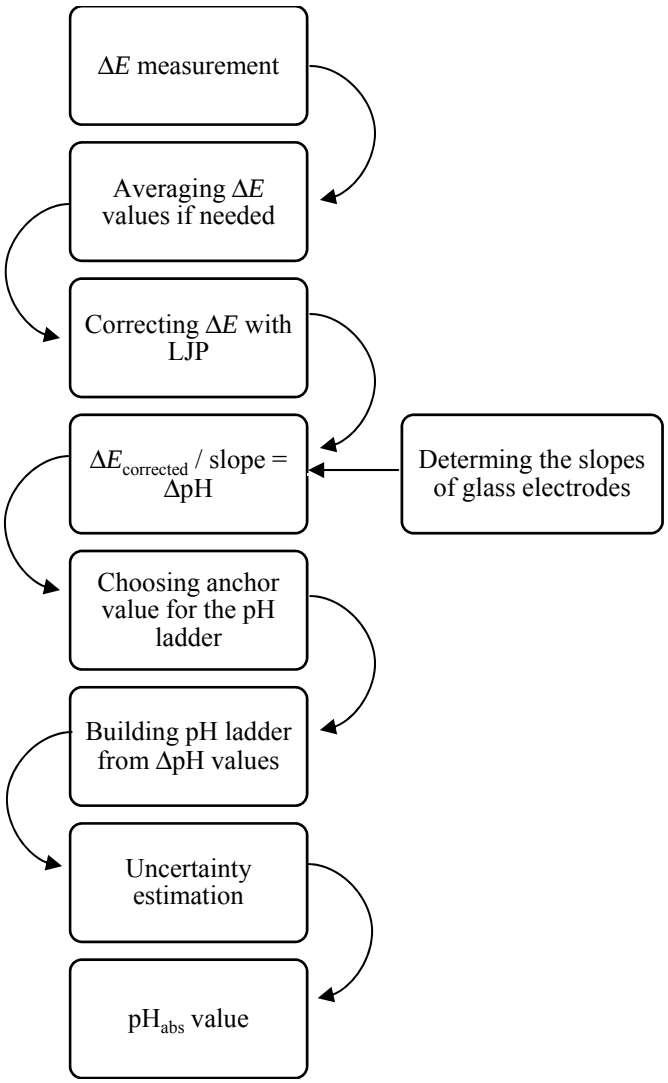


Figure 2. First salt bridge used (Paper I).

This salt bridge had quite large volume and had problems with mixing of solutions. At the beginning (Paper I) the glass electrodes were immersed in the solutions and after stabilization (15 min) the potential reading was taken. Then the electrodes were taken out, washed, dried and again immersed in solutions but with switched (swapped) positions and the measurement was repeated. The sign of the reading changed, when electrodes were switched between the solutions. Two measurements were made with same solutions and then all solutions were renewed. Absolute values of four measurements were averaged to obtain one potential difference. The average potential difference was corrected for LJP and then divided by the average slope to get a pH_{abs} difference (Eqs. 13 and 14). Workflow of obtaining pH_{abs} values is depicted below (Scheme 2).



Scheme 2. Workflow of assigning unified acidities (pH_{abs} values).

After testing different configurations glass siphone was used as salt bridge and Radiometer Pt electrode was replaced with Pt wire (Figure 3). This enabled to reduce the volume of the salt bridge. In addition two measurement results were averaged instead of four. These changes improved the reproducibility and internal consistency of the scale (see section 6.3). This procedure was used in Paper I and Paper II.

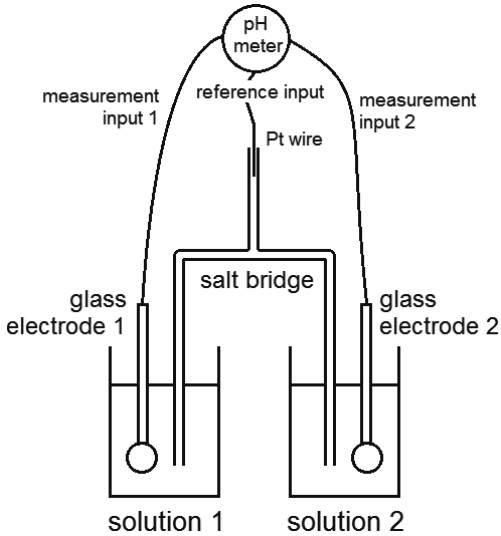


Figure 3. Cell used in Paper I and Paper II.

The density of used ILs was higher than the density of solutions measured. Therefore, if IL bridge electrolyte was used then the cell in Figure 3 was unsuitable, because the IL did not stay in the salt bridge. Consequently there was a need for a cell where the salt bridge was below the measurement solutions. Such cell is presented in Figure 4. In addition, the pH meter was replaced with a potentiostat which allowed discarding the auxiliary electrode, thus simplifying the design of the cell and reducing the noise level. The stabilization time was prolonged to 30 min. Only one 30 min measurement cycle was done for one ΔpH_{abs} without averaging.

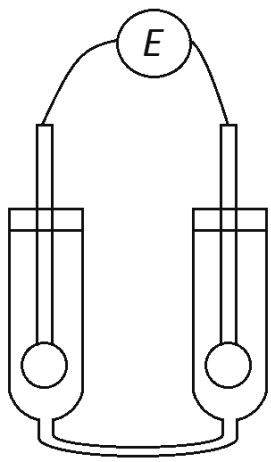


Figure 4. Cell used with ionic liquids in salt bridge (Paper III).

5.5. Liquid junction potential

In all measurements the ionic strength of measured electrolytes was significantly lower than that of the salt bridge electrolytes. Therefore it can be assumed that the ionic component of LJP is only due to salt bridge ions Et_4N^+ and ClO_4^- or the ions of IL. The LJP values were calculated for the junctions (B, S) between the bridge electrolyte solution (B) and the different solutions (S) in contact with it (see scheme in section 4.5). The assumptions and calculation details are given in the supporting information of Paper I. Table 3 gives the LJP values for each junction. The main trends in LJP are that (a) LJP is larger for junctions with MeOH than for MeCN when water percentage is the same and (b) the higher is the organic component content the smaller the LJP.

In case of IL the phase boundary potentials were assumed to cancel out because the measured solutions were made with similar concentrations in the same solvent.

Table 3. Calculated LJP values between salt bridge (0.05 M Et_4NClO_4 in MeCN) and solvent mixtures used in mobile phase acidity measurements (Paper I and Paper II).

Solution pair (S – B)	LJP / mV
water – bridge solution	−79.8
MeCN/water 20/80 – bridge solution	−57.4
MeCN/water 40/60 – bridge solution	−35.2
MeCN/water 45/55 – bridge solution	−31.5
MeCN/water 50/50 – bridge solution	−27.9
MeCN/water 70/30 – bridge solution	−15.1
MeCN/water 75/25 – bridge solution	−12.9
MeCN/water 80/20 – bridge solution	−10.9
MeOH/water 50/50 – bridge solution	−48.5
MeOH/water 80/20 – bridge solution	−31.8

5.6. Slopes of the glass electrodes

Slopes of the glass electrodes were measured in water and in MeCN/water 80/20 with four different methods, three of which apply a reference electrode. The experimental details are given in the supporting information of Paper I. The mean slope −58.1 mV with standard deviation 0.7 mV and standard deviation of the mean 0.3 mV was used in all calculations of pH_{abs} values. Electrodes were chosen so that the intercept of the calibration curves were very similar and therefore there was no need to take intercepts into account in calculating potential difference into pH difference.

5.7. Method validation

The acidity differences of aqueous buffers with known pH value ($pH_{reference}$) were measured as described in section 5.4 and Paper I and a "ladder" was built to assign a pH value to the measured buffers. The assigned values were compared to the known values. Aqueous KCl and later $N_{2225}NTf_2$ and $C_6mimNTf_2$ were used as salt bridge electrolytes.

In addition a second method – UV/Vis spectrophotometry – was used to validate the potentiometric unified acidity measurements. The ΔpH_{abs} of five pairs of buffer solutions (an organic base and its salt in MeCN) were measured with IL in salt bridge as described in section 5.4 and Paper III. The bases were quinoline, isoquinoline, quinazoline, 5,6-benzoquinoline and acridine. The counter-ion was $CF_3SO_3^-$. The overall concentrations of the bases were in the range from $9.0 \cdot 10^{-4}$ to $1.6 \cdot 10^{-2}$ M. The salt-bridge electrolyte was $C_6mimNTf_2$ and LJPs can be assumed to cancel out in these experiments. The ΔpH_{abs} of five base pairs were measured. For comparison the ΔpK_a values calculated from values in Table 1 in Paper III were used. No "ladder" was built in this validation experiment.

In order to compare these two methods the measured ΔpH were converted into ΔpK_a values (Paper III):

$$\Delta pK_a = \Delta pH_{abs} - \log\frac{[B_1]}{[B_1H^+]} + \log\frac{[B_2]}{[B_2H^+]} \qquad (21)$$

where B_i is base and B_iH^+ its conjugate acid.

The ratios of the neutral and protonated forms needed for the calculations were determined by UV/Vis spectrophotometry. The potentiometric measurements of the relative basicity were made outside of the glovebox and the water content in the solutions was 140–200 ppm, determined by Karl-Fischer titration. As has been demonstrated earlier[67], this water content is still tolerable in case of ionic equilibria of neutral bases and their cations.

5.8. Measurement uncertainty

The measurement uncertainty was estimated by two approaches. The standard uncertainty estimates u_{RW} obtained by approach 1 (long-term within-laboratory reproducibilities) can be used to evaluate the internal consistency of the measurement method. Approach 2 takes additionally into account the uncertainty of the LJP estimation, uncertainty of the aqueous reference points and the uncertainty of electrode slopes. The combined standard uncertainty estimates u_C obtained by approach 2, applicable only to $pH_{abs}^{H_2O}$ values, can be used to compare the acidities of the solutions measured in the context of this unified scale with the

acidities in aqueous solutions obtained using conventional pH measurement. Measurement uncertainty estimation is described in detail in supporting information of Paper I.

5.9. Testing ionic liquids as salt bridge electrolytes

Tetrabutylammonium acetate with acetic acid (Bu_4NOAc + AcOH) buffers were used to compare the potential measurement results with different salt bridge electrolytes: ILs or 0.05 M Et_4NClO_4 in MeCN with 0.5% (w/w) water. Buffers were made in water and MeCN with 0.5% (w/w) water. A small amount of water was added to acetonitrile to keep the water content constant when measuring in open air. The measurements were made with the pH meter.

5.10. Ionization degree measurements

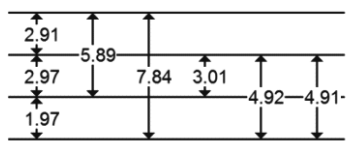
Degrees of ionization of 2-nitrophenol, sorbic acid, benzoic acid, 2,4-dinitrophenol, 2,6-dimethylpyridine, benzimidazole, 1-naphthylamine were measured spectrophotometrically as described in Paper I. The measurements were carried out to test if $pH_{abs}^{H_2O}$ values can be used to estimate ionization degrees. Origin and purity of used chemicals are described in Paper I.

6. RESULTS AND DISCUSSION

6.1. Method validation

Example results of aqueous buffer pH measurements (cell from Figure 3 was used) are shown in Table 4. The ladder is anchored to a standard aqueous buffer with pH value 7.00 and the consistency standard deviation of the scale in Table 4 is 0.02. Later the same experiments were repeated with ILs, which gave a slightly better consistency standard deviation, but at the same time a bit larger difference between reference and experimental pH values. The results clearly show that the method is suitable for measuring pH.

Table 4. A comparison between known pH values ($pH_{reference}$) and experimentally assigned pH values ($pH_{abs}^{H_2O}$) measured at (25±1) °C with saturated KCl water solution in the salt bridge.

$pH_{reference}$ [a]	$pH_{abs}^{H_2O}$	Measured ΔpH_{abs}
9.98	9.95	
7.00	7.00	
4.03	3.98	
2.08	2.00	

[a] Aqueous buffer with known pH value.

It was deemed important to also validate the method with measurements based on a completely independent technique. This was done by comparing ΔpK_a values calculated from ΔpH_{abs} values with spectrophotometric ΔpK_a results. The results are presented in Table 5. The root mean square difference reveals good agreement between the methods, thereby demonstrating the mutual consistency of these methods based on fundamentally different principles. This can be regarded as evidence of validity of both methods. These experiments were done as proof of concept and were not intended as pK_a measurements. Further development is needed for carrying out rigorous pK_a measurement using similar differential potentiometric method. ΔpH_{abs} values were calculated into ΔpK_a values only to enable comparison with spectrophotometric data. The spectrophotometric ΔpK_a measurements are well established in the group of Leito et al.[30], thus the spectrophotometric ΔpK_a values are expected to be highly reliable. The maximum ΔpK_a that can be measured with spectrophotometric method is up to 1.5 units. Potentiometric method does not have such a limit and can be used as a complementary method to spectrophotometry in cases where a reference base or acid with suitably close pK_a value is difficult to find.

Table 5. Comparison of the ΔpK_a results of spectrophotometric and potentiometric methods.

Base 1	Base 2	ΔpK_a[a] (spectro-photometric)	ΔpK_a[a] (potentio-metric)	Difference
Quinoline	Isoquinoline	0.73	0.70	0.03
Quinazoline	Isoquinoline	3.50	3.56	–0.06
Quinazoline	5,6-benzoquinoline	2.77	2.85	–0.08
5,6-benzoquinoline	Acridine	0.71	0.72	–0.01
Quinoline	Acridine	0.71	0.82	–0.11
			RMS[b] difference:	**0.09**

[a] $\Delta pK_a = pK_a(\text{Base 2}) - pK_a(\text{Base 1})$.
With potentiometry the ΔpK_a was calculated from measured ΔpH_{abs} by directly comparing the solutions of the bases and with spectrophotometry the ΔpK_a was calculated as differences of the pK_a values of the bases from Paper III.

[b] RMS is root mean square.

6.2. Ionic liquids as salt bridge electrolytes

The comparison of potential for different salt bridge electrolytes is shown in Figure 5. The potential for salt bridge electrolyte Et_4NClO_4 in MeCN with 0.5% (w/w) water, which is corrected with LJP calculated according to Izutsu's approach, could be considered to be the closest to the true value.[50] The measurements with ILs have not been corrected for junction potential[65] due to lack of information about input values at the current stage. However, the uncorrected potential values of both ILs are close to the corrected potential value for the Et_4NClO_4 salt bridge. The measured potential differences for the IL salt bridges are some tens of mV from the corrected potential value of Et_4NClO_4 salt bridge in MeCN, but this difference is small compared to the near 70 mV (uncorrected) difference for Et_4NClO_4 in MeCN. This suggests that junction potential (LJP for liquid-liquid junctions or phase boundary potential for immiscible IL interfaces) is larger in the case of Et_4NClO_4 in MeCN salt bridge than in case of salt bridges with the tested ILs. The small phase boundary potential means that the chosen ILs are a good starting point for salt bridge electrolytes and with further investigation more appropriate ILs could be found.

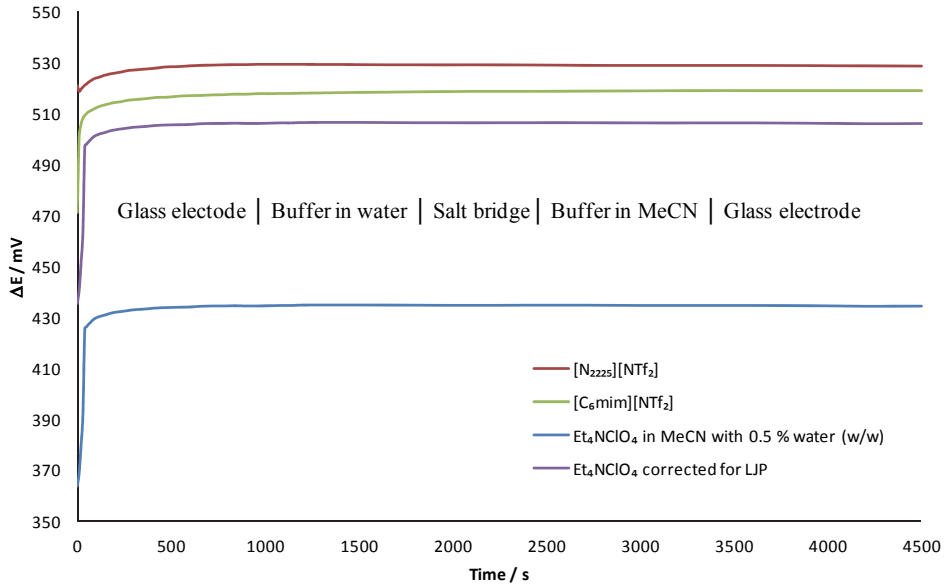


Figure 5. Comparison of potential difference measured and corrected for liquid junction potential (LJP) between (Bu_4NOAc + AcOH) buffers in water and MeCN with 0.5% (w/w) water with different salt bridge electrolytes noted in the figure.

6.3. Acidity of mobile phases

The acidity values of liquid chromatography mobile phases, expressed as $pH_{abs}^{H_2O}$, is the first outcome of the experimental realization of unified acidity concept. The results have been published in two articles. In Paper I 79 (56 with old and 23 with new cell design) relative acidity measurements were made with the consistency standard deviation of 0.14 pH units. In addition 13 measurements were done separately to evaluate $pH_{abs}^{H_2O}$ values calculated from ${}_{w}^{w}pH$ values by interpolation method. In Paper II 52 measurements were made only with the new cell design and the consistency standard deviation was 0.01 pH units, which shows how beneficial the improvements were. All in all, the $pH_{abs}^{H_2O}$ values of 43 acetonitrile- and 6 methanol-containing mobile phases were measured and the resulting $pH_{abs}^{H_2O}$ scale of all the measured mobile phases is visualized in Table 6 and Figure 6.

The $pH_{abs}^{H_2O}$ values have a physical meaning related to solvated proton's chemical potential and can be used for direct comparison of acidities of mobile phases with different composition. This allows determining the effect that solvent, solvent fraction and aqueous phase composition have on the mobile phase acidity. The previous ways of describing mobile phase acidity did not enable such comparison.

There are two counteracting effects that determine $pH_{abs}^{H_2O}$ of the mobile phases. On one hand, adding methanol or acetonitrile decreases the basicity of the solvent, thereby making the solution containing the same concentration of solvated protons more acidic. MeCN is a less basic solvent than methanol and therefore has a stronger effect. On the other hand, adding MeOH or MeCN changes the pK_a values of the acids and bases that are used for buffering the mobile phases. Both solvents, especially MeCN, significantly suppress dissociation of acids, thereby decreasing the concentration of solvated protons and thus increasing $pH_{abs}^{H_2O}$. For example MeCN/ 1 mM NH_3 80/20 has $pH_{abs}^{H_2O}$ 10.47 and the respective methanol mobile phase MeOH/ 1 mM NH_3 80/20 has $pH_{abs}^{H_2O}$ value of 8.89. At the most acidic end of the scale comparison of MeCN/ 0.1% HCOOH 50/50 with $pH_{abs}^{H_2O}$ 4.39 and MeCN/ 0.1% HCOOH 50/50 with $pH_{abs}^{H_2O}$ 3.89 shows similar trend although the difference is not constant.

Table 6. Unified acidities (pH_{abs} and $pH_{abs}^{H_2O}$) of all measured liquid chromatography mobile phases together with uncertainties (Paper I and Paper II).

Mobile phase		pH_{abs} [a]	$pH_{abs}^{H_2O}$ [b]	u_{RW} [c]	u_c [d]
MeCN/ 1 mM NH_3	80/20	204.0	10.47	0.06	0.15
MeCN/ 1 mM NH_3	50/50	203.6	10.07	0.06	0.15
MeCN/ pH 9.01	80/20	203.2	9.69	0.01	0.14
MeCN/ pH 7.03	80/20	202.9	9.41	0.01	0.14
MeCN/ pH 7.82	80/20	202.9	9.39	0.01	0.14
MeCN/ pH 6.50	80/20	202.9	9.39	0.02	0.14
MeCN/ pH 5.48	80/20	202.7	9.22	0.01	0.14
MeCN/ pH 6.05	80/20	202.7	9.16	0.01	0.14
MeCN/ pH 5.03	80/20	202.5	8.99	0.01	0.14
MeCN/ pH 5	80/20	202.4	8.93	0.07	0.15
MeOH/ 1 mM NH_3	80/20	202.4	8.89	0.05	0.15
MeOH/ 1 mM NH_3	50/50	202.2	8.70	0.05	0.15
MeCN/ pH 4.66	80/20	202.2	8.65	0.01	0.14
MeCN/ pH 5.50	80/20	202.1	8.59	0.01	0.14
MeCN/ pH 5.00	80/20	201.9	8.43	0.01	0.14
MeCN/ pH 5.50	75/25	201.9	8.41	0.01	0.14
MeCN/ pH 5.50	70/30	201.8	8.23	0.01	0.14
MeCN/ pH 5.00	75/25	201.7	8.18	0.01	0.14
MeCN/ pH 4.50	80/20	201.6	8.10	0.01	0.14
MeCN/ pH 4.02	80/20	201.5	7.95	0.01	0.14
MeCN/ pH 5.00	70/30	201.5	7.94	0.01	0.14
MeCN/ pH 4.50	75/25	201.3	7.80	0.01	0.14

Table 6. Continuation

Mobile phase		pH_{abs} [a]	$pH_{abs}^{H_2O}$ [b]	u_{RW} [c]	u_c [d]
MeCN/ pH 4.00	80/20	201.2	7.64	0.01	0.14
MeCN/ pH 3.77	80/20	201.0	7.52	0.02	0.14
MeCN/ pH 4.50	70/30	201.0	7.50	0.01	0.14
MeCN/ pH 5	50/50	201.0	7.50	0.07	0.15
MeOH/ pH 5	80/20	201.0	7.49	0.06	0.15
MeCN/ pH 5.50	50/50	201.0	7.49	0.01	0.14
MeCN/ pH 4.00	75/25	200.8	7.32	0.01	0.14
MeCN/ pH 5.50	45/55	200.8	7.30	0.01	0.14
MeCN/ pH 3.50	80/20	200.7	7.18	0.01	0.14
MeCN/ pH 5.50	40/60	200.6	7.11	0.01	0.14
MeCN/ pH 5.00	50/50	200.6	7.05	0.01	0.14
MeCN/ pH 4.00	70/30	200.6	7.03	0.01	0.14
MeCN/ pH 3.50	75/25	200.4	6.86	0.01	0.14
MeCN/ pH 5.00	45/55	200.4	6.85	0.01	0.14
MeCN/ pH 3.07	80/20	200.3	6.82	0.01	0.14
MeCN/ pH 5.00	40/60	200.1	6.62	0.01	0.14
MeCN/ pH 3.50	70/30	200.1	6.57	0.01	0.14
MeCN/ pH 4.50	50/50	200.1	6.56	0.01	0.14
MeOH/ pH 5	50/50	200.0	6.47	0.06	0.15
MeCN/ pH 4.50	45/55	199.9	6.36	0.01	0.14
MeCN/ pH 4.50	40/60	199.7	6.14	0.01	0.14
MeCN/ 0,1% HCOOH	80/20	198.9	5.37	0.07	0.15
MeOH/ 0,1% HCOOH	80/20	198.3	4.79	0.05	0.15
MeCN/ 0,1% HCOOH	50/50	197.9	4.39	0.05	0.15
MeOH/ 0,1% HCOOH	50/50	197.4	3.89	0.04	0.14
MeCN/ 0.1% TFA	80/20	197.2	3.63	0.01	0.14
MeCN/ 0.1% HCOOH	20/80	196.8	3.27	0.04	0.14

[a] Unified acidities.

[b] Unified acidities expressed on aqueous pH scale for better comparison with conventional pH values.

[c] Uncertainty estimation according to approach 1. Can be used to compare acidities within the scale.

[d] Uncertainty estimation according to approach 2. Applicable only to $pH_{abs}^{H_2O}$ values and can be used to compare with acidities of aqueous solutions with conventional pH values.

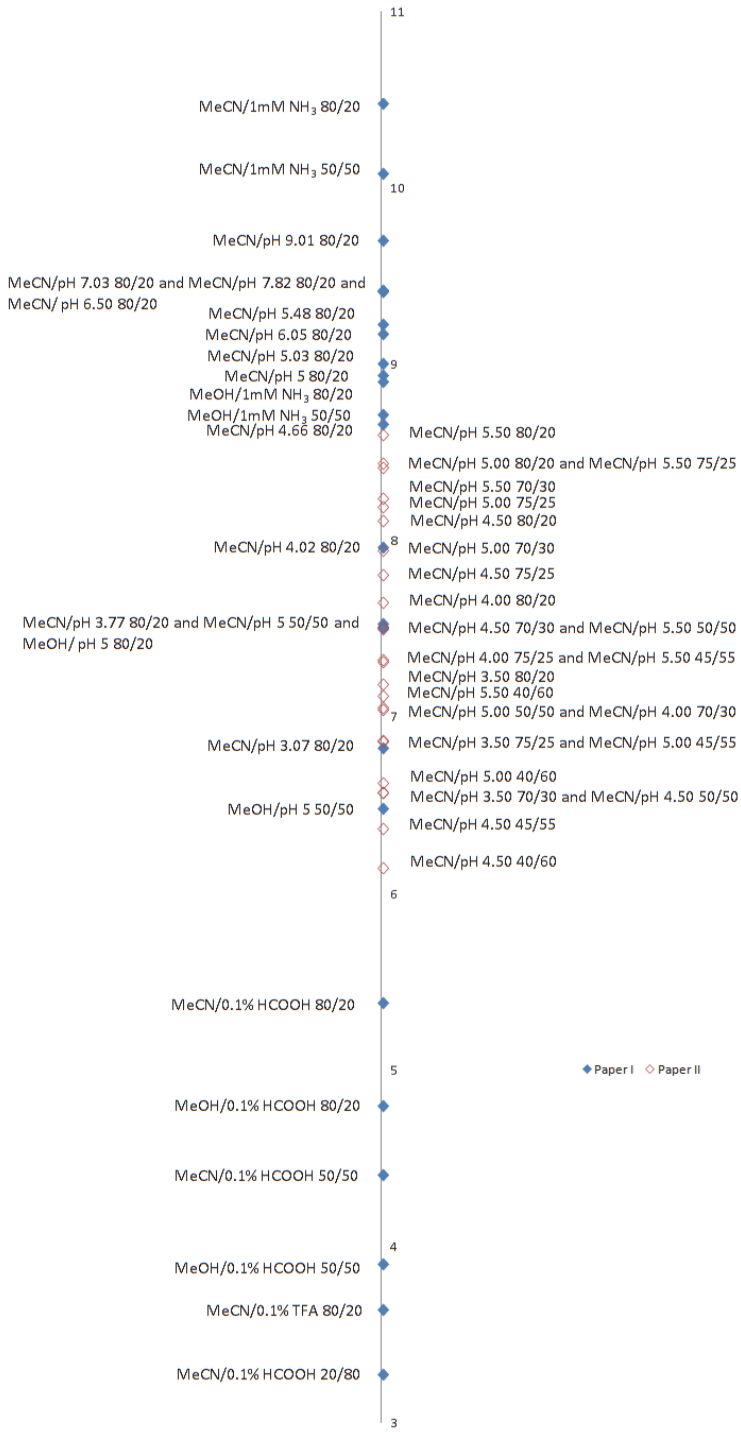


Figure 6. Unified acidities expressed on aqueous pH scale of all measured liquid chromatography mobile phases.

The effect of volume fraction of organic solvent on the solution acidity depends on the acid type. With neutral acids (e.g., HCOOH) the effect is strong. Water favours the separation of charges formed on dissociation more than the used two organic solvents, and increasing the water content in the mixture increases the acidity of the solution. In the case of cationic acid (e.g., NH_4^+), the number of charged particles remains the same on dissociation and the effect of volume fraction of water is weaker. The effect is stronger with MeCN than with MeOH. The effect of added organic solvent on bases (e.g., ammonia) is weaker but still present. This effect can be seen when comparing the acidities of aqueous phases 1 mM NH_3, pH 5 and 0.1% HCOOH with 80% or 50% organic solvent (MeCN or MeOH). In case of neutral acids (mobile phases with pH 5 and 0.1% HCOOH) the effect of decreasing the organic solvent from 80% to 50% decreases the $pH_{abs}^{H_2O}$ about 0.9 to 1.0 pH units for MeOH and 1.0 to 1.4 pH units for MeCN. In case of aqueous phase 1 mM NH_3 the effect is only 0.2 pH units for MeOH and 0.4 pH units for MeCN.

The main trends in acidity of mobile phases are:

(a) solutions containing methanol are more acidic than the respective acetonitrile-containing solutions of similar composition and
(b) the larger the volume fraction of the organic phase the less acidic the solution is.

As said before, the change in pK_a values of the acids and bases used for buffering has a strong effect on pH of the mobile phase, e.g. in the mobile phase MeCN/ pH 5 80/20. Let's discuss more about this mobile phase. There are two aqueous phases with different compositions, but have the same ${}_{w}^{w}pH$ 5. In Paper I this aqueous pH 5 is achieved by adding HCOOH to 5 mM CH_3COONH_4 and the mobile phase MeCN/ pH 5 80/20 is measured twice with different cell designs. The $pH_{abs}^{H_2O}$ values were 8.99 and 8.93. After reproducibility is taken into account, the acidities of these mobile phases can be considered the same. In Paper II the aqueous phase without the acetate ion was used and the $pH_{abs}^{H_2O}$ value for MeCN/ pH 5 80/20 was about half unit less – 8.43. The same is true with MeCN/ pH 4 80/20 where the pH difference is 0.3 units between different compositions – with or without acetate ion. The acidities of the mobile phases with same ${}_{w}^{w}pH$, but varying compositions are clearly different and it is due to different pK_a of acetic and formic acid.

As shown in Figure 6, mobile phases with different composition can have similar or essentially the same acidities. This is especially the case of mobile phases used in Paper II, where the ${}_{w}^{w}pH$ is varied between 3.50 to 5.50 and organic solvent fraction from 40% to 80%. The overall range is 2.45 pH units for the 24 mobile phases measured in Paper II. Decreasing water phase pH and the acetonitrile content have similar effect on mobile phase pH – both increase the acidity of mobile phase. Therefore mobile phases with lower ${}_{w}^{w}pH$ and higher MeCN percentage will have similar acidities with mobile phases with

higher ${}^{w}_{w}\mathrm{pH}$ and lower MeCN content. To exemplify these effects let's take MeCN/ pH 5.50 80/20 as a starting point with $\mathrm{pH}^{\mathrm{H_2O}}_{\mathrm{abs}}$ value 8.59. Decreasing the water phase aqueous pH to 3.50 should increase the acidity and indeed for MeCN/ pH 3.50 80/20 the $\mathrm{pH}^{\mathrm{H_2O}}_{\mathrm{abs}}$ value is 7.18. Now if we keep the ${}^{w}_{w}\mathrm{pH}$ constant at 5.50 and instead lower the acetonitrile percentage to 40%, we see the similar effect that the mobile phase acidity increases and for MeCN/ pH 5.50 40/60 the $\mathrm{pH}^{\mathrm{H_2O}}_{\mathrm{abs}}$ value is 7.11. Although mobile phases MeCN/ pH 3.50 80/20 and MeCN/ pH 5.50 40/60 have very different compositions their acidities are similar, because decreasing the aqueous phase pH and the acetonitrile content have similar effect on acidity as said previously.

A very interesting case is the $\mathrm{pH}^{\mathrm{H_2O}}_{\mathrm{abs}}$ value 7.5, that can be achieved with five different compositions – MeCN/ pH 5 50/50, MeCN/ pH 3.77 80/20, MeOH/ pH 5 80/20 from Paper I and MeCN/ pH 4.50 70/30, MeCN/ pH 5.50 50/50 from Paper II. This acidity can be achieved by mixing water phases with different compositions and ${}^{w}_{w}\mathrm{pH}$ with different organic solvents in different ratios. This means that the analyst can choose appropriate solvent and aqueous phase for the specific target, while maintaining the needed acidity.

Dissociation degree measurements (Paper I) showed that the combination of aqueous pK_a values and $\mathrm{pH}^{\mathrm{H_2O}}_{\mathrm{abs}}$ values can be successfully used to evaluate the ionization behavior of neutral bases (since their pK_a values do not change significantly with solvent composition), but in case of neutral acids the change of their pK_a values with solvent composition change is strong and has to be taken into account.

SUMMARY

The aims of the study were to develop an experimental method to measure unified pH values, validate the method and measure the absolute acidity of several liquid chromatography mobile phases as the first experimental realization of the unified acidity concept. All the aims were successfully fulfilled.

Differential potentiometry with two metal-contact glass electrodes were chosen as the appropriate method for unified acidity measurements. The cell design was constantly improved during the work and two suitable designs are now in use with salt bridge above or under the measurement solutions, depending on the densities of all solutions used.

Liquid junction potentials cannot be neglected in cases when relative acidities between solutions made in different solvents are measured. The Isutzu's three component approach was used to calculate the liquid junction potentials in mobile phase acidity measurements. In validation experiments, where acidities in the standard buffer solutions were compared, the liquid junction potentials and phase boundary potentials were assumed to cancel out. Two ionic liquids were tested as salt bridge electrolytes – $N_{2225}NTf_2$ and $C_6mimNTf_2$ – and were used in experiments where junction potential was assumed to cancel out, because at this stage the phase boundary potentials cannot yet be calculated.

The method was validated with two separate approaches. The first validation was measuring the differences of acidities of aqueous buffers with known pH values and evaluating their pH by the "ladder-approach". There was good agreement between experimental and reference values. The second validation was comparison of potentiometric ΔpK_a values with the spectrophotometric ΔpK_a results where again good agreement was found. The agreement between methods with fundamentally different principles is an evidence of validity of both methods.

The first experimental realization of the unified acidity concept led to the measurement of 25 mobile phases in Paper I and later supplemented with 24 mobile phases in Paper II. The main trends in acidity of mobile phases are (a) solutions containing methanol are more acidic than the respective acetonitrile-containing solutions of similar composition and (b) the larger the volume fraction of organic phase the less acidic the solution. The ionization behaviour of bases in mobile phases can be evaluated with combination of aqueous pK_a values and $pH_{abs}^{H_2O}$ values, but in case of acids change of their pK_a values with change in solvent composition has to be taken into account.

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SUMMARY IN ESTONIAN

Üldistatud happelisuse skaala katseline teostus ja rakendused

Töö eesmärk oli välja töötada ja valideerida eksperimentaalne meetod üldistatud pH väärtuste mõõtmiseks. Üldistatud happelisuse skaala esmase eksperimentaalse teostusena mõõdeti vedelikkromatograafia mobiilfaaside üldistatud happelisusi. Töö eesmärk täideti edukalt.

Üldistatud happelisusi mõõdeti diferentsiaalpotentsiomeetriaga, kasutades kahte klaaselektroodi. Töö käigus täiustati pidevalt mõõteraku ehitust ning mõõtmisteks kasutati kahte eri konstruktsiooniga mõõterakku. Olenevalt kasutatud lahuste tihedusest oli soolasild ülevalpool või allpool mõõtelahuseid.

Mõõtes happelisusi erinevates solventides valmistatud lahuste vahel, tuleb arvesse võtta difusioonipotentsiaali. Mobiilfaaside happelisuste hindamisel kasutati difusioonipotentsiaali arvutamiseks Izutsu teooriat. Valideerimisel kasutati standard puhverlahuseid happelisuse erinevuste mõõtmistel ja lähtuti eeldusest, et difusioonipotentsiaal taandub välja. Soolasilla elektolüütidena katsetati kahte ioonset vedelikku: $N_{2225}NTf_2$ ja $C_6mimNTf_2$. Ioonsete vedelike kasutamisel eeldati, et difusioonipotentsiaali ei pea arvestama. Käesoleval ajal pole veel välja töötatud kasutatavat teooriat ioonsete vedelike piirpinna potentsiaalide arvutamiseks.

Valideerimiseks kasutati kahte erinevat lähenemist. Esimeses valideerimismeetodis mõõdeti teadaoleva pH-ga vesilahuste happelisuste erinevusi ning määrati nende pH „redelimeetodi“ abil. Eksperimentaalsete ning referents-pH väärtuste hea kooskõla näitas, et diferentsiaalpotentsiomeetria sobib happelisuste erinevuste mõõtmiseks. Teises valideerimismeetodis võrreldi diferentsiaalpotentsiomeetria meetodi tulemustest arvutatud ΔpK_a väärtusi spektrofotomeetriliste ΔpK_a tulemustega. Hea kooskõla kahe erineva põhimõttega meetodi tulemuste vahel kinnitab, et mõlemad meetodid on rakendatavad.

Üldistatud happelisuse mõõtmismetoodikat kasutati esmakordselt 25 mobiilfaasi happelisuse hindamisel (Artikkel I), millele hiljem lisandusid 24 mobiilfaasi happelisuse hinnangud (Artikkel II). Põhilised suundumused mobiilfaasi happelisuse puhul on: (a) metanooli sisaldavad mobiilfaasid on happelisemad kui vastavad sarnase koostisega atsetonitriili sisaldavad mobiilfaasid ning (b) lahus on seda vähem happeline, mida suurem on orgaanilise lahusti osakaal. Üldjuhul saab mobiilfaasis aluste ionisatsiooni hinnata vesilahuse pK_a väärtuste ning $pH_{abs}^{H_2O}$ väärtuste abil, samas hapete puhul tuleb arvesse võtta ka pK_a muutust lahusti vahetumisel.

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Erialane enesetäiendus ja konverentsid:

18.–22.06.2017 – *45th International Symposium on high performance liquid phase separations and related techniques* (HPLC 2017), Praha, Tšehhi. Posterettekanne: "*Unified pH of LC mobile phases*".

07.–08.03.2017 – TÜ ja TTÜ doktorikooli "Funktsionaalsed materjalid ja tehnoloogiad" teaduskonverents, Tartu, Eesti. Suuline ettekanne: "*Unified pH of liquid chromatography mobile phases*".

25.–26.08.2016 – *International Electrosynthesis Workshop*, Riia, Läti.

12.–16.01.2015 – *Bath Electrochemistry Winter School*, Bath, UK.

2014 – uurimistöö Freiburgi Ülikoolis (4 kuud) professor Krossingu uurimisrühmas.

15.–28.07.2012 – *Measurement Science in Chemistry 5th Summer School*, Fatima, Portugal.

15.05.2012 – *Biohit's Pipetting Academy*

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