3-[4-(2-Hydroxyethyl)-1-piperazinyl]propanesulfonic Acid (HEPPS) and the pH of Its Buffer Solutions from (278.15 to 328.15) K

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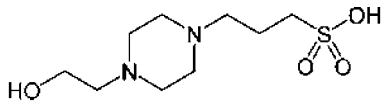
The values of the second dissociation constant pK_2 and related thermodynamic quantities of the ampholyte 3-[4-(2-hydroxyethyl)-1-piperazinyl]propanesulfonic acid (HEPPS) have already been published at temperatures from T = (278.15 to 328.15) K. The pH values of two equimolal buffer solutions and eight buffer solutions with ionic strengths similar to that of plasma in blood (I = 0.16 mol·kg^{-1}) have been experimentally determined and then corrected at 12 temperatures from T = (278.15 to 328.15) K using the extended Debye−Hückel equation. The liquid junction potentials (E_j) between the buffer solutions of HEPPS and the saturated KCl solution of the calomel electrode at (298.15 and 310.15) K have been estimated by measurement with the flowing junction cell. These values of E_j have been used to ascertain the operational pH values at T = (298.15 and 310.15) K. The zwitterionic buffer HEPPS was proven to be useful through experimentation as a pH standard well within the region close to blood serum.

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. Previously, the authors have reported the pK_2 values of [(2-hydroxyethyl)amino]acetic acid (HEPPS)[3] at temperatures from T = (278.15 to 328.15) K including T = 310.15 K. In the present investigation, the ampholyte 3-[4-(2-hydroxyethyl)-1-piperazinyl]propanesulfonic acid, HEPPS, takes on the following structure:



3-[4-(2-Hydroxyethyl)-1-piperazinyl]propanesulfonic acid

(HEPPS)

Bates and his associates[4] have reported the results of the pH values for buffer compounds tris(hydroexymethyl)aminomethane (TRIS), *N*-tris(hydroxymethyl)methyl-2-amino-ethanesulfonic acid (TES) at (298.15 and 310.15) K. Roy et al.[5,6] published the results of *N*-[tris(hydroxymethyl)methyl-3-amino]propanesulfonic acid (TAPS) at (278.15 to 328.15) K. As a continuation of previous work on HEPPS,[3] the authors have investigated 10 buffer solutions (two without NaCl and eight with NaCl) in the temperature range of (278.15 to 328.15) K.

To guarantee accuracy and reproducibility, the glass electrode pH meter assembly at a point close to the pH of blood (that is, between 7 and 8) can be obtained within the framework of the National Institute of Standards and Technology/National Bureau of Standards (NIST/NBS) by using physiological phosphate pH buffer as a primary standard.[7,8] The physiological phosphate buffer standard has a determined pH value of 7.415 at T = 298.15 K and 7.395 at T = 310.15 K and has been internationally used for standardization at or close to the pH of physiological fluids.

Various attempts to establish a suitable primary reference standard at an ionic strength of 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}). A few mentionable problems exist with the use of physiological phosphate solutions, such as: (i) phosphates interact unfavorably with biological media, (ii) phosphate precipitates with cations in human blood (namely Mg^{2+} and Ca^{2+}), and (iii) the temperature coefficient of blood is (−0.015 pH·K^{-1}) as compared to 1:3.5 phosphate standard (−0.0028 pH·K^{-1}).[8] The compound HEPPS is not expected to have any undesirable side effects (no precipitation), but the possibility of complex formation with cations such as Ca^{2+} and Mg^{2+} exists. The authors have attempted to minimize this with a high concentration of chloride−base ratio for an isotonic saline solution.

Good and his associates[1,2] introduced a series of new hydrogen ion buffers for use in the physiological pH range. The authors took the liberty of citing some published works by various investigators for zwitterionic compounds that are structurally similar with a view of comparing the effects of substituents on pH values. Wu and co-workers[9] have published the values of pK_2 and pH of the zwitterionic buffer HEPES, and a second zwitterionic buffer, 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid (MOPSO).[10] Both HEPES and MOPSO buffers have been certified by the NIST and NBS as primary reference standards. Roy et al.[11] reported results for pK_2 and pH for 3-(*N*-morpholino)propanesulfonic acid (MOPS) and 4-*N*-(morpholino)butanesulfonic acid (MOBS).[12] The pH of these solutions closely matches that of the common biological media. In 1973, Bates et al.[13] suggested the use of tris(hydroxymethyl)methylglycine (TRICINE) as a secondary buffer standard for

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Table 1. Cell Potential, E, of Cell I (in V): Pt(s); H_2(g), 101.325 kPa |HEPPS (m_1), NaHEPPS (m_2), NaCl (m_3)| AgCl(s), Ag(s)

(m_1, m_2, m_3)/(mol·kg^{-1})			E/V										
m_1	m_2	m_3	T/K = 278.15	283.15	288.15	293.15	298.15	303.15	308.15	310.15	313.15	318.15	323.15	328.15
0.04	0.04	0.005	0.81569	0.81965	0.82329	0.82664	0.82995	0.83299	0.83578	0.83695	0.83837	0.84071	0.84259	0.90012
0.04	0.04	0.010	0.79997	0.80362	0.80702	0.81017	0.81314	0.81586	0.81834	0.81929	0.82062	0.82249	0.82431	0.88167
0.04	0.04	0.015	0.79086	0.79433	0.79760	0.80062	0.80341	0.80593	0.80823	0.80914	0.81033	0.81211	0.81368	0.87138
0.04	0.04	0.020	0.78494	0.78830	0.79144	0.79430	0.79702	0.79946	0.80167	0.80247	0.80359	0.80525	0.80666	0.86400
0.06	0.06	0.005	0.81665	0.82057	0.82430	0.82789	0.83115	0.83415	0.83695	0.83795	0.83951	0.84188	0.84381	0.85648
0.06	0.06	0.010	0.80074	0.80438	0.80780	0.81103	0.81401	0.81671	0.81922	0.82012	0.82146	0.82338	0.82512	0.83704
0.06	0.06	0.015	0.79151	0.79499	0.79822	0.80124	0.80405	0.80659	0.80889	0.80973	0.81095	0.81278	0.81423	0.82570
0.06	0.06	0.020	0.78522	0.78860	0.79170	0.79454	0.79722	0.79964	0.80177	0.80255	0.80371	0.80523	0.80668	0.81792

Table 2. Cell Potential of Cell I (in V): Pt(s); H_2(g), 101.325 kPa |HEPPS (m_1), NaHEPPS (m_2), NaCl (m_3)| AgCl(s), Ag(s)

(m_1, m_2, m_3)/(mol·kg^{-1})			E/V										
m_1	m_2	m_3	T/K = 278.15	283.15	288.15	293.15	298.15	303.15	308.15	310.15	313.15	318.15	323.15	328.15
0.01	0.02	0.14	0.75664	0.75950	0.76212	0.76448	0.76662	0.76842	0.77010	0.77074	0.77159	0.77253	0.77352	0.77415
0.02	0.04	0.12	0.76034	0.76324	0.76588	0.76834	0.77063	0.77249	0.77424	0.77484	0.77582	0.77695	0.77788	0.77842
0.03	0.06	0.10	0.76431	0.76723	0.76993	0.77236	0.77457	0.77656	0.77842	0.77911	0.78012	0.78135	0.78235	0.78319
0.04	0.08	0.08	0.76993	0.77300	0.77584	0.77852	0.78081	0.78279	0.78458	0.78533	0.78633	0.78753	0.78856	0.78834
0.05	0.05	0.11	0.74624	0.74884	0.75118	0.75331	0.75524	0.75693	0.75840	0.75892	0.75971	0.76056	0.76126	0.76166
0.06	0.06	0.10	0.74816	0.75081	0.75321	0.75545	0.75741	0.75899	0.76051	0.76113	0.76209	0.76312	0.76405	0.76490
0.07	0.07	0.09	0.75093	0.75358	0.75599	0.75830	0.76029	0.76197	0.76350	0.76405	0.76494	0.76589	0.76675	0.76725
0.08	0.08	0.08	0.75401	0.75670	0.75916	0.76140	0.76335	0.76526	0.76679	0.76740	0.76843	0.76958	0.77066	0.77175

the physiological range of pH 7.2 to 8.5. The pH of 0.06 *m* TRICINE + 0.02 *m* NaTRICINE buffer solution at $T = 328.15$ K is 7.407, matching exactly the pH of blood. Goldberg et al.[14] inscribed a relevant review article of the thermodynamic quantities of the biological buffers. This article suggested that the results for pK_2 are available in the literature for HEPPS. To the authors' knowledge, no pH values of HEPPS have been reported.

To provide accurate and reproducible pH values for physiological pH standards, the authors have studied the buffer compound, HEPPS, with the following compositions on the scale of molality (m), where m = mol·kg^{-1} and I is the ionic strength in the unit of mol·kg^{-1}:

(a) HEPPS (0.04 mol·kg^{-1}) + NaHEPPS (0.04 mol·kg^{-1}), I = 0.04 mol·kg^{-1}

(b) HEPPS (0.06 mol·kg^{-1}) + NaHEPPS (0.06 mol·kg^{-1}), I = 0.06 mol·kg^{-1}

(c) HEPPS (0.01 mol·kg^{-1}) + NaHEPPS (0.02 mol·kg^{-1}) + NaCl (0.14 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

(d) HEPPS (0.02 mol·kg^{-1}) + NaHEPPS (0.04 mol·kg^{-1}) + NaCl (0.12 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

(e) HEPPS (0.03 mol·kg^{-1}) + NaHEPPS (0.06 mol·kg^{-1}) + NaCl (0.10 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

(f) HEPPS (0.04 mol·kg^{-1}) + NaHEPPS (0.08 mol·kg^{-1}) + NaCl (0.08 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

(g) HEPPS (0.05 mol·kg^{-1}) + NaHEPPS (0.05 mol·kg^{-1}) + NaCl (0.11 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

(h) HEPPS (0.06 mol·kg^{-1}) + NaHEPPS (0.06 mol·kg^{-1}) + NaCl (0.10 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

(i) HEPPS (0.07 mol·kg^{-1}) + NaHEPPS (0.07 mol·kg^{-1}) + NaCl (0.09 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

(j) HEPPS (0.08 mol·kg^{-1}) + NaHEPPS (0.08 mol·kg^{-1}) + NaCl (0.08 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

These concentrations are also referenced in terms of m_1, m_2, and m_3, which denote the molality of the buffer (HEPPS), the buffer salt (NaHEPPS), and the chloride ion, respectively. The detailed procedure for the preparation of these buffer solutions for HEPPS is described in the following section.

Experimental Section

HEPPS was obtained from Sigma Chemical Co. (St. Louis, Missouri). The details of the purification by further crystallization as well as the assay have been reported in an earlier paper.[5] The assay showed that the purity of HEPPS was 0.9994 mass fraction. All buffer solutions were prepared by weighing the buffer substance HEPPS, recrystallized NaCl (ACS reagent grade), a standard solution of NaOH to prepare NaHEPPS, and finally calculated amounts of CO_2-free doubly distilled water. Corrections were applied for all masses used.

The cell design, the preparation of the hydrogen electrodes using a chloroplatinic acid solution, the thermal electrolytic silver−silver chloride electrodes,[15] the purification procedure for hydrogen gas, and the solution preparation procedure have been described previously.[3,11] Details about the control of the temperature (within ± 0.005 K) using a digital platinum resistance thermometer (Guildline model 9540), a digital multimeter (Hewlett-Packard 2000 multimeter), and other experimental procedures will also be found elsewhere.[3]

Methods and Results

The values of cell voltage for the calculations of pH are given in Tables 1 and 2. The cell voltage data for cell I containing two equimolal buffer solutions and eight buffer solutions in which NaCl had been added to make $I = 0.16$ mol·kg^{-1}, have been corrected to a hydrogen pressure of 101.325 kPa. The values of the cell voltage at $T = 298.15$ K are the result of averaging cell voltage readings taken at the beginning, middle, and end of the temperature sequence. Duplicate cells usually gave readings, on average, within 0.04 mV in the temperature range T = (278.15 to 328.15) K. For the two equimolal buffer solutions (a and b), the cell voltage values are presented in Table 1, whereas for isotonic buffer solutions (c through j), the cell voltage data are listed in Table 2.

pH of the HEPPS Buffer. The conventional standard pH values have been evaluated by the method of Bates et al.[13,15−17] for 10 standard buffer solutions, stated in the introduction section, (a to j). For accurate calculations of the pH values for

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

T	0.04 m HEPPS + 0.04 m NaHEPPS	0.06 m HEPPS + 0.06 m NaHEPPS
K	$I = 0.04$ m	$I = 0.06$ m
278.15	8.233	8.255
283.15	8.166	8.187
288.15	8.099	8.121
293.15	8.033	8.059
298.15	7.971	7.997
303.15	7.910	7.934
308.15	7.848	7.874
310.15	7.827	7.849
313.15	7.791	7.815
318.15	7.734	7.759
323.15	7.676	7.701
328.15	7.618	7.643

[a] $(m_1 = m_2)/(\text{mol}\cdot\text{kg}^{-1})$.

the 10 buffer solutions, the following cell I is used for the collection of cell voltage data:

$$\text{Pt(s); } H_2\text{(g)}, p^\circ = 101.325\text{ kPa}|\text{HEPPS } (m_1) + \text{NaHEPPS } (m_2) + \text{NaCl } (m_3)|\text{AgCl(s), Ag(s)} \quad \text{(I)}$$

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

The flowing junction cell II, was used for the evaluation of the liquid junction potential at the contact between the less saturated buffer solution and the more saturated KCl solution shown with a double vertical line.

$$\text{Pt(s); } H_2\text{(g), } p^\circ = 101.325\text{ kPa}|\text{HEPPS } (m_1) + \text{NaHEPPS } (m_2), \text{NaCl } (m_3)||\text{ KCl(satd)}|Hg_2Cl_2\text{(s), Hg(l)} \quad \text{(II)}$$

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

For cell III, 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.

$$\text{Pt(s); } H_2\text{(g)}, p^\circ = 101.325\text{ kPa}|\text{phosphate buffer}||\text{KCl(satd)}|Hg_2Cl_2\text{(s), Hg(l)} \quad \text{(III)}$$

It should be emphasized that the difference in values of 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 HEPPS from cell II were obtained[9,11] using the flowing junction cell. The equation for the calculation of E_j^{II} is:

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

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

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

where x refers to the unknown buffer HEPPS + NaHEPPS, 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 3 resembles the following:

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

It is important to mention that eq 3 is more common, as δE_j is typically assumed to be 0.

To calculate the pH(s) values for all 10 buffer solutions, calculations of the values of the acidity function $p(a_H\gamma_{Cl})$ were made in the temperature range $T = (278.15$ to $328.15)$ K from the cell voltage (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[13,15,17] for cell I claims the form:

$$p(a_H\gamma_{Cl}) = \frac{E - E^\circ}{k} + \log_{10} m_{Cl} \quad (4)$$

where k is the Nernst slope.

Table 4. $p(a_H\gamma_{Cl})$ of (HEPPS + NaHEPPS) Buffer Solutions from (278.15 to 328.15) K Computed for Isotonic Saline Solution[a]

T	0.01 m HEPPS + 0.02 m NaHEPPS + 0.14 m NaCl	0.02 m HEPPS + 0.04 m NaHEPPS + 0.12 m NaCl	0.03 m HEPPS + 0.06 m NaHEPPS + 0.10 m NaCl	0.04 m HEPPS + 0.08 m NaHEPPS + 0.08 m NaCl	0.05 m HEPPS + 0.05 m NaHEPPS + 0.11 m NaCl	0.06 m HEPPS + 0.06 m NaHEPPS + 0.10 m NaCl	0.07 m HEPPS + 0.07 m NaHEPPS + 0.09 m NaCl	0.08 m HEPPS + 0.08 m NaHEPPS + 0.08 m NaCl
K	$I = 0.16$ m							
278.15	8.626	8.626	8.619	8.624	8.333	8.326	8.331	8.335
283.15	8.558	8.558	8.550	8.556	8.264	8.258	8.261	8.265
288.15	8.492	8.491	8.482	8.489	8.196	8.190	8.193	8.197
293.15	8.426	8.426	8.416	8.425	8.130	8.125	8.128	8.130
298.15	8.363	8.364	8.351	8.360	8.066	8.061	8.064	8.065
303.15	8.299	8.299	8.288	8.295	8.003	7.996	7.999	8.003
308.15	8.237	8.238	8.227	8.231	7.941	7.934	7.940	7.940
310.15	8.214	8.213	8.204	8.208	7.917	7.912	7.913	7.916
313.15	8.178	8.179	8.170	8.172	7.882	7.879	7.879	7.884
318.15	8.116	8.120	8.110	8.111	7.822	7.821	7.819	7.826
323.15	8.060	8.062	8.052	8.052	7.765	7.767	7.763	7.773
328.15	8.004	8.003	7.997	7.994	7.707	7.716	7.706	7.724

[a] $(m_1, m_2, m_3)/(\text{mol}\cdot\text{kg}^{-1})$.

Table 5. Values of pH for HEPPS + NaHEPPS Buffer Solutions from (278.15 to 323.15) K[a]

T	0.04 *m* HEPPS + 0.04 *m* NaHEPPS	0.06 *m* HEPPS + 0.06 *m* NaHEPPS
K	I = 0.04 *m*	I = 0.06 *m*
278.15	8.156	8.165
283.15	8.089	8.097
288.15	8.021	8.031
293.15	7.955	7.969
298.15	7.892	7.905
303.15	7.830	7.842
308.15	7.769	7.781
310.15	7.747	7.757
313.15	7.711	7.722
318.15	7.653	7.665
323.15	7.594	7.606
328.15	7.541	7.551

[a] (m_1, m_2, m_3)/(mol·kg^{-1}).

From the plot of $p(a_H\gamma_{Cl})$ 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 for two equimolal buffers listed above (a and b) are given in Table 3. The acidity function values, $p(a_H\gamma_{Cl})$ for the eight isotonic buffers (c to j) reside in Table 4 from T = (278.15 to 328.15) K. The uncertainty (mean deviation) generated in this plot extrapolation for the values in Table 3 was recorded at slightly greater than 0.001 from the lines inscribed. Conventional pH(s) values determined from the cell voltage of cells without liquid junction for the solution without the presence of the chloride ion were determined by the equation:

$$\mathrm{pH(x)} = p(a_H\gamma_{Cl})^\circ + \log_{10}\gamma^\circ_{Cl} \quad (5)$$

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 5 was inspected in a previously published work.[11] The pH values obtained from the liquid junction cell are indicated by pH, whereas the "conventional" pH calculated from eq 5 is designated as pH(s).

The "pH convention," commonly known as Bates−Guggenheim convention,[16] is expressed by the following equation:

$$-\log_{10}\gamma^\circ_{Cl} = \frac{A\sqrt{I}}{1 + 1.5\sqrt{I}} \quad (6)$$

The International Union of Pure and Applied Chemistry[17] has recommended this convention. It has been assumed that eq 6 is valid for I = (0 to 0.1) mol·kg^{-1}. For I > 0.1 mol·kg^{-1}, there is no widely accepted (agreed-upon) convention. Perhaps a linear dependent Cl term from eq 6 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"[9,11] based on an extended Debye−Hückel eq 7 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 $\log_{10}\gamma^\circ_{Cl}$ for all of the buffer−chloride solutions. The following equation is preferred:

$$\log_{10}\gamma^\circ_{Cl} = -\frac{A\sqrt{I}}{1 + Ba^\circ\sqrt{I}} + CI \quad (7)$$

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 of the experimental temperatures,[9] corresponding to an ion-size parameter, a° of 4.2 Å.[11] The empirical equation given below for the calculation of the parameter C[9,11] was obtained from a curve-fitting method:

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

where $C_{298.15}$ = 0.032 kg·mol^{-1} at T = 278.15 K and T is the Kelvin temperature.[8]

The values of pH(s), listed in Tables 5 and 6, respectively, for the equimolal buffer solutions of HEPPS with NaCl were computed from eqs 1 to 3 and 5 and are represented by the following equations:

For HEPPS (0.04 mol·kg^{-1}) + NaHEPPS (0.04 mol·kg^{-1}):

$$\mathrm{pH(s)} = 7.893 - 1.2599\cdot10^{-2}(T/\mathrm{K} - 298.15) + 2.86\cdot10^{-5}(T/\mathrm{K} - 298.15)^2 \quad (9)$$

For HEPPS (0.06 mol·kg^{-1}) + NaHEPPS (0.06 mol·kg^{-1}):

$$\mathrm{pH(s)} = 7.904 - 1.2512\cdot10^{-2}(T/\mathrm{K} - 298.15) + 2.46\cdot10^{-5}(T/\mathrm{K} - 298.15)^2 \quad (10)$$

where (278.15 ≤ T ≤ 328.15) K. The standard deviations of regression for the pH(s) of the chloride-free buffer solutions, obtained from the fits with eqs 7 and 8, are 0.0009 and 0.0009, respectively.

Table 6. Values of pH for HEPPS (m_1) + NaHEPPS (m_2) + NaCl (m_3) Buffer Solutions from (278.15 to 328.15) K Computed Using Equations 4 to 7[a]

T	0.01 *m* HEPPS + 0.02 *m* NaHAPPS + 0.14 *m* NaCl	0.02 *m* HEPPS + 0.04 *m* NaHEPPS + 0.12 *m* NaCl	0.03 *m* HEPPS + 0.06 *m* NaHEPPS + 0.10 *m* NaCl	0.04 *m* HEPPS + 0.08 *m* NaHEPPS + 0.08 *m* NaCl	0.05 *m* HEPPS + 0.05 *m* NaHEPPS + 0.11 *m* NaCl	0.06 *m* HEPPS + 0.06 *m* NaHEPPS + 0.10 *m* NaCl	0.07 *m* HEPPS + 0.07 *m* NaHEPPS + 0.09 *m* NaCl	0.08 *m* HEPPS + 0.08 *m* NaHEPPS + 0.08 *m* NaCl
K	I = 0.16 m							
278.15	8.501	8.501	8.493	8.498	8.207	8.201	8.205	8.210
283.15	8.433	8.432	8.424	8.430	8.138	8.132	8.136	8.140
288.15	8.366	8.365	8.357	8.363	8.070	8.064	8.067	8.071
293.15	8.302	8.301	8.291	8.300	8.005	8.000	8.003	8.006
298.15	8.236	8.237	8.224	8.233	7.939	7.935	7.938	7.938
303.15	8.172	8.172	8.161	8.168	7.876	7.869	7.873	7.876
308.15	8.110	8.110	8.099	8.103	7.813	7.807	7.810	7.812
310.15	8.086	8.086	8.076	8.080	7.789	7.784	7.785	7.789
313.15	8.050	8.051	8.041	8.044	7.754	7.751	7.751	7.756
318.15	7.987	7.990	7.981	7.981	7.693	7.692	7.690	7.697
323.15	7.930	7.931	7.922	7.921	7.634	7.636	7.633	7.643
328.15	7.873	7.871	7.865	7.863	7.576	7.584	7.575	7.593

[a] (m_1, m_2, m_3)/(mol·kg^{-1}).

Table 7

Cell Voltage of Cell II[a] for HEPPS Buffer				
(m_1, m_2, m_3)/(mol·kg^{-1})			E/V	
m_1	m_2	m_3	T = 298.15 K	T = 310.15 K
0.02	0.04	0.12	0.72918	0.72855
0.03	0.06	0.10	0.72835	0.73064
0.06	0.06	0.10	0.71145	0.71325
0.08	0.08	0.08	0.71153	0.71345

Cell Voltage of Cell III[a]		
	E/V	
cell III	T = 298.15 K	T = 310.15 K
0.008695 *m* KH_2PO_4 + 0.03043 *m* Na_2HPO_4	0.68275	0.69144

[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 HEPPS Buffer at (298.15 and 310.15) K Computed Using Equation 1

	E^a_j/mV	
system	T = 298.15 K	T = 310.15 K
physiological phosphate (0.008695 *m* KH_2PO_4 + 0.03043 *m* NaCl)	2.6	2.9
0.02 *m* HEPPS + 0.04 *m* NaHEPPS + 0.12 *m* NaCl	0.4	0.6
0.03 *m* HEPPS + 0.06 *m* NaHEPPS + 0.10 *m* NaCl	0.4	0.6
0.06 *m* HEPPS + 0.06 *m* NaHEPPS + 0.10 *m* NaCl	0.6	0.8
0.08 *m* HEPPS + 0.08 *m* NaHEPPS + 0.08 *m* NaCl	0.5	0.7

[a] $E_j = E + E^\circ_{SCE} - k$pH from eq 1; E is the cell voltage from Table 7; k = Nernst slope with values of 0.059156 units at 298.15 K and 0.061538 units 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, respectively; E°_{SCE} = standard electrode potential of the saturated calomel electrode = (−0.2415 and −0.2335) V at (298.15 and 310.15) K, respectively.

For eight buffer solutions containing NaCl at an isotonic saline media at a total ionic strength of $I = 0.16$ mol·kg^{-1}, the values of cell voltage are given in Table 2 and the values of pa_H(s) listed in Table 6 are manifested in the following equations:

HEPPS (0.01 mol·kg^{-1}) + NaHEPPS (0.02 mol·kg^{-1}) + NaCl (0.14 mol·kg^{-1}):

$$\text{pH(s)} = 8.236 - 1.2804\cdot10^{-2}(T/\text{K} - 298.15) + 2.24\cdot10^{-5}(T/\text{K} - 298.15)^2 \quad (11)$$

HEPPS (0.02 mol·kg^{-1}) + NaHEPPS (0.04 mol·kg^{-1}) + NaCl (0.12 mol·kg^{-1}):

$$\text{pH(s)} = 8.236 - 1.2766\cdot10^{-2}(T/\text{K} - 298.15) + 2.11\cdot10^{-5}(T/\text{K} - 298.15)^2 \quad (12)$$

HEPPS (0.03 mol·kg^{-1}) + NaHEPPS (0.06 mol·kg^{-1}) + NaCl (0.10 mol·kg^{-1}):

$$\text{pH(s)} = 8.226 - 1.2834\cdot10^{-2}(T/\text{K} - 298.15) + 2.80\cdot10^{-5}(T/\text{K} - 298.15)^2 \quad (13)$$

HEPPS (0.04 mol·kg^{-1}) + NaHEPPS (0.08 mol·kg^{-1}) + NaCl (0.08 mol·kg^{-1}):

$$\text{pH(s)} = 8.232 - 1.2918\cdot10^{-2}(T/\text{K} - 298.15) + 1.94\cdot10^{-5}(T/\text{K} - 298.15)^2 \quad (14)$$

HEPPS (0.05 mol·kg^{-1}) + NaHEPPS (0.05 mol·kg^{-1}) + NaCl (0.11 mol·kg^{-1}):

$$\text{pH(s)} = 7.940 - 1.2858\cdot10^{-2}(T/\text{K} - 298.15) + 2.51\cdot10^{-5}(T/\text{K} - 298.15)^2 \quad (15)$$

HEPPS (0.06 mol·kg^{-1}) + NaHEPPS (0.06 mol·kg^{-1}) + NaCl (0.10 mol·kg^{-1}):

$$\text{pH(s)} = 7.933 - 1.2748\cdot10^{-2}(T/\text{K} - 298.15) + 3.63\cdot10^{-5}(T/\text{K} - 298.15)^2 \quad (16)$$

HEPPS (0.07 mol·kg^{-1}) + NaHEPPS (0.07 mol·kg^{-1}) + NaCl (0.09 mol·kg^{-1}):

$$\text{pH(s)} = 7.937 - 1.2872\cdot10^{-2}(T/\text{K} - 298.15) + 2.72\cdot10^{-5}(T/\text{K} - 298.15)^2 \quad (17)$$

HEPPS (0.08 mol·kg^{-1}) + NaHEPPS (0.08 mol·kg^{-1}) + NaCl (0.08 mol·kg^{-1}):

$$\text{pH(s)} = 7.938 - 1.2826\cdot10^{-2}(T/\text{K} - 298.15) + 4.16\cdot10^{-5}(T/\text{K} - 298.15)^2 \quad (18)$$

where T is the temperature in Kelvin. The standard deviations for the regression of the "observed" results from eqs 11 to 18 are 0.0009, 0.0010, 0.0007, 0.0012, 0.0006, 0.0017, 0.0009, and 0.0015, respectively.

The operational pH values at T = (298.15 and 310.15) K were evaluated from cells with liquid junctions, cells II and III, by means of the flowing junction cell.[9,11] The cell voltage values of cells II and III at T = (278.15 and 310.15) K are given in Table 7. The values of E_j were obtained by using eq 1 and are listed in Table 8. There does not exist a commonly used experimental method for the determination of single-ion activity coefficient, $\log_{10}\gamma^\circ_{Cl}$. The common equation for the calculation of $\log_{10}\gamma^\circ_{Cl}$ is based on the Bates−Guggenheim convention and can be used up to $I = 0.1$ mol·kg^{-1}.[7,16−18] The error associated with the pH(s) values can be attributed to these factors: (i) assumption for the calculation of the $\log_{10}\gamma^\circ_{Cl}$ (± 0.004), (ii) extrapolation to $p(a_H\gamma_{Cl})^\circ$ at to $m_{Cl} = 0$ (less than ± 0.002),

Table 9. Values of pH at (298.15 and 310.15) K for HEPPS Buffer Solutions Using Data for E_j Corrections from Table 8

cell II			pH						
(m_1, m_2, m_3)/(mol·kg^{-1})			T = 298.15 K				T = 310.15 K		
m_1	m_2	m_3	I	without[a] E_j corr	with[b] E_j corr	calc[c]	without[a] E_j corr	with[b] E_j corr	calc[c]
0.02	0.04	0.12	0.16	8.200	8.237	8.237	7.998	8.035	8.086
0.03	0.06	0.10	0.16	8.186	8.223	8.224	8.031	8.075	8.076
0.06	0.06	0.10	0.16	7.900	7.934	7.935	7.749	7.783	7.784
0.08	0.08	0.08	0.16	7.902	7.937	7.938	7.752	7.788	7.789

[a] pH = 7.415 + [(E/V − 0.68275)/0.059156] at 298.15 K, and pH = 7.395 + [(E/V − 0.69144)/0.061538] at 310.15 K; the cell voltage values (Table 7) are (0.68275 and 0.69144) V at (298.15 and 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 6 and 7.

and (iii) error in the experimental cell voltage measurement (± 0.02 mV). Thus, the overall estimated uncertainty is ± 0.006 for buffers without the presence of NaCl and ± 0.012 for buffer solutions with the ionic strength $I = 0.16$ mol·kg^{-1}. Errors in the values of E_j are irrelevant to the values of pH(s) determined from cell I without liquid junction; however, the δE_j of eq 2 does affect the operational pH values which are listed in Table 9 at room and body temperatures. These are recommended as useful secondary pH standards to calibrate electrodes for pH measuring assembly in physiological pH range. The consistency of the four sets of experiments listed in Table 9 gives credence to the reliability of the pH values of HEPPS buffer solutions.

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Literature Cited

(1) Good, N. E.; Winget, G. D.; Winter, W.; Connolly, T. N.; Izawa, S.; Singh, R. M. M. Hydrogen Ion Buffers for Biological Research. *Biochemistry* **1966**, *5*, 467–477.

(2) Ferguson, W. J.; Braunschweiger, K. I.; Braunschweiger, W. R.; Smith, J. R.; McCormick, J. J.; Wasmann, C. C.; Jarvis, N. P.; Bell, D. H.; Good, N. E. Hydrogen Ion Buffers for Biological Research. *Anal. Biochem.* **1980**, *104*, 300–310.

(3) Roy, R. N.; Moore, C. P.; Lord, P.; Mrad, D.; Good, W. S.; Niederschmidt, J. A.; Roy, L. N.; Kuhler, K. M. Thermodynamic Constants of N-(2-Hydroxyethyl) Piperazine-N′-3-Propanesulfonic Acid (HEPPS) and (3-[N-Morpholino])-2-hydroxypropanesulfonic acid (MOPSO) from the temperatures 278.15 to 328.15 K. *J. Chem. Thermodyn.* **1997**, *29*, 1323–1331.

(4) Bates, R. G.; Vega, C. A.; White, D. R., Jr. Standards for pH measurements in isotonic saline media of ionic strength I = 0.16. *Anal. Chem.* **1978**, *50*, 1295−1300.

(5) Roy, R. N.; Roy, L. N.; LeNoue, S. R.; Denton, C. E.; Simon, A. N.; Richards, S. J.; Moore, A. C.; Roy, C. N.; Redmond, R. R.; Bryant, P. A. Thermodynamic Constants of N-[tris(hydroxymethyl)methyl-3-amino]propanesulfonic Acid (TAPS) from the Temperatures 278.15 K to 328.15 K. *J. Chem. Thermodyn.* **2006**, *38*, 413–417.

(6) Roy, L. N.; Roy, R. N.; Denton, C. E.; LeNoue, S. R.; Himes, C. A.; Richards, S. J.; Simon, A. N.; Roy, C. N.; Somal, V. S. Buffer Standards for the Physiological pH of the Zwitterionic Compound, TAPS, from 5 to 55 °C. *J. Solution Chem.* **2006**, *35*, 551–566.

(7) Bower, V. E.; Paabo, M.; Bates, R. G. A Standard for the Measurement of the pH of Blood and Other Physiological Media. *J. Res. Nat. Bur. Stand.* **1961**, *65A*, 267–2701.

(8) Durst, R. A.; Staples, B. R. Tris/Tris HCl: Standard Buffer for Use in the Physiological pH Range. *Clin. Chem. (Washington, DC, U.S.)* **1972**, *18*, 206–208.

(9) Feng, D.; Koch, W. F.; Wu, Y. C. Second Dissociation Constant and pH of N-(2-Hydroxyethyl)piperazine-N′-2-ethanesulfonic Acid from 0 to 50 °C. *Anal. Chem.* **1989**, *61*, 1400–1405.

(10) Wu, Y. C.; Berezansky, P. A.; Feng, D.; Koch, W. F. Second Dissociation Constant of 3-(N-Morpholino)-2-hydroxypropanesulfonic Acid and pH of Its Buffer Solutions. *Anal. Chem.* **1993**, *65*, 1084–1087.

(11) Roy, R. N.; Mrad, D. R.; Lord, P. A.; Carlsten, J. A.; Good, W. S.; Allsup, P.; Roy, L. N.; Kuhler, K. M.; Koch, W. F.; Wu, Y. C. Thermodynamics of the Second Dissociation Constant and Standards for pH of 3-(N-Morpholino)propanesulfonic Acid (MOPS) from 5 to 55 °C. *J. Solution Chem.* **1998**, *27*, 73–87.

(12) Roy, R. N.; Roy, L. N.; Grant, J. G.; Cummins, M. P.; Tabor, B. J.; Richards, S. J.; Himes, C. A.; Lively, B. R.; Blackwell, P. L.; Simon, A. N. Second Dissociation Constants of 4-[N-morpholino]butane-sulfonic Acid and N-[2-hydroxyethyl]piperazine-N'-4-butanesulfonic Acid from 5 to 55°C. *J. Solution Chem.* **2002**, *31*, 861–872.

(13) Bates, R. G.; Roy, R. N.; Robinson, R. A. Buffer Standards of Tris(hydroxymethyl)methylglycine ("Tricine") for the Physiological Range pH 7.2 to 8.5. *Anal. Chem.* **1973**, *45*, 1663–1666.

(14) Goldberg, R. N.; Kishore, N.; Lennen, R. M. Thermodynamic Quantities for the Ionization Reactions of Buffers. *J. Phys. Chem. Ref. Data* **2002**, *31*, 231–370.

(15) Bates, R. G. *Determination of pH*, 2nd ed.; Wiley: New York, 1973; Chapter 4, p 10.

(16) Bates, R. G.; Vega, C. A.; White, D. R., Jr. Standards for pH Measurements in Isotonic Saline Media of Ionic Strength I = 0.16. *Anal. Chem.* **1978**, *50*, 1295–1300.

(17) Bates, R. G.; Guggenheim, E. A. Report on the Standardization of pH and Related Terminology. *Pure Appl. Chem.* **1960**, *1*, 163–168.

(18) Buck, R. P.; Rondinini, S.; Covington, A. K.; Baucke, F. G. K.; Brett, C. M. A.; Camões, M. F.; Milton, M. J. T.; Mussini, T.; Naumann, R.; Pratt, K. W.; Spitzer, P.; Wilson, G. S. Measurement of pH. Definition, Standards, and Procedures. *Pure Appl. Chem.* **2002**, *74*, 2169–2200.

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