Buffer Standards for the Biochemical pH of 3-(*N*-Morpholino)-2-hydroxypropanesulfonic Acid from (278.15 to 328.15) K

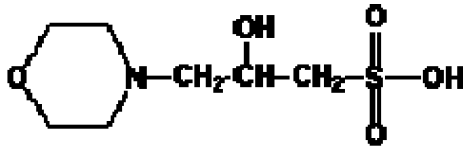
Lakshmi N. Roy, Rabindra N. Roy,* Cole E. Denton, Sean R. LeNoue, Chandra N. Roy, Shahaf Ashkenazi, Michael S. Fuge, Joshua T. Wollen, Jessica M. Stegner, Kathleen A. Allen, and Meagan A. Harmon

Hoffman Department of Chemistry, Drury University, Springfield, Missouri 65802

The values of the second dissociation constant pK_2 and related thermodynamic quantities of the ampholyte 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid (MOPSO) have been previously determined at temperatures from (278.15 to 328.15) K. In this study, the pH values of two buffer solutions without NaCl and three buffer solutions with NaCl having ionic strengths ($I = 0.16$ mol·kg^{-1}) similar to those in blood plasma, have been evaluated at 12 temperatures from (278.15 to 328.15) K using an extended form of the Debye−Hückel equation, since the Bates−Guggenheim convention is valid up to $I = 0.1$ mol·kg^{-1}. The liquid junction potentials (E_j) between the buffer solutions of MOPSO and the saturated KCl solution of the calomel electrode at (298.15 and 310.15) K have been estimated by measurement with a flowing junction cell. These values of E_j have been used to ascertain the operational pH values at (298.15 and 310.15) K. Three buffer solutions of MOPSO are recommended as useful reference solutions for pH measurements in saline media of ionic strength $I = 0.16$ mol·kg^{-1}.

Introduction

The buffer substances recommended by Good et al.[1,2] have proven very useful for the measurement of the pH of blood and the control of pH in the region close to that of physiological solutions. Very recently, we have reported the pH values of 3-[(1,1-dimethyl-2-hydroxymethyl)amino]-2-hydroxypropanesulfonic acid (AMPSO)[3] at temperatures from (278.15 to 328.15) K including 310.15 K. The zwitterionic buffer *N*-[tris(hydroxymethyl)methyl-3-amino]propanesulfonic acid (TAPS)[4] has also been recommended for use as a physiological buffer at (298.15 and 310.15) K. In the present investigation, we are interested in providing reliable pH values for the ampholyte 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid (MOPSO), which has the following structure:



3-(*N*-morpholino)-2-hydroxypropanesulfonic acid

MOPSO

Bates and his associates[5] reported pH values of *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) at (298.15 and 310.15) K. These buffer solutions are recommended as standard buffers for pH measurements.

For the highest reproducibility and accuracy, the glass electrode pH meter assembly at a point close to the pH of blood (7.407) can be obtained within the framework of the National Institute of Standards and Technology (NIST/NBS) by using physiological phosphate pH buffer as a primary standard.[6] The pH of this physiological phosphate buffer standard is 7.415 at 298.15 K and 7.395 at 310.15 K, and has been internationally used for standardization at or close to the pH of the clinical sample.

Various attempts to establish a suitable primary reference standard at an isotonic saline solution, $I = 0.16$ mol·kg^{-1}, and near the pH of blood plasma have been met with difficulty. The commonly accepted physiological phosphate standard solutions are mixtures of KH_2PO_4 (0.008695 mol·kg^{-1}) and Na_2HPO_4 (0.03043 mol·kg^{-1}). The problems associated with the use of the physiological phosphate solutions are (i) phosphates interact unfavorably with biological media, (ii) phosphate precipitates with blood ingredients (Mg^{2+} and Ca^{2+}), and (iii) the temperature coefficient of blood is −0.015 pH unit·K^{-1} as compared to that of the 1:3.5 phosphate standard (−0.0028 pH unit·K^{-1}).[7] The compound MOPSO is not expected to have any undesirable side effects (no precipitation with Ca^{2+} and Mg^{2+}), but the possibility of complex formation with cations such as Ca^{2+} and Mg^{2+} exists. We have attempted to minimize it with a high sodium chloride:buffer concentration ratio for an isotonic saline solution of $I = 0.16$ mol·kg^{-1}.

Good and his associates[1,2] introduced a series of new hydrogen ion buffers for use in the physiological pH range. They took the liberty of citing some published works by various investigators for structurally related zwitterionic buffer compounds with a view to comparing the effects of substituents on pK_2 and pH values. Wu and co-workers[8] have published the values of pK_2 and pH of the zwitterionic buffer *N*-(2-hydroxyethyl)piperazine-*N*-2-ethanesulfonic acid (HEPES) and a second zwitterionic buffer, MOPSO.[9] Roy et al.[10] reported results for pK_2 and pH for 3-(*N*-morpholino)propanesulfonic acid (MOPS) and 4-*N*-(morpholino)butanesulfonic acid (MOBS).[11] The pH values of these solutions closely match those of common biological media. In 1973, Bates et al.[12] suggested the use of tris(hydroxymethyl)methylglycine (TRICINE) as a secondary buffer standard for the physiological pH range of 7.2 to 8.5.

* Corresponding author. E-mail: rroy@drury.edu.

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Table 1. Cell Potential of Cell A (V): Pt(s), H_2(g), 101.325 kPa|MOPSO (m_1), NaMOPSO (m_2), NaCl (m_3)|AgCl(s), Ag(s)

m_1	m_2	m_3												
mol·kg^{-1}	mol·kg^{-1}	mol·kg^{-1}	278.15 K	283.15 K	288.15 K	293.15 K	298.15 K	303.15 K	308.15 K	310.15 K	313.15 K	318.15 K	323.15 K	328.15 K
0.02	0.06	0.005	0.78522	0.78765	0.79014	0.79235	0.79454	0.79663	0.79830	0.79933	0.80035	0.80232	0.80380	0.80535
0.02	0.06	0.010	0.76992	0.77207	0.77410	0.77617	0.77823	0.78003	0.78121	0.78229	0.78327	0.78461	0.78576	0.78689
0.02	0.06	0.015	0.76144	0.76351	0.76535	0.76706	0.76951	0.77114	0.77179	0.77318	0.77395	0.77514	0.77614	0.77704
0.02	0.06	0.020	0.75575	0.75787	0.75966	0.76130	0.76347	0.76500	0.76522	0.76684	0.76750	0.76854	0.76941	0.77017
0.02	0.04	0.005	0.77350	0.77583	0.77798	0.78017	0.78180	0.78395	0.78552	0.78626	0.78709	0.78859	0.78954	0.79070
0.02	0.04	0.010	0.75766	0.75980	0.76168	0.76337	0.76456	0.76681	0.76821	0.76883	0.76953	0.77118	0.77149	0.77203
0.02	0.04	0.015	0.74873	0.75053	0.75232	0.75389	0.75495	0.75704	0.75830	0.75885	0.75946	0.76139	0.76116	0.76182
0.02	0.04	0.020	0.74283	0.74447	0.74609	0.74752	0.74821	0.75063	0.75175	0.75220	0.75274	0.75489	0.75422	0.75478

Table 2. Cell Voltage of Cell A (V): Pt(s), H_2 (g), 101.325 kPa|MOPSO (m_1), NaMOPSO (m_2), NaCl (m_3)|AgCl(s), Ag(s)

m_1	m_2	m_3												
mol·kg^{-1}	mol·kg^{-1}	mol·kg^{-1}	278.15 K	283.15 K	288.15 K	293.15 K	298.15 K	303.15 K	308.15 K	310.15 K	313.15 K	318.15 K	323.15 K	328.15 K
0.01	0.03	0.13	0.71369	0.71524	0.71636	0.71726	0.71798	0.71858	0.71905	0.71918	0.71932	0.71944	0.71945	0.71934
0.02	0.06	0.10	0.71951	0.72100	0.72226	0.72333	0.72429	0.72504	0.72568	0.72592	0.72616	0.72651	0.72666	0.72680
0.04	0.04	0.12	0.74072	0.74249	0.74407	0.74558	0.74704	0.74812	0.74920	0.74955	0.75007	0.75078	0.75132	0.75171

The pH of the 0.06 *m* TRICINE + 0.02 *m* sodium TRICINEate buffer solution at 310.15 K is 7.407, matching exactly the pH of blood. Goldberg et al.,[13] in their excellent review article of the thermodynamic quantities of biological buffers, indicated that the results for pK_2 are available in the literature for MOPSO. To the authors' knowledge, no reliable pH values of MOPSO for the buffer compositions under study have been reported.

To provide accurate and reproducible pH values for physiological pH standards, we have studied the buffer compound MOPSO with the following compositions on the scale of molality (m), where $m = 1$ mol·kg^{-1} and I is the ionic strength in units of mol·kg^{-1}: (a) MOPSO (0.02 mol·kg^{-1}) + NaMOPSO (0.06 mol·kg^{-1}), $I = 0.06$ mol·kg^{-1}; (b) MOPSO (0.02 mol·kg^{-1}) + NaMOPSO (0.04 mol·kg^{-1}), $I = 0.04$ mol·kg^{-1}; (c) MOPSO (0.01 mol·kg^{-1}) + NaMOPSO (0.03 mol·kg^{-1}) + NaCl (0.13 mol·kg^{-1}), $I = 0.16$ mol·kg^{-1}; (d) MOPSO (0.02 mol·kg^{-1}) + NaMOPSO (0.06 mol·kg^{-1}) + NaCl (0.10 mol·kg^{-1}), $I = 0.16$ mol·kg^{-1}; (e) MOPSO (0.04 mol·kg^{-1}) + NaMOPSO (0.04 mol·kg^{-1}) + NaCl (0.12 mol·kg^{-1}), $I = 0.16$ mol·kg^{-1}. The detailed procedure for the preparation of these buffer solutions for MOPSO is described in the following section.

Experimental Section

MOPSO was purchased from the Sigma Chemical Co. (St. Louis, MO). The purification procedure (using further crystallization) and the assay have been reported in a previous paper.[5] The assay showed that the MOPSO buffer used was (99.91 to 99.97) % pure. All buffer solutions were prepared by massing the MOPSO, NaCl (ACS reagent grade), a standard solution of NaOH for the preparation of NaMOPSO, and calculated amounts of CO_2-free doubly distilled water. Buoyancy corrections were made for all masses used to prepare the solutions.

The cell design, preparation procedure of the hydrogen electrodes using chloroplatinic acid, silver−silver chloride thermal, electrolytic electrodes,[14] hydrogen gas purification, and preparation of the solutions have been described previously.[3,10] Details about the control of temperature (within ± 0.005 K)[3] using a digital platinum resistance thermometer (Guildline model 9540), a digital voltmeter (Hewlett-Packard 2000 multimeter), and other experimental procedures are also found elsewhere.[3]

Methods and Results

The values of the cell potential for the calculations of pH are given in Tables 1 and 2 for cell A containing two buffer solutions lacking NaCl and three buffer solutions in which NaCl had been added to make $I = 0.16$ mol·kg^{-1}, respectively. These values have been corrected to a hydrogen pressure of 101.325 kPa. At 298.15 K, cell potential values are the average of at least two readings at the beginning, the middle, and sometimes the end of the temperature sequence. Duplicate cells usually gave readings on the average within 0.04 mV in the temperature range of (278.15 to 328.15) K.

pH of the MOPSO Buffer. The conventional standard pH values have been evaluated by the method of Bates et al.[3,9,10,15−17] for five standard buffer solutions, described in the Introduction (a to e). For accurate calculations of the second dissociation constants, pK_2, and pH values of the five buffer solutions, the following cell (A) is used for the collection of cell potential data:

$$\mathrm{Pt(s), H_2(g), 101.325\ kPa|MOPSO}\ (m_1) + \mathrm{NaMOPSO}\ (m_2) + \mathrm{NaCl}\ (m_3)|\mathrm{AgCl(s), Ag(s)} \quad \text{(A)}$$

where m_1, m_2, and m_3 indicate the molalities of the respective species and 1 atm = 101.325 kPa in SI units. Cell A is known as the Harned-type cell.

The flowing junction cell (B), was used for the evaluation of the liquid junction potential at the contact between the buffer solution and the heavier saturated KCl solution shown with a double vertical line:

$$\mathrm{Pt(s), H_2(g), 101.325\ kPa|MOPSO}\ (m_1) + \mathrm{NaMOPSO}\ (m_2) + \mathrm{NaCl}\ (m_3)||\mathrm{KCl(satd), Hg_2Cl_2(s), Hg(l)} \quad \text{(B)}$$

where the abbreviations s, l, and g denote the solid, liquid, and gaseous states, respectively. In routine laboratory measurements, the hydrogen electrode is commonly replaced by a glass electrode. For cell B, the values of the standard electrode potential, E°_{SCE}, of the saturated calomel electrode were taken as (−0.2415 and −0.2335) V at (298.15 and 310.15) K, respectively. These values are periodically rechecked with experiments.

For cell C, the phosphate salts were NIST standard reference materials with the composition KH_2PO_4 (0.008695 mol·kg^{-1}) + Na_2HPO_4 (0.03043 mol·kg^{-1}), and its solutions are recommended for pH measurements in physiological solutions:

$$\mathrm{Pt(s), H_2(g), 101.325\ kPa|phosphate\ buffer||KCl(satd), Hg_2Cl_2(s), Hg(l)} \quad \text{(C)}$$

It should be emphasized that the difference in values between the liquid junction potential when one solution (the

pH standard) is replaced by another (the unknown) is important. The values of the liquid junction potential, E_j, for the physiological phosphate solutions and other buffer solutions of MOPSO from cell B were obtained[8,10] using the flowing junction cell. The equation for the calculation of E_j[10] is

$$E_j = E + E^\circ_{SCE} - k(pH) \quad (1)$$

where $E^\circ_{SCE} = -0.2415$ V, $k = 0.059156$, and the pH is 7.415 (physiological phosphate buffer solution) at 298.15 K and $E^\circ_{SCE} = -0.2335$, $k = 0.061538$, and the pH is 7.395 at 310.15 K. The operational definition of pH, designated as pH(x), is

$$pH(x) = pH(s) + \frac{E_x - E_s + \delta E_j}{k} \quad (2)$$

where "x" refers to the unknown buffer MOPSO + NaMOPSO, "s" is the reference solution (NBS/NIST physiological phosphate buffer) of known pH, and $\delta E_j = E_{j(s)} - E_{j(x)}$. If $\delta E_j = 0$, then eq 2 takes the form

$$pH(x) = pH(s) + \frac{E_x - E_s}{k} \quad (3)$$

It is important to mention that eq 2 is more common, as δE_j (the difference) is all that is needed, not E_j (mV).

To calculate pH(s) values for all five buffer solutions, calculations for the acidity function $p(a_H\gamma_{Cl})$ values were made in the temperature range of (278.15 to 328.15) K, from the cell potential (E) listed in Tables 1 and 2, the molality of the chloride ion, and E°, the standard potential of the silver−silver chloride electrode.[3] The Nernst equation[12,14,17] for cell A is given by

$$p(a_H\gamma_{Cl}) = \frac{E - E^\circ}{k} + \log m_{Cl} \quad (4)$$

where k is the Nernst slope.

From the plot of the acidity function, $p(a_H\gamma_{Cl})$, for each buffer solution against the molality of the chloride ion employing linear regression analysis, the intercept, $p(a_H\gamma_{Cl})^\circ$, at $m_{Cl} = 0$ was obtained. These values of $p(a_H\gamma_{Cl})^\circ$ for two chloride-free buffer solutions listed above are given in Table 3. The uncertainty (mean deviation) introduced in this type of graphical extrapolation appeared to be slightly greater than 0.001 from the lines drawn. For three buffer solutions in the presence of NaCl (c to e), the values of $p(a_H\gamma_{Cl})$ are entered in Table 4 from (278.15 to 328.15) K.

Table 3. $p(a_H\gamma_{Cl})^\circ$ of MOPSO + NaMOPSO Buffer Solutions from (278.15 to 328.15) K Obtained by Extrapolation for Chloride-Free Solutions[a]

T/K	0.02 *m* MOPSO + 0.04 *m* NaMOPSO (I = 0.04 *m*)	0.02 *m* MOPSO + 0.06 *m* NaMOPSO (I = 0.06 *m*)
278.15	7.455	7.662
283.15	7.374	7.575
288.15	7.293	7.496
293.15	7.219	7.420
298.15	7.142	7.344
303.15	7.073	7.275
308.15	7.002	7.208
310.15	6.978	7.181
313.15	6.937	7.142
318.15	6.868	7.084
323.15	6.807	7.022
328.15	6.743	6.964

[a] $m = 1$ mol·kg^{-1}.

Table 4. $p(a_H\gamma_{Cl})$ of MOPSO + NaMOPSO Buffer Solutions from (278.15 to 328.15) K Computed Using Equations *4*, *5*, *6*, and *7*[a]

T/K	0.01 *m* MOPSO + 0.03 *m* NaMOPSO + 0.13 *m* NaCl (I = 0.16 *m*)	0.02 *m* MOPSO + 0.06 *m* NaMOPSO + 0.10 *m* NaCl (I = 0.16 *m*)	0.04 *m* MOPSO + 0.12 *m* NaMOPSO + 0.04 *m* NaCl (I = 0.16 *m*)
278.15	7.803	7.794	7.781
283.15	7.725	7.713	7.698
288.15	7.645	7.634	7.617
293.15	7.566	7.557	7.541
298.15	7.491	7.483	7.470
303.15	7.417	7.411	7.397
308.15	7.346	7.341	7.327
310.15	7.318	7.314	7.300
313.15	7.277	7.273	7.260
318.15	7.210	7.208	7.194
323.15	7.144	7.143	7.130
328.15	7.080	7.081	7.066

[a] $m = 1$ mol·kg^{-1}.

Conventional pH(s) values determined from the cell potential of cells without a liquid junction for the solution without the presence of the chloride ion were determined by the equation

$$pH(s) = p(a_H\gamma_{Cl})^\circ + \log \gamma^\circ_{Cl} \quad (5)$$

where the single-ion activity coefficient, γ°_{Cl}, cannot be measured experimentally. The estimation of γ°_{Cl} for the calculation of pH(s) by eq 6 has been outlined before.[10] The pH values obtained from the liquid junction cell are indicated by pH, whereas the "conventional" pH calculated from eq 6 is designated as pH(s). The "pH convention", commonly known as the Bates−Guggenheim convention,[18] is expressed by the following equation:

$$-\log \gamma^\circ_{Cl} = \frac{A\sqrt{I}}{1 + 1.5\sqrt{I}} \quad (6)$$

The International Union of Pure and Applied Chemistry[19] has recommended this convention. It has been assumed that eq 6 is valid up to $I = 0.1$ mol·kg^{-1}. For $I > 0.1$ mol·kg^{-1}, there is no widely accepted (agreed-upon) convention. Perhaps a linear dependent CI term from eq 7 along with a variation of the ion size parameter as a function of temperature would provide a more logical choice when $I > 0.1$ mol·kg^{-1}.

Thus, a pH convention[8,10] based on an extended Debye−Hückel equation[8] has been assumed to be more logical when $I > 0.1$ mol·kg^{-1} up to $I = 1.0$ mol·kg^{-1} in the calculation of $\log \gamma^\circ_{Cl}$ for all of the buffer−chloride solutions. The following equation is preferred:

$$\log \gamma^\circ_{Cl} = -\frac{A\sqrt{I}}{1 + Ba^\circ\sqrt{I}} + CI \quad (7)$$

where I is the ionic strength of the buffer solution, A and B are the Debye−Hückel constants, C is an adjustable parameter, Ba° was assumed to be 1.38 kg$^{1/2}$·mol$^{-1/2}$ for all the experimental temperatures, corresponding to an (ion size parameter) a° of 4.2 Å.[8,10] The empirical equation given below for the calculation of the parameter C[8,10] was obtained from a curve-fitting method:

$$C = C_{298.15} + (6.2\cdot10^{-4})(T - 298.15) - (8.7\cdot10^{-6})\cdot(T - 298.15)^2 \quad (8)$$

where $C_{298.15} = 0.032$ kg·mol^{-1} [8] at 298.15 K and T is the absolute temperature.

Table 5. pH(s) of MOPSO + NaMOPSO Buffer Solutions from (278.15 to 328.15) K Computed Using Equations *4*, *5*, *6*, and *7*[a]

T/K	0.02 *m* MOPSO + 0.06 *m* NaMOPSO (*I* = 0.06 *m*)	0.02 *m* MOPSO + 0.04 *m* NaMOPSO (*I* = 0.04 *m*)
278.15	7.572	7.378
283.15	7.484	7.297
288.15	7.405	7.215
293.15	7.329	7.141
298.15	7.252	7.065
303.15	7.182	7.994
308.15	7.115	7.922
310.15	7.088	7.898
313.15	7.049	7.857
318.15	6.990	6.787
323.15	6.927	6.725
328.15	6.869	6.661

[a] $m = 1$ mol·kg^{-1}.

Table 6. pH(s) of MOPSO + NaMOPSO Buffer Solutions from (278.15 to 328.15) K Computed Using Equations *4*, *5*, *6*, and *7*[a]

T/K	0.01 *m* MOPSO + 0.03 *m* NaMOPSO +0.13 *m* NaCl (*I* = 0.16 *m*)	0.02 *m* MOPSO + 0.06 *m* NaMOPSO +0.10 *m* NaCl (*I* = 0.16 *m*)	0.04 *m* MOPSO + 0.12 *m* NaMOPSO +0.04 *m* NaCl (*I* = 0.16 *m*)
278.15	7.677	7.669	7.655
283.15	7.599	7.588	7.572
288.15	7.519	7.508	7.492
293.15	7.441	7.432	7.416
298.15	7.364	7.357	7.343
303.15	7.290	7.284	7.270
308.15	7.219	7.213	7.200
310.15	7.190	7.186	7.172
313.15	7.148	7.144	7.131
318.15	7.080	7.078	7.065
323.15	7.014	7.013	7.999
328.15	7.949	6.950	7.934

[a] $m = 1$ mol·kg^{-1}.

The values of pH(s), listed in Table 5 for two buffer solutions of MOPSO without NaCl, were computed from eqs 4, 5, 6, 7, and 8 and are represented by the following equations:

$$\mathrm{pH(s)} = 7.254 - (1.4578\cdot10^{-2})(T - 298.15) + (5.98\cdot10^{-5})(T - 298.15)^2 \quad (9)$$

for MOPSO (0.02 mol·kg^{-1}) + NaMOPSO (0.06 mol·kg^{-1}) and

$$\mathrm{pH(s)} = 7.006 - (1.4714\cdot10^{-2})(T - 298.15) + (4.12\cdot10^{-5})(T - 298.15)^2 \quad (10)$$

for MOPSO (0.02 mol·kg^{-1}) + NaMOPSO (0.04 mol·kg^{-1}), where 278.15 K $\leq T \leq$ 328.15 K. The standard deviations of regression for the pH(s) of the chloride-free buffer solutions, obtained from the fits with eqs 12 and 13, are 0.0019 and 0.0015, respectively.

For three buffer solutions containing NaCl at an isotonic saline medium total ionic strength of $I = 0.16$ mol·kg^{-1}, the values of pH(s) calculated using eqs 4, 5, 6, 7, and 8 and from the acidity function data listed in Table 4 are entered in Table 6. These values of pH(s) are expressed by the following equations:

$$\mathrm{pH(s)} = 7.365 - (1.4968\cdot10^{-2})(T - 298.15) + (3.7\cdot10^{-5})(T - 298.15)^2 \quad (11)$$

for MOPSO (0.01 mol·kg^{-1}) + NaMOPSO (0.03 mol·kg^{-1}) + NaCl (0.13 mol·kg^{-1})

$$\mathrm{pH(s)} = 7.357 - (1.4773\cdot10^{-2})(T - 298.15) + (4.04\cdot10^{-5})(T - 298.15)^2 \quad (12)$$

for MOPSO (0.02 mol·kg^{-1}) + NaMOPSO (0.06 mol·kg^{-1}) + NaCl (0.10 mol·kg^{-1}), and

$$\mathrm{pH(s)} = 7.343 - (1.4739\cdot10^{-2})(T - 298.15) + (3.93\cdot10^{-5})(T - 298.15)^2 \quad (13)$$

for MOPSO (0.04 mol·kg^{-1}) + NaMOPSO (0.04 mol·kg^{-1})

Table 7. Cell Voltages of Cell B for MOPSO Buffer and Cell C

Cell Voltage for Cell B				
m_1	m_2	m_3	*E*/V	
mol·kg^{-1}	mol·kg^{-1}	mol·kg^{-1}	298.15 K	310.15 K
0.02	0.06	0.10	0.67722	0.69514
0.04	0.04	0.12	0.67633	0.67556
0.01	0.03	0.13	0.67745	0.67672

Cell Voltage for Cell C[a]		
	E/V	
cell C	298.15 K	310.15 K
0.008695 *m* KH_2PO_4 + 0.03043 *m* Na_2HPO_4	0.68275	0.69147

[a] Corrected to a hydrogen pressure of 101.325 kPa for physiological phosphate buffer solutions (primary reference standard buffer) at (298.15 and 310.15) K.

Table 8. Values of the Liquid Junction Potentials for MOPSO at (298.15 and 310.15) K

system	E_j[a]/mV	
	298.15 K	310.15 K
physiological phosphate (0.008695 *m* KH_2PO_4 + 0.03043 *m* NaCl)	2.6	2.9
0.02 *m* MOPSO + 0.06 *m* NaMOPSO + 0.10 *m* NaCl	0.5	0.7
0.04 *m* MOPSO + 0.04 *m* NaMOPSO + 0.12 *m* NaCl	0.5	0.8
0.01 *m* MOPSO + 0.03 *m* NaMOPSO + 0.13 *m* NaCl	0.4	0.8

[a] $E_j = E + E^{\circ}_{CE} - k(\mathrm{pH})$ from eq 1, E is the cell voltage from Table 5, k = Nernst slope with values of 0.059156 at 298.15 K and 0.061538 at 310.15 K;, the pH of the primary reference standard phosphate buffer is 7.415 and 7.395 at (298.15 and 310.15) K, and E°_{CE}= electrode potential of the saturated calomel electrode = (−0.2415 and −0.2335) V at (298.15 and 310.15) K, respectively.

Table 9. Values of pH at (298.15 to 310.15) K for MOPSO Buffer Solutions

cell B				pH					
m_1	m_2	m_3	I	298.15 K			310.15 K		
mol·kg^{-1}	mol·kg^{-1}	mol·kg^{-1}	mol·kg^{-1}	without[a] E_j corr	with[b] E_j corr	calcd[c]	without[a] E_j corr	with[b] E_j corr	calcd[c]
0.02	0.06	0.10	0.16	7.322	7.357	7.357	7.149	7.185	7.186
0.04	0.04	0.12	0.16	7.307	7.342	7.343	7.137	7.171	7.172
0.01	0.03	0.13	0.16	7.326	7.363	7.364	7.156	7.190	7.190

[a] pH = 7.415 + (E − 0.68275)/0.059156 at 298.15 K and pH = 7.395 + (E − 0.69144)/0.061538 at 310.15 K; the cell potential values (Table 7) are (0.68275 and 0.69144) V at (298.15 to 310.15) K for the physiological phosphate buffer standard solution. [b] Values obtained from eq 2 and E_j data of Table 8. [c] Obtained from Tables 5 and 6.

+ NaCl (0.12 mol·kg^{-1}), where T is the temperature (K). The standard deviations for regression of the "observed" results from eqs 11, 12, and 13 are 0.0009, 0.0004, and 0.0014, respectively.

The operational pH values at (298.15 and 310.15) K were evaluated from cells with a liquid junction (B and C) by means of the flowing junction cell.[8,10] The cell potential values of cells B and C at (298.15 and 310.15) K are given in Table 7. The values of E_j listed in Table 8 were obtained by using eq 1. The widely used equation for the calculation of log γ°_{Cl} is based on the Bates−Guggenheim convention,[3,5,7,8] and is valid up to I = 0.1 mol·kg^{-1}.[6,17−19] The combined standard uncertainty for the pH(s) values was accounted for by combining the various known sources of error: (i) assumption for the calculation of log γ°_{Cl} using eq 7 (± 0.004 pH unit), (ii) extrapolation of the $p(a_H\gamma_{Cl})^\circ$ plot for chloride-free solutions (less than ± 0.002 pH unit), and (iii) error in the experimental measurement from the multimeter (± 0.02 mV). Thus, the overall estimated uncertainty is ± 0.006 and ± 0.012 pH unit for buffers without the presence of NaCl and with ionic strength I = 0.16 mol·kg^{-1}, respectively. Errors in the values of E_j are irrelevant to the values of pH(s) determined from cell A without a liquid junction; however, δE_j of eq 2 does affect the operational pH values listed in Table 9 at (298.15 and 310.15) K. These are recommended as useful secondary pH standards for calibrating electrodes for the pH measuring assembly in the range of physiological interest. The consistency of the three sets of experiments listed in Table 9 lends credence to the pH values of MOPSO buffer solutions.

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