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## Improved pH measurement of mobile phases in reversed-phase liquid chromatography†

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Mobile phase pH is a critically important parameter in reversed-phase liquid chromatographic (RPLC) separations involving analytes that display acidic or basic properties in the pH range used for the mobile phase. The main problem in measuring mobile phase pH lies in the fact that RPLC mobile phases are typically aqueous–organic mixtures. In addition to experimental difficulties, the pH values refer to different aqueous–organic compositions that cannot be correctly compared. Given this situation, the unified pH ( $w_{\text{abs}}$  pH, also termed as  $\text{pH}_{\text{abs}}^{\text{H}_2\text{O}}$ ) based on the absolute chemical potential of the solvated proton has been proposed as a rigorous way of characterising mobile phase acidity that is fully inter-comparable between mobile phases of any composition. Here we report the  $w_{\text{abs}}$  pH values of 78 reversed-phase liquid chromatography–mass spectrometry mobile phases that were carefully measured by potential differences in a symmetric cell with two glass electrode half-cells and almost ideal ionic liquid triethylammonium bis((trifluoromethyl)sulfonyl)imide [N<sub>2225</sub>][NTf<sub>2</sub>] salt bridge with multiple overlapping measurements. The system of altogether 300  $\Delta w_{\text{abs}}$  pH values was anchored to the pH value of standard pH 7.00 aqueous buffer solution. The consistency standard deviation of the whole set of measurements was 0.09 pH units. In addition to the differential potentiometric reference method, simpler measurement methods that use double junction reference or double junction combined electrodes were tested and were found suitable for routine laboratories if high accuracy is not required.

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### 1. Introduction

Liquid chromatography (LC), especially its reversed-phase (RP) variant, and its combination with mass spectrometry (LC-MS) are the workhorses in proteomics,<sup>1</sup> metabolomics,<sup>2</sup> environmental analysis<sup>3</sup> *etc.* Mobile phase pH is a critically important parameter in separations involving analytes that display acidic or basic properties in the pH range used for the mobile phase (meaning the majority of analytes) and is used in retention modelling.<sup>4,5</sup> pH is also crucial for LC column stability, and manufacturers state the operational pH ranges of columns but usually refer to the aqueous pH values.<sup>6</sup>

The difficulty in measuring mobile phase pH is that RPLC mobile phases are typically aqueous–organic mixtures, and mobile phase composition changes during the run if gradient elution is used (which is the case in most separations). The pH quoted for mobile phases is usually the pH of the aqueous

component (denoted as  $w_{\text{pH}}$ )<sup>7</sup> or the pH of the mixed mobile phase measured with pH electrode calibrated in aqueous standards (denoted as  $s_{\text{pH}}$ ).<sup>7</sup> Neither of these is rigorous in the context of RPLC.

$w_{\text{pH}}$  characterises the pH of only the aqueous component of the mobile phase. Obviously, the acidity will change upon adding the organic component. True, if the buffer system in the mobile phase and the analytes are of a similar chemical nature, then the change will at least partially be offset by the parallel changes in the  $\text{pK}_{\text{a}}$  values of the analytes and buffer substance.  $s_{\text{pH}}$  expresses pH in the actual mobile phase, but the way it is measured neglects the potentially quite significant liquid junction potential (LJP) difference between the aqueous calibration buffers and aqueous–organic mobile phases and therefore such a measurement may yield a value that is significantly biased from the actual pH (in terms of  $\text{H}^+$  activity) in the solution.

The solvent-specific pH scales ( $s_{\text{pH}}$ ) do not have such flaws but are rarely used due to (a) lack of calibration standards, (b) limited knowledge of the  $\text{pK}_{\text{a}}$  values of analytes in mixed solvents and (c) (in the case of gradient elution) changing mobile phase composition during elution (meaning that in principle at any moment in the gradient run a different pH scale holds).<sup>7</sup>  $\text{pK}_{\text{a}}$  values of analytes in different solvents can be

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quite well estimated<sup>8–12</sup> and if pH is known for the eluent composition in the beginning, in the middle and at the end of the gradient, then pH can be reasonably estimated in other parts of the gradient profile. There still remains the issue of lack of calibration standards;  $s_p\text{pH}$  does not solve the comparability problem, and all the pH values are still solvent-specific.

The comparability problem can be solved by the unified acidity scale ( $\text{pH}_{\text{abs}}$  scale), which is based on solvent-independent standard state (absolute chemical potential of the solvated proton) and thus enables rigorous comparability of  $\text{pH}_{\text{abs}}$  values between any solvent systems.<sup>13</sup> The  $\text{pH}_{\text{abs}}$  scale has been experimentally realised *via* measurement of potential difference between two glass electrodes immersed in two solutions (connected *via* a salt bridge) which  $\text{pH}_{\text{abs}}$  values are compared and LC mobile phases were the first systems where it was used.<sup>14</sup> For convenience, the  $\text{pH}_{\text{abs}}$  scale was shifted and aligned with the aqueous pH scale to be termed  $^w_{\text{abs}}\text{pH}$  (also termed as  $\text{pH}_{\text{abs}}^{\text{H}_2\text{O}}$ ).<sup>14</sup> This way of writing means that the  $^w_{\text{abs}}\text{pH}$  values measured in any solvent/medium are directly comparable to the aqueous pH scale. *i.e.*  $^w_{\text{abs}}\text{pH}$  7.0 measured in any medium refers to the same chemical potential of the solvated proton as in aqueous solution with the conventional pH of 7.0. This also means that the acidities of LC mobile phases with various solvent compositions can be directly compared to the conventional aqueous pH scale.<sup>14</sup>

While rigorous in its essence, measurement of  $^w_{\text{abs}}\text{pH}$  is typically carried out *via* differential potentiometry, which involves liquid junctions. It is, therefore, still influenced by the LJP. The first  $^w_{\text{abs}}\text{pH}$  measurement method<sup>14</sup> used a bridge electrolyte of 0.05 M  $\text{Et}_4\text{NClO}_4$  solution in MeCN and a sophisticated (but still approximate) LJP estimation approach. This LJP estimation approach needed experimental data from the literature. In the (frequent) cases of non-availability of data, estimates were used instead. This led to high uncertainties of the estimated LJP values and, therefore, high uncertainties of the  $^w_{\text{abs}}\text{pH}$  values.

The more advanced measurement method<sup>15</sup> employed in this work uses a novel ionic liquid salt bridge (ILSB) with triethylamylammonium bis(trifluoromethyl)sulfonylimide  $[\text{N}_{2225}][\text{NTf}_2]$ . Because of the high similarity of the involved ions – both by mobilities and solvation Gibbs energies in a wide variety of solvents – this IL has been demonstrated to effectively cancel the LJP out within standard deviation of 6.3 mV, corresponding to 0.11 pH units,<sup>16–19</sup> thus, significantly simplifying the measurement and decreasing the uncertainty of the obtained  $^w_{\text{abs}}\text{pH}$  values. The ILSB approach can be used with essentially any solvent or mixture. Another advantage is that in the case of ILSB, the shape of the junction does not matter, unlike in the case of the traditional KCl salt bridge.<sup>20</sup>

Any commercially available glass electrode half-cell is suitable for differential potentiometric measurement<sup>21</sup> and any instrument capable of measuring potentials in the millivolt range, like a pH meter, potentiostat and electrometer, is suitable if the input impedance is  $>10^{13} \Omega$ .<sup>15</sup>

The method based on measuring potential differences between two glass electrodes might be too complex for routine

laboratories. Therefore, the same approach was applied in a more conventional and simpler-to-use manner using a glass electrode half-cell with a double junction reference electrode or a double junction combined pH electrode. The outer compartment of the reference electrode was filled with the above-mentioned ionic liquid. In contrast, the inner compartment was filled with the traditional 3 M or a saturated aqueous solution of KCl.

In this paper, we present  $^w_{\text{abs}}\text{pH}$  values of a large number of LC mobile phases measured with improved method. Some earlier published values were remeasured to compare the previous values obtained with LJP estimation<sup>14</sup> to the more accurate values obtained with the new method utilising the specially designed ILSB, which enables cancellation of LJP.<sup>19</sup> In addition, the new  $^w_{\text{abs}}\text{pH}$  measurement approach was applied with a double junction combined pH electrode to demonstrate the easy applicability of the method adaption by practitioners in routine laboratories.

## 2. Experimental

### 2.1. Materials and instrumentation

Different types of electrodes were used depending on the electrochemical cell. Electrodes from different manufacturers were used to test the robustness of the method. The used electrodes and instruments are given in Table 1.

Aqueous standard buffers  $4.01 \pm 0.02$  (Mettler Toledo),  $7.00 \pm 0.02$  (Mettler Toledo),  $9.00 \pm 0.02$  (Hach),  $10.01 \pm 0.02$  (Mettler Toledo) were used for calibrating the electrodes (Table 1) and for anchoring the  $^w_{\text{abs}}\text{pH}$  values. The ionic liquid  $[\text{N}_{2225}][\text{NTf}_2]$  (99%, Iolitec) and KCl (p.a., LachNer) were used as salt bridge electrolytes.

Chemicals for mobile phases: acetonitrile (CHROMASOLV,  $\geq 99.9\%$ , for LC-MS, Honeywell Riedel-de-Haën), methanol (CHROMASOLV, for HPLC,  $\geq 99.9\%$ , Honeywell Riedel-de-Haën), formic acid (puriss, p.a., ACS reagent, Reag. Ph. Eur,  $\geq 98\%$ , Honeywell Fluka), ammonium hydroxide solution (eluent additive for LC-MS,  $\geq 25\%$  in  $\text{H}_2\text{O}$ , Honeywell Fluka), ammonium acetate (BioUltra, for molecular biology,  $\geq 99.0\%$ ), 1,1,1,4,4,4-hexafluoro-2,3-bis(trifluoromethyl)butane-2,3-diol (perfluoropinacol, PP), 1,1,1,3,3,3-hexafluoro-2-methylisopropanol (HFTB), 2,2,2-trifluoroethanol (TFE) were LC-MS grade and obtained from Sigma-Aldrich, 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) was obtained from Fluka. All chemicals were used without further purification.

The aqueous phase compositions are given in Table 2. The used ammonia concentration is not common in liquid chromatography mobile phases but was used to compare results obtained with the previously used method.<sup>14</sup>

$^w_{\text{abs}}\text{pH}$  values were measured with Oakton WD-35801-09 connected with Evikon E6115 pH meter or Elmetron EPP-1 connected with Elmetron CP-411 pH meter. The combined pH electrode was calibrated daily with standard buffers with pH values of 7.00 and 4.01 or 10.01, depending on the pH values of the prepared aqueous solutions.



**Table 1** List of used glass electrodes, electrochemical cells and instruments

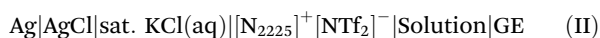
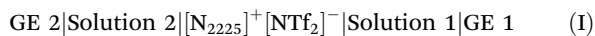
Electrode type	Electrode	Diaphragm	Used in cell	Instrument
A Half-cell, solid metal contact	EST-0601, Izmeritel'naya Tekhnika	None	I	Metrohm 713 pH meter; Keysight B2987A
B Half-cell, liquid filled	DG300-SC, Mettler-Toledo	None	II.1	Keysight B2987A
C Half-cell, liquid filled	6.0150.100, Metrohm	None	II.1	Keysight B2987A
D Double junction combined electrode	6.0269.100, Metrohm	Fixed ground-joint	II.2	Elmetron CP-411 pH meter; Metrohm 744 pH meter
E Double junction combined electrode	WD-35805-09, Oakton	Flushable PTFE	II.2	Elmetron CP-411 pH meter; Metrohm 744 pH meter

**Table 2** Aqueous phase compositions and  $w_{\text{abs}}$ pH values

Aqueous phase composition	Short name	$w_{\text{abs}}$ pH
0.1 vol% HCOOH	None	2.68
1 mM NH <sub>3</sub>	None	9.75
5 mM CH <sub>3</sub> COONH <sub>4</sub> titrated with HCOOH or NH <sub>4</sub> OH	Acetate	5.00; 6.00; 7.00; 9.50
0.1 vol% HCOOH titrated with NH <sub>4</sub> OH	Formate	4.00; 4.50; 5.00; 5.50
5 mM 1,1,1,3,3,3-hexafluoro-2-methyl-2-propanol titrated with NH <sub>4</sub> OH	HFTB	5.00; 6.00; 7.00; 9.50
50 mM Na <sub>2</sub> HPO <sub>4</sub> + 50 mM KH <sub>2</sub> PO <sub>4</sub>	Phosphate	7.50
5 mM perfluoropinacol titrated with NH <sub>4</sub> OH	PP	5.00; 6.00; 7.00
5 mM 2,2,2-trifluoroethanol titrated with NH <sub>4</sub> OH	TFE	8.50; 9.00; 10.00
5 mM 1,1,1,3,3,3-hexafluoro-2-propanol titrated with NH <sub>4</sub> OH	HFIP	8.50; 9.00; 10.00

## 2.2. Methods

$w_{\text{abs}}$ pH measurements were performed with two methods. One was the potential measurement between two glass electrodes (GE) (cell I). This is the so-called “reference method” for  $w_{\text{abs}}$ pH. The other method, aiming to be the method of choice for routine use, measures the potential between a glass and a double-junction reference electrode (cell II) or a double junction combined pH electrode. This method is termed the “routine method”. The above-mentioned cells are as follows:



The reference method with the cell I used a Metrohm 713 pH meter or a Keysight B2987A electrometer to measure the potential difference between two glass electrodes immersed in the two solutions that are compared, separated by a salt bridge containing the ionic liquid  $[\text{N}_{2225}]^+[\text{NTf}_2]^-$  (see Fig. S2 and S3 in ESI†).

A Pt wire was used as an auxiliary electrode for measurements with Metrohm 713 pH meter.<sup>14</sup> Metal solid-contact glass electrodes (electrode A, Table 1) were used. Radiometer K401 saturated calomel reference electrode was used to calibrate the metal solid-contact glass electrodes. Measurements were thermostated at 25.0 °C ( $\pm 1.0$  °C) using a thermostat MLW U2C and later Julabo Corio CD-200F. These measurements were carried out in a special water-jacketed glass cell<sup>15</sup> from Gebr. Rettberg (Fig. S1†), which was placed in a Faraday cage (VistaShield, Gamry).

Measurements with the cell I give “relative”  $w_{\text{abs}}$ pH values ( $\Delta w_{\text{abs}}$ pH values). When overlapping measurements are in

excess, these values can be combined into a “ladder”. A least-squares minimization technique<sup>15</sup> assigns “absolute”  $w_{\text{abs}}$ pH values to mobile phases. One of the solutions with a known pH value is used as the “anchor” solution. In this case, it is the standard aqueous buffer solution with a pH value of 7.00. MS Excel Solver is used to carry out the least-squares minimization.

Although every effort was made to choose GE 1 and GE 2 with as close a slope and intercept as possible, there are still some minor differences between them. The most straightforward approach to minimize the effect of these differences is taking an average of two measurements where the electrode positions have been switched between the solutions.<sup>15</sup> Although simple, this approach is labour-intensive.

Therefore, this was not done with all measurements but with a set of 31 mobile phase pairs measured with both polarities. Minimizations were done with potentials from measurements of Solution 1–Solution 2, Solution 2–Solution 1 and the average potential of both polarities. The root mean square difference between the three obtained sets of values was 0.04. Also, averaging did not give better consistency than using individual differences. Thus, making the main bulk of measurements with only one polarity of the electrodes was considered sufficient.

The aim was to measure a large  $w_{\text{abs}}$ pH data pool to validate the method and define quality requirements for the measured data. Data points were collected at 3 s (Metrohm) or 10 s (Keysight) intervals. Initially, the data for one measurement series was collected for 30 minutes and later for 60 minutes. Most of the data was collected for 60 min. One measurement was 10 h to test the stability. In all cases, only the points from a continuous 15 min time interval giving the most stable



signals were used for averaging to get the  $\Delta\text{pH}_{\text{abs}}$  values displayed on the arrows in Fig. S7.† The quality criteria for stable reading were set retrospectively to retain only reliable measurements. The criteria are given in section 3.1.

The routine method (Fig. S4†) uses cell II to measure the potential difference between a glass and a double junction reference electrode. These measurements were made at room temperature, around 21 °C in our case, and a magnetic stirrer was used for mixing.

The method using cell II can be operated with any mainstream pH meter. This method was realised (a) separately with a glass half-cell pH electrode (electrodes B and C, Table 1) together with a double junction Metrohm Ag/AgCl reference electrode 6.0729.100 (DJ ref) designated as cell II.1 (see Fig. S4A†), and (b) as a combined electrode with double junction pH electrodes D and E (Table 1), designated as cell II.2 (see Fig. S4B†). Electrodes B and C were used because glass electrodes with metal contact (electrode A) are challenging to obtain.

Measurements with cell II were intended to be similar to routine measurements. Measurements with cell II.1 were carried out with Keysight B2987A because it enables the connection of a separate glass and a reference electrode. This method is new and there is no information about the temporal nature of the data. Points were collected for 600 s with 5 s intervals to ensure that the plateau had been reached. Points from 285 s to 315 s were averaged where the stable reading had been reached. Glass electrode half-cell measurements against a reference electrode reach a plateau faster than measurements against another glass electrode half-cell. Most measurements with cell II.2 were carried out during 30 s to 120 s with an Elmetron CP-411 pH meter to mimic routine pH measurements and were single values. CP-411 resolution is lower. Some of the measurements with cell II.2 were done with Metrohm 744 pH meter to investigate the behaviour of combined electrodes.

The inner solution of the double junction reference electrode was saturated aqueous KCl solution, and the outer filling was  $[\text{N}_{2225}][\text{NTf}_2]$ . Combined electrodes were filled with 3 M aqueous KCl and  $[\text{N}_{2225}][\text{NTf}_2]$ . In the case of an ionic liquid salt bridge, the LJP does not depend on the shape of the junction.<sup>20</sup> Cell II.1 has been previously tested with TRIS-buffered artificial seawater<sup>22</sup> and water–ethanol mixtures.<sup>23,24</sup> This is the first successful use of cell II.2. Two-point calibration<sup>25</sup> using standard aqueous buffers was used to measure  $w_{\text{abs}}\text{pH}$  values.

### 3. Results and discussion

#### 3.1. Overview of the results

Overall, the  $w_{\text{abs}}\text{pH}$  values of 78 mobile phases were measured with cell I (Table 3), connected by  $\Delta\text{pH}_{\text{abs}}$  measurements conducted over two years. Based on the typical measurement results, the maximum standard deviation of the used data points of 1 mV and maximum drift of 4 mV h<sup>-1</sup> were set as

**Table 3** Unified acidity values that were obtained in this work and comparison with previously published values

Mobile phase <sup>a</sup>	$w_{\text{abs}}\text{pH}^b$	Earlier value
MeCN/1 mM NH <sub>3</sub> 80/20	10.81	10.47 <sup>c</sup>
MeOH/pH 10 (TFE) 25/75	10.49	9.73 <sup>d</sup>
MeOH/pH 10 (HFIP) 25/75	10.29	9.87 <sup>d</sup>
MeCN/1 mM NH <sub>3</sub> 50/50	10.21	8.70 <sup>c</sup>
MeOH/pH 9.5 (HFTB) 25/75	10.14	
Aqueous buffer pH 10.01	10.05	
MeOH/1 mM NH <sub>3</sub> 80/20	9.95	8.89 <sup>c</sup>
MeOH/pH 9.5 (acetate) 25/75	9.93	
MeOH/pH 9.5 (PP) 25/75	9.93	
MeOH/1 mM NH <sub>3</sub> 20/80	9.92	
MeOH/1 mM NH <sub>3</sub> 50/50	9.90	10.07 <sup>c</sup>
MeCN/1 mM NH <sub>3</sub> 20/80	9.85	
MeOH/pH 9 (TFE) 25/75	9.61	7.38 <sup>d</sup>
MeOH/pH 9 (HFIP) 25/75	9.54	9.18 <sup>d</sup>
		9.62 <sup>e</sup>
MeOH/pH 9 (HFTB) 25/75	9.39	9.20 <sup>d</sup>
		9.40 <sup>e</sup>
MeOH/pH 8.5 (HFIP) 25/75	9.30	8.83 <sup>d</sup>
		9.36 <sup>e</sup>
MeOH/pH 8.5 (TFE) 25/75	9.23	7.23 <sup>d</sup>
MeCN/pH 5 (acetate) 80/20	9.14	8.99 <sup>c</sup>
MeOH/pH 8.5 (HFTB) 25/75	8.98	8.85 <sup>d</sup>
		9.03 <sup>e</sup>
MeOH/pH 7.5 (phosphate) 50/50	8.98	
Aqueous buffer pH 9.00	8.96	
MeOH/pH 9 (acetate) 25/75	8.96	8.70 <sup>d</sup>
		9.00 <sup>e</sup>
MeOH/pH 9 (PP) 25/75	8.91	8.68 <sup>e</sup>
		8.87 <sup>e</sup>
		8.59 <sup>f</sup>
MeCN/pH 5.5 (formate) 80/20	8.63	
MeOH/pH 7 (HFTB) 80/20	8.52	
MeCN/pH 5 (formate) 80/20	8.50	8.43 <sup>f</sup>
MeOH/pH 8.5 (acetate) 25/75	8.49	8.17 <sup>d</sup>
		8.48 <sup>e</sup>
MeOH/pH 8.5 (PP) 25/75	8.40	8.20 <sup>d</sup>
		8.47 <sup>e</sup>
MeOH + 5 mM HFTB/pH 7 (HFTB) 80/20	8.37	
MeCN/pH 4.5 (formate) 80/20	8.23	8.10 <sup>f</sup>
MeOH/pH 5 (acetate) 90/10	8.11	
MeOH/pH 7.5 (phosphate) 20/80	7.96	
MeOH + 5 mM HFTB/pH 7 (HFTB) 40/60	7.86	
MeCN/pH 4 (formate) 80/20	7.84	7.64 <sup>f</sup>
MeOH/pH 7 (HFTB) 40/60	7.83	
MeOH/pH 7 (HFTB) 25/75	7.79	
MeOH/pH 5 (acetate) 80/20	7.70	
MeOH/pH 7 (PP) 25/75	7.65	
MeOH/pH 5.5 (formate) 80/20	7.57	
MeOH/pH 7 (HFTB) 5/95	7.48	
MeOH + 5 mM HFTB/pH 7 (HFTB) 5/95	7.46	
MeOH/pH 7 (acetate) 25/75	7.34	
MeOH/pH 6 (HFTB) 25/75	7.33	
MeOH/pH 5 (formate) 80/20	7.22	
MeCN/pH 5 (acetate) 50/50	7.19	7.50 <sup>c</sup>
MeCN/pH 5.5 (formate) 50/50	7.09	7.49 <sup>f</sup>
MeOH/pH 6 (PP) 25/75	7.02	
Aqueous buffer pH 7.00	7.00	
MeOH/pH 6 (acetate) 25/75	6.82	
MeOH/pH 5 (HFTB) 25/75	6.82	
MeCN/pH 5.5 (formate) 40/60	6.78	7.11 <sup>f</sup>
MeCN/pH 4 (formate) 75/25	6.69	7.32 <sup>f</sup>
MeCN/pH 5 (formate) 50/50	6.59	7.05 <sup>f</sup>
MeOH/pH 5.5 (formate) 50/50	6.56	
MeCN/pH 4 (formate) 70/30	6.53	7.03 <sup>f</sup>
MeOH/pH 5 (acetate) 50/50	6.50	6.47 <sup>c</sup>
MeOH/pH 5 (PP) 25/75	6.23	
MeCN/pH 5 (formate) 40/60	6.19	6.62 <sup>f</sup>
MeOH/pH 5 (formate) 50/50	6.08	
MeCN/pH 4.5 (formate) 50/50	6.06	6.56 <sup>f</sup>



Table 3 (Contd.)

Mobile phase <sup>a</sup>	$w_{\text{abs}}\text{pH}^b$	Earlier value
MeOH/pH 5 (acetate) 25/75	6.05	
MeCN/pH 4 (formate) 60/40	5.98	
MeCN/pH 4.5 (formate) 40/60	5.76	6.14 <sup>f</sup>
MeOH/pH 5.5 (formate) 20/80	5.75	
MeOH/pH 5 (acetate) 20/80	5.50	
MeCN/0.1% HCOOH 80/20	5.48	5.37 <sup>c</sup>
MeOH/pH 5 (acetate) 10/90	5.36	
MeOH/pH 5 (formate) 20/80	5.29	
MeCN/pH 4 (formate) 40/60	5.25	
MeOH/0.1% HCOOH 90/10	4.95	
MeCN/pH 4 (formate) 25/75	4.73	
MeOH/0.1% HCOOH 80/20	4.69	4.79 <sup>c</sup>
0.1% HCOOH MeOH/0.1% HCOOH 90/10	4.60	
0.1% HCOOH MeOH/0.1% HCOOH 80/20	4.36	
MeCN/0.1% HCOOH 50/50	3.97	4.39 <sup>c</sup>
Aqueous buffer pH 4.01	3.96	
MeOH/0.1% HCOOH 50/50	3.68	3.89 <sup>c</sup>
0.1% HCOOH MeOH/0.1% HCOOH 50/50	3.60	
MeOH/0.1% HCOOH 20/80	2.95	
0.1% HCOOH MeOH/0.1% HCOOH 20/80	2.88	
MeOH/0.1% HCOOH 10/90	2.74	
0.1% HCOOH MeOH/0.1% HCOOH 10/90	2.68	

<sup>a</sup> Composition given as organic phase/aqueous phase. The pH values indicated in compositions refer to the aqueous phase, before mixing.

<sup>b</sup> This work and the most accurate value. <sup>c</sup> Ref. 14. <sup>d</sup> Ref. 26. <sup>e</sup> Ref. 27. <sup>f</sup> Ref. 28.

quality limits. Measurement series that failed to meet these criteria were omitted from data analysis. Eventually, 300 measurement series out of the overall 427 were used. 90% of measurements done with MeOH mixtures passed the quality control, but only 60% of MeCN-based mixtures passed. This is due to specific experimental problems related to mobile phases with 70% to 80% acetonitrile by volume. Fig. S7 in ESI† presents the full set of measurement results.

Mobile phases with acetonitrile (MeCN) are more complicated to measure than the ones with methanol (MeOH) because the ionic liquid (IL) solubility is better in MeCN. The most difficult-to-measure mobile phases were those with 70% to 80% acetonitrile by volume. With mobile phase composition in this region, droplets were formed on the IL-solution interface (Fig. S5†), and their movement disturbed the signal stability. This results in the corresponding mobile phase  $w_{\text{abs}}\text{pH}$  measurements more often failing the quality check and being left out from least squares minimization (Fig. S6†).

Assigning absolute  $w_{\text{abs}}\text{pH}$  values to individual solutions using the obtained  $\Delta\text{pH}_{\text{abs}}$  values *via* least-squares minimization is the same as in literature<sup>15,29</sup> except for the modified data analysis.<sup>30</sup>

The complete set of  $\Delta\text{pH}_{\text{abs}}$  measurement results are shown in Fig. S7.† As described above, the  $w_{\text{abs}}\text{pH}$  values were anchored to an aqueous standard buffer with conventional pH (and thus also  $w_{\text{abs}}\text{pH}$ ) equal to 7.00. The  $w_{\text{abs}}\text{pH}$  values of the other three aqueous standard buffers were allowed to change during the least squares minimization. The good agreement between their reference values and the values assigned as a result of minimization (3.96 *vs.* 4.01; 8.96 *vs.* 9.00 and 10.05 *vs.* 10.00) gives additional evidence that the measurement

method works well. The consistency standard deviation<sup>15</sup> of the whole set of measurements was 0.09 pH units. The uncertainties are in the range of 0.12 to 0.13, the largest contribution coming from LJP cancellation.<sup>29</sup>

When the same solution is on both sides of cell I, which means no pH difference between the solutions, the measured pH difference can be up to 0.07 instead of zero for all tested mobile phases.

### 3.2. Relations between $w_{\text{abs}}\text{pH}$ values and other ways of expressing pH of mobile phases

There is a weak correlation between  $w_{\text{w}}\text{pH}$  values and  $w_{\text{abs}}\text{pH}$  values when different buffers and organic solvent compositions are involved (Fig. 1A)—the changes in the  $\text{pK}_{\text{a}}$  values of the buffer components with adding the organic solvent cause this. The  $\text{pK}_{\text{a}}$  changes more in the case of neutral acids compared to cationic acids. However, there is a reasonable correlation between  $w_{\text{w}}\text{pH}$  values and  $w_{\text{abs}}\text{pH}$  values within a fixed organic solvent percentage (Fig. 1B) because the change in  $\text{pK}_{\text{a}}$  is constant for the given solvent composition.

There is a correlation between  $s_{\text{w}}\text{pH}$  values and  $w_{\text{abs}}\text{pH}$  values. Still, they do not differ simply by a constant value as was previously assumed<sup>26</sup> (Fig. 2A). Some of the  $s_{\text{w}}\text{pH}$  measurements

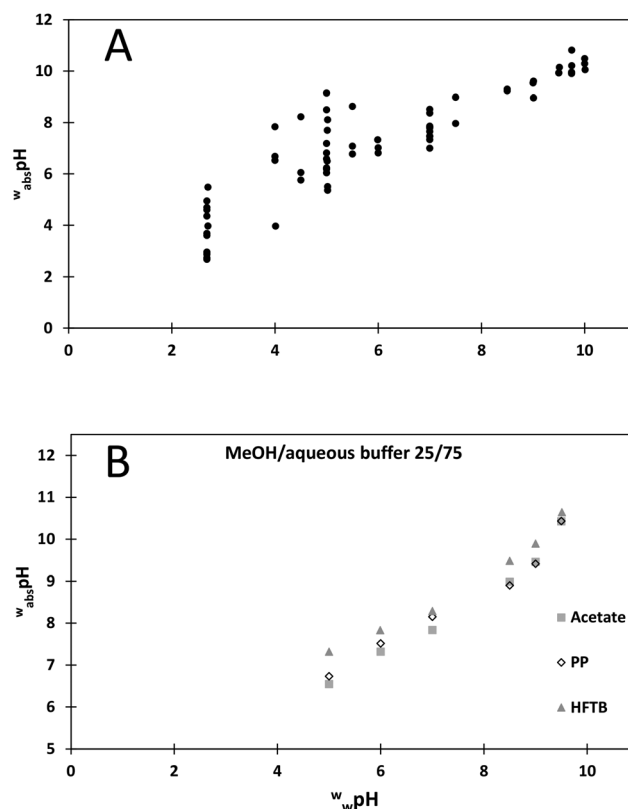


Fig. 1 (A) Relation between  $w_{\text{w}}\text{pH}$  values and  $w_{\text{abs}}\text{pH}$  values (cell I) in all measured mobile phases. (B) Relation between  $w_{\text{w}}\text{pH}$  values and  $w_{\text{abs}}\text{pH}$  values (cell I) in mobile phases with 25% MeOH and varying buffer component. PP is perfluoropinacol, HFTB is 1,1,1,3,3,3-hexafluoro-2-methyl-2-propanol.



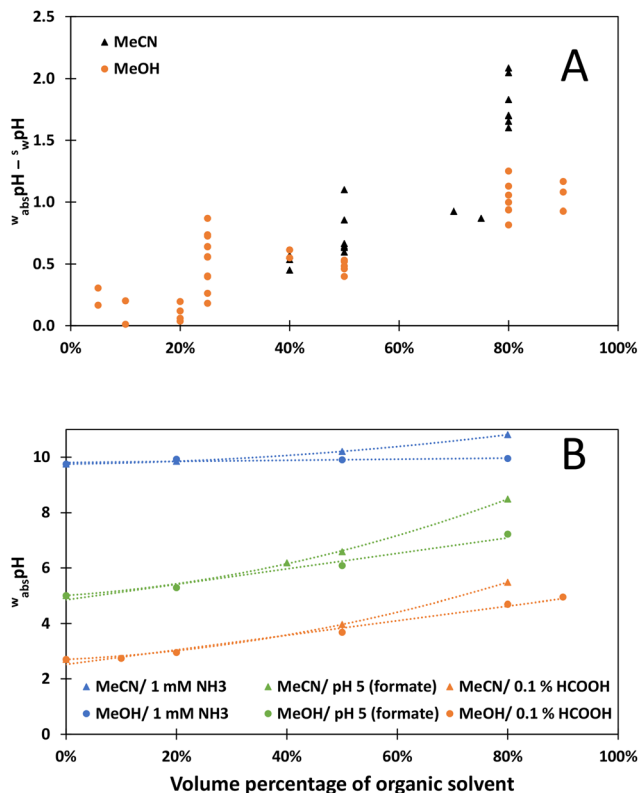


Fig. 2 (A) Relation between  $s_w\text{pH}$  and  $w_{\text{abs}}\text{pH}$  values (cell I). (B) Dependence of the  $w_{\text{abs}}\text{pH}$  value on organic component volume percentage.

were repeated, and the values differed up to 0.3 pH units. Solubility issues might have caused this difference. KCl solubility in methanol<sup>31</sup> and acetonitrile<sup>31</sup> and their aqueous mixtures<sup>32</sup> is substantially lower than in water, and KCl might partially precipitate in the diaphragm of the combined electrode during measurements. These findings suggest that the  $\delta$  parameter<sup>33–35</sup> proposed for such pH conversions is not a constant value, and the difference seems more significant for MeCN-based mobile phases.

The dependence of mobile phase acidity on the organic component volume percentage (Fig. 2B) is the same as previously reported.<sup>14,36,37</sup> The change in pH is similar for both MeCN and MeOH-based mobile phases up to 50 volume per cent of the organic component. At higher percentages than 50% by volume of organic component, the acidity changes linearly upon adding MeOH, but not when adding MeCN. Interestingly, the ammonia-based mobile phases have rather similar  $w_{\text{abs}}\text{pH}$ . This might be because changes in the  $\text{pK}_a$  compensate for the changes in solvent acidity.

Changes in  $\text{pK}_a$  are very similar for carboxylic acids. Therefore, the pH changes of acetate and formate buffers should be very similar when adding the same amount of the same organic phase. The difference between acetate and formate buffer pH 5 is due to the concentrations of the used acetate (5 mM) and formate buffers (26.5 mM).

According to the  $w_{\text{abs}}\text{pH}$  values, all aqueous–organic mobile phases are more basic than the respective aqueous phases. The most extreme example is acetate buffer with  $w_{\text{abs}}\text{pH}$  5, which after adding 80% MeCN in volume, becomes a basic solution with  $w_{\text{abs}}\text{pH}$  9.14. The reason for this is a significant increase in  $\text{pK}_a$  values of carboxylic acids when MeCN is added to water. Both the aqueous pH and the volume per cent of the aqueous phase play a role in mobile phase acidity. Different mobile phase compositions can achieve similar  $w_{\text{abs}}\text{pH}$  value. For example, the pH of MeCN/pH 5 (formate) 50/50, MeCN/pH 4 (formate) 70/30, MeOH/pH 5.5 (formate) 50/50 and MeOH/pH 5 (acetate) 50/50 are all in the range of 6.50 to 6.59.

The possibility of measuring  $w_{\text{abs}}\text{pH}$  values of mobile phases with ordinary pH measurement equipment was investigated. Although the reference method cell I provides good accuracy, it is complicated to set up (it needs a specialised glass cell) and operate. Moreover, to obtain the  $w_{\text{abs}}\text{pH}$  of a solution, it is necessary to measure it against at least two reference solutions, increasing the workload. Therefore, it is not expected that routine laboratories will widely adopt it.

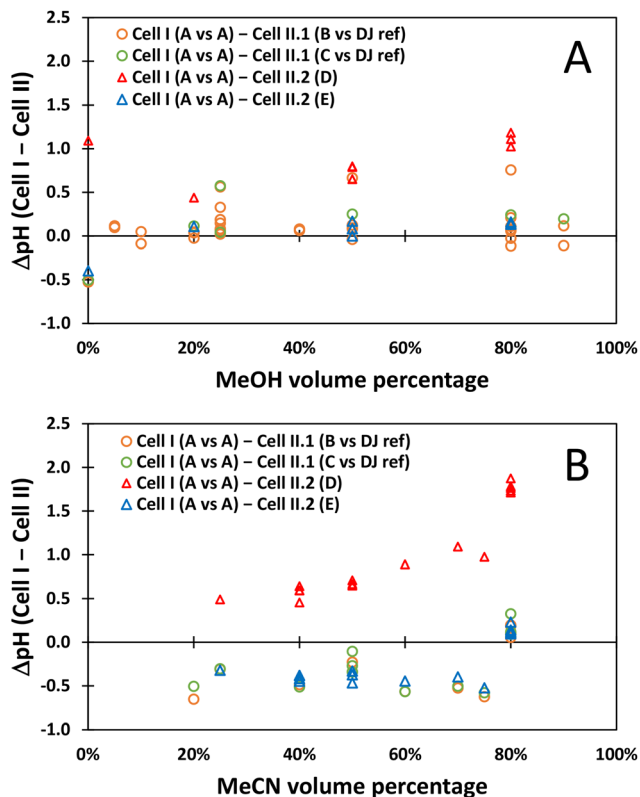
Measurements with cell II were done to find an alternative for the reference method (cell I) suitable for routine laboratories. Cell II uses a glass electrode half-cell and a double junction reference electrode or a double junction combined pH electrode. Measurements are done the conventional way – the potential of the glass electrode against the reference electrode is measured. The cell was calibrated using the standard aqueous buffers. 45 mobile phases out of 78 were measured with cell II.1 (glass and a reference electrode), and 23 with cell II.2 (combined electrode). Fluoroalcohol-based mobile phases were left out. The differences between the results obtained with the two cells are presented in Fig. 3.

The results show that the suitability of cell II depends on electrodes and the measured system. Cell II.1 worked somewhat better but occasionally displayed discrepancies from the cell I results, amounting to around 0.5 pH units and, in a few cases, even more. Cell II.2 performance differs strongly: electrode D is unsuitable for these measurements, while electrode E behaves the same as cell II.1.

The reasons for the discrepancies might be the different types of the junction, possibly insufficient electrical contact, the temperature difference (cell I was thermostated at 25 °C; cell II was at room temperature, not thermostated), stirring was used with cell II but not with the cell I. We have not studied the double junction electrodes enough to confidently state that double junction electrodes are, as a rule, suitable for this work. However, the present results give an indication that these electrodes may work. The reason for discrepancies between cells needs further investigation.

Measurements with a separate glass electrode half-cell and a double junction reference electrode (B or C vs. DJ ref, cell II.1) agree satisfactorily with the results of cell I, and there is no difference between glass electrodes. In the case of the double junction combined pH electrode (D or E, cell II.2), the agreement depends on the used electrode. Results with elec-





**Fig. 3** Difference between results obtained with cell I and II. (A) MeOH-based mobile phases. (B) MeCN-based mobile phases. Glass electrodes are given in Table 1. DJ ref is a double junction reference electrode.

trode E agree with the results of cell I and with measurements made with separate electrodes (cell II.1).

Electrode D gave unacceptable results. Some measurements were repeated with a second pH meter. There was no difference in results with electrode E between the two pH meters, but with electrode D the results differed by up to 0.2 pH units. This indicates that the diaphragm type of the reference electrode plays an important role.

Electrode E has a flushable PTFE junction, and the DJ ref has a moveable ground-joint diaphragm. Hence the wetting of the diaphragm is ensured. Electrode D has a fixed ground-joint, and the IL's leakage is insufficient for good contact between IL and the test solution.

These results show that  $w_{\text{abs}}^{\text{pH}}$  can be routinely measured with a double junction combined pH electrode if high accuracy is not required. Still, care must be taken when choosing the junction of the reference electrode. The larger the leakage rate of the diaphragm, the better the connection between the solutions and the more accurate the results.

## 4. Conclusions

Overall 427 relative measurements were made to assign unified acidity ( $w_{\text{abs}}^{\text{pH}}$ ) values to 78 mobile phases. Based on the predefined quality limits (standard deviation not more

than 1 mV and drift not more than  $4 \text{ mV h}^{-1}$ ), 300 relative measurements out of 427 were used in the data assignment of pH values. A novel method based on a double junction reference electrode filled with ionic liquid was tested to be adequate for routine measurements. This concept was tested as a double junction combined electrode for the first time. Results show that the design of the junction is an important factor in deciding if the electrode can be used for unified acidity measurements. The effect of electrode design on the quality of results needs further investigation.

## Author contributions

Agnes Heering: conceptualization, methodology, writing – original draft preparation, investigation, formal analysis, writing – review & editing; Markus Lahe: methodology, investigation; Martin Vilbaste: investigation, formal analysis, writing – review & editing; Jaan Saame: investigation; John Paulo Samin: investigation, formal analysis; Ivo Leito: conceptualization, resources, formal analysis, writing – review & editing.

## Data availability

The data sets of this work are available at <https://doi.org/10.5281/zenodo.7913050>.

## Conflicts of interest

There are no conflicts to declare.

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## References

- 1 R. Aebersold and M. Mann, *Nature*, 2003, **422**, 198–207.
- 2 H. G. Gika, G. A. Theodoridis, R. S. Plumb and I. D. Wilson, *J. Pharm. Biomed. Anal.*, 2014, **87**, 12–25.
- 3 M. Petrovic, M. Farré, M. L. de Alda, S. Perez, C. Postigo, M. Köck, J. Radjenovic, M. Gros and D. Barcelo, *J. Chromatogr. A*, 2010, **1217**, 4004–4017.



- 4 C. Zisi, A. Pappa-Louisi and P. Nikitas, *J. Chromatogr. A*, 2020, **1617**, 460823.
- 5 M. J. den Uijl, P. J. Schoenmakers, B. W. J. Pirok and M. R. van Bommel, *J. Sep. Sci.*, 2021, **44**, 88–114.
- 6 T. Alvarez-Segura, X. Subirats and M. Rosés, *Anal. Chim. Acta*, 2019, **1050**, 176–184.
- 7 M. Rosés, *J. Chromatogr. A*, 2004, **1037**, 283–298.
- 8 A. Kütt, S. Selberg, I. Kaljurand, S. Tshepelevitsh, A. Heering, A. Darnell, K. Kaupmees, M. Piirsalu and I. Leito, *Tetrahedron Lett.*, 2018, **59**, 3738–3748.
- 9 X. Subirats, M. Rosés and E. Bosch, *Sep. Purif. Rev.*, 2007, **36**, 231–255.
- 10 F. Rived, I. Canals, E. Bosch and M. Rosés, *Anal. Chim. Acta*, 2001, **439**, 315–333.
- 11 J. L. Beltrán, N. Sanli, G. Fonrodona, D. Barrón, G. Özkan and J. Barbosa, *Anal. Chim. Acta*, 2003, **484**, 253–264.
- 12 F. Z. Erdemgil, S. Şanlı, N. Şanlı, G. Özkan, J. Barbosa, J. Guiteras and J. L. Beltrán, *Talanta*, 2007, **72**, 489–496.
- 13 D. Himmel, S. K. Goll, I. Leito and I. Krossing, *Angew. Chem., Int. Ed.*, 2010, **49**, 6885–6888.
- 14 A. Suu, L. Jalukse, J. Liigand, A. Kruve, D. Himmel, I. Krossing, M. Rosés and I. Leito, *Anal. Chem.*, 2015, **87**, 2623–2630.
- 15 A. Heering, D. Stoica, F. Camões, B. Anes, D. Nagy, Z. Nagyné Szilágyi, R. Quendera, L. Ribeiro, F. Bastkowski, R. Born, J. Nerut, J. Saame, S. Lainela, L. Liv, E. Uysal, M. Roziková, M. Vičarová and I. Leito, *Symmetry*, 2020, **12**, 1150.
- 16 V. Radtke, A. Ermantraut, D. Himmel, T. Koslowski and I. Leito, *Angew. Chem., Int. Ed.*, 2018, **57**, 2344–2347.
- 17 A. Ermantraut, V. Radtke, N. Gebel, D. Himmel, T. Koslowski and I. Leito, *Angew. Chem., Int. Ed.*, 2018, **57**, 2348–2352.
- 18 V. Radtke, N. Gebel, D. Priester, A. Ermantraut, M. Bäuerle, D. Himmel, R. Stroh, T. Koslowski, I. Leito and I. Krossing, *Chem. – Eur. J.*, 2022, e202200509.
- 19 V. Radtke, D. Priester, A. Heering, C. Müller, T. Koslowski, I. Leito and I. Krossing, *Chem. – Eur. J.*, 2023, e202300609.
- 20 *Handbook of Reference Electrodes*, ed. G. Inzelt, A. Lewenstam and F. Scholz, Springer-Verlag, Berlin, Heidelberg, 2013.
- 21 A. Heering, F. Bastkowski and S. Seitz, *J. Sens. Sens. Syst.*, 2020, **9**, 383–389.
- 22 S. Lainela, I. Leito, A. Heering, G. Capitaine, B. Anes, F. Camões and D. Stoica, *Water*, 2021, **13**, 2522.
- 23 L. Deleebeeck, A. Snedden and D. Stoica, *Anal. Chim. Acta: X*, 2022, **10**, 100085.
- 24 F. Bastkowski, A. Heering, E. Uysal, L. Liv, I. Leito, R. Quendera, L. Ribeiro, L. Lisa Deleebeeck, A. Snedden, D. Nagy, Z. N. Szilágyi, F. Camões, B. Anes, M. Roziková and D. Stoica, *Anal. Bioanal. Chem.*, 2024, **416**, 461–465.
- 25 F. G. K. Baucke, *Anal. Bioanal. Chem.*, 2002, **374**, 772–777.
- 26 R. Veigure, K. Lossmann, M. Hecht, E. Parman, R. Born, I. Leito, K. Herodes and K. Kipper, *J. Chromatogr. A*, 2020, **1613**, 460667.
- 27 K. Lossmann, R. Hecht, J. Saame, A. Heering, I. Leito and K. Kipper, *J. Chromatogr. A*, 2022, **1666**, 462850.
- 28 P. Liigand, A. Heering, K. Kaupmees, I. Leito, M. Girod, R. Antoine and A. Kruve, *J. Am. Soc. Mass Spectrom.*, 2017, **28**, 2124–2131.
- 29 R. J. N. Bettencourt da Silva, J. Saame, B. Anes, A. Heering, I. Leito, T. Naykki, D. Stoica, L. Deleebeeck, F. Bastkowski, A. Snedden and M. F. Camões, *Anal. Chim. Acta*, 2021, **1182**, 338923.
- 30 A. Heering, I. Leito, M. Lahe, M. Vilbaste and J. P. Samin, *protocols.io*, 2023, DOI: [10.17504/protocol](https://doi.org/10.17504/protocol).
- 31 Y. Marcus, *Solubility and solvation in mixed solvent systems*, 1990, vol. 62.
- 32 M. Li, D. Constantinescu, L. Wang, A. Mohs and J. Gmehling, *Ind. Eng. Chem. Res.*, 2010, **49**, 4981–4988.
- 33 R. G. Bates, *Determination of pH; Theory and Practice*, John Wiley & Sons, New York, London, Sydney, Toronto, 2nd edn, 1973.
- 34 C. B. Castells, C. Ràfols, M. Rosés and E. Bosch, *J. Chromatogr. A*, 2003, **1002**, 41–53.
- 35 L. G. Gagliardi, C. B. Castells, C. Ràfols, M. Rosés and E. Bosch, *Anal. Chem.*, 2007, **79**, 3180–3187.
- 36 D. v. McCalley, *J. Chromatogr. A*, 2017, **1483**, 71–79.
- 37 X. Subirats, E. Bosch and M. Rosés, *J. Chromatogr. A*, 2007, **1138**, 203–215.

