Open Access

Buffer Standards for the Physiological pH of *N*-(2-Hydroxyethyl) piperazine-*N'*-4-butanesulfonic Acid (HEPBS) from 5 to 55 °C

Lakshmi N. Roy, Rabindra N. Roy*, Kathleen A. Allen, Casey J. Mehrhoff, Isaac B. Henson, Jessica M. Stegner and Zachary M. Downs

Hoffman Department of Chemistry, Drury University, 900 N. Benton Ave., Springfield, Missouri 65802, USA

Abstract: The pH values of six buffer solutions of equal compositions on the molal scale and eight buffer solutions having ionic strengths ($I = 0.16\ \mathrm{mol\cdot kg^{-1}}$) similar to the concentration of blood plasma have been evaluated at 12 temperatures from 5 to 55 °C using the Bates-Guggenheim convention and extended Debye-Hückel equation. The values of E_j for the buffer solution of HEPBS have been obtained from the flowing junction cell measurement. These values of E_j have been used to ascertain the operational pH values at 25 and 37 °C for HEPBS buffer solution. The pH values at 25 and 37 °C are 7.415 and 7.395, respectively, for physiological phosphate buffer solutions. The zwitterionic buffer HEPBS was shown to be useful as a secondary pH standard in the region close to that of blood serum.

Keywords: Buffers, Emf, HEPBS, Ionic strength,·pH, Zwitterions.

1. INTRODUCTION

Recently, we have reported the pK_2 values of *N*-(2-hydroxyethyl)piperazine-*N'*-4-butanesulfonic acid (HEPBS) [1] at temperatures from 5 to 55 °C, including 37 °C. The buffer substances recommended by Good *et al.* [2, 3] are useful for the control of pH in the region close to that of physiological solutions. The structure of HEPBS is shown in Fig. (**1**).

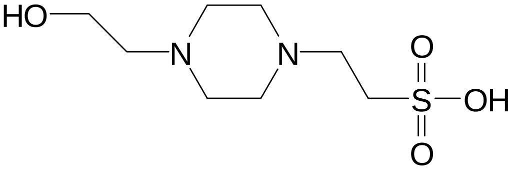

Fig. (1). *N*-(2-hydroxyethyl)piperazine-*N'*(4-butanesulfonic acid) (HEPBS)

Bates and his associates [4] reported pK_2 values and related thermodynamic quantities from 5 to 55 °C, at 5 °C intervals. These values are in very good agreement with those of Roy and associates [1]. Calibration of the electrodes with the pH meter at pH near 7 is routine in clinical laboratories. The NBS "blood buffer", KH_2PO_4 (0.008695) + Na_2HPO_4 (0.03043 $\mathrm{mol\cdot kg^{-1}}$) has been universally adopted as a reference standard solution. The limitations of the 'blood phosphate buffer' are: (a) no resistance of the sample matrix of the blood ingredients [5], resulting in precipitation with bivalent cations, and (b) the temperature coefficient of the blood does not match with that of the phosphate buffer.

The buffer substances recommended by Good [2, 3] are useful for pH measurement and control of acidity in the pH range 6-8. Roy and coworkers [6] have reported the pK_2 data and pH values of N-substituted amino acid buffer, bis-[(2-hydroxyyethyl)amino]acetic acid (BICINE), and the values of pH for the buffer TRICINE [7]. The values of pK_2 and pH for the buffer HEPES have been published by Feng and coworkers [8]. The thermodynamic quantities (pK_2 and pH) of physiological buffers MOPS and MOPSO have been published by Roy and coworkers [9], and Wu and associates [10], respectively. These buffer solutions lie in the physiological pH range 6-8. Bates and coworkers [11] recommended TRICINE buffer as a primary standard for pH measurement. Goldberg and associates [12] published pK_2 data and related thermodynamic quantities for the ionization reactions of 68 biological buffer compounds. They clearly indicated that no data for pK_2 and pH are available for the buffer material HEPBS.

Hence, we are prompted to study the following composition of HEPBS buffer on the molal scale of concentration expressed in $\mathrm{mol\cdot kg^{-1}}$. Exact values of the molalities of buffer, HEPBS, NaHEPBS, and NaCl are put in brackets for the following compositions: (a) HEPBS (0.02) + NaHEPBS (0.02), (b) HEPBS (0.04) + NaHEPBS (0.08), (c) HEPBS (0.08) + NaHEPBS (0.08), (d) HEPBS (0.04) + NaHEPBS (0.04), (e) HEPBS (0.05) + NaHEPBS (0.05), (f) HEPBS (0.06) + NaHEPBS (0.06), (g) HEPBS (0.02) + NaHEPBS (0.04) + NaCl (0.12); (h) HEPBS (0.03) + NaHEPBS (0.06) + NaCl (0.10), (i) HEPBS (0.04) + NaHEPBS (0.08) + NaCl (0.08), (j) HEPBS (0.03) + NaHEPBS (0.09) + NaCl (0.07), (k) HEPBS (0.04) + NaHEPBS (0.04) + NaCl (0.12), (l) HEPBS (0.05) + NaHEPBS (0.05) + NaCl (0.11), (m) HEPBS (0.06) + NaHEPBS (0.06) + NaCl (0.10), (n) HEPBS (0.08) + NaHEPBS (0.08) + NaCl (0.08).

---

*Address correspondence to this author at the Hoffman Department of Chemistry, Drury University, 900 N. Benton Ave., Springfield, Missouri 65802, USA; Tel: 417-873-7247; Fax: 417-873-7856;
E-mail: rroy@drury.edu

1876-505X/12

2. EXPERIMENTAL

HEPBS, purchased from the Sigma Chemical Co., was recrystallized twice from 85 wt. % ethanol. The sample was vacuum dried. The purified compound was assayed by titration with a standard solution of NaOH. The purity of the sample was 99.94%. All buffer solutions for the cell measurements were prepared by weight methods by adding weighted amounts of conductivity water, buffer material, NaCl, and NaOH standard solution. Thinly coated platinum black hydrogen electrodes and the thermal-electrolytic silver-silver chloride electrodes were prepared as described in the literature [1, 9].

For accurate calculations of pH values of the fourteen buffer solutions, the following cell (A) without liquid junction was used for emf measurement:

Pt(s), H_2(g, 1 atm) | HEPBS (m_1) + NaHEPBS (m_2) + NaCl (m_3) | AgCl(s), Ag(s) (A)

in which species in solution are expressed in molalities. The liquid junction potential, E_j , was measured using a cell of the type:

Pt(s), H_2(g, 1 atm) | HEPBS (m_1) + NaHEPBS (m_2), NaCl (m_3) || KCl(satd) | Hg_2Cl_2(s), Hg(l) (B)

in which (s), (l), and (g) indicate three different phases. The emf values of the following cell (C) with the flowing junction set-up were measured for NIST standard phosphate buffer KH_2PO_4 (0.008695) + Na_2HPO_4 (0.03043):

Pt(s), H_2(g, 1 atm) | phosphate buffer || KCl(satd) | Hg_2Cl_2 (s), Hg(l) (C)

The cells (B) and (C) yield E_j values for HEPBS, and NIST standard phosphate buffer, respectively. The Eqs. [8, 9] used to calculate the liquid junction potential are given below:

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

in which data for E°_{SCE}, k, and pH for NIST phosphate standard buffer at 25 and 37°C are available in the literature [6]. The operational definition of pH, abbreviated pH(x), is given below:

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

in which x indicates HEPBS + NaHEPBSate buffer, s refers to pH standard phosphate solution, and $\delta E_j = E_{j(s)} - E_{j(x)}$. Without liquid junction contribution, the simlipfied Eq. 3 is obtained:

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

3. METHODS AND RESULTS

The electromotive force values of the galvanic cell (A) after the usual pressure correction to 1 atm for all experimental buffer solutions and temperatures are entered in Tables **1** (without Cl^-) and 2 (with Cl^-), respectively.

The pa_H values of all buffer solutions including isotonic saline solutions (I = 0.16) have been calculated by using the standard methods of Bates *et al*. [9, 11,13]. The values of $-\log(\gamma_H\gamma_{Cl}m_H)$ or $p(a_H\gamma_{Cl})$ known as the acidity function for six buffer solutions with Cl^- ion, and 12 temperatures were derived from Eq. (4), given below:

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

The linear plot of $p(a_H\gamma_{Cl})$ versus molality of chloride ion, m_{Cl^-}, yields values of $p(a_H\gamma_{Cl})^\circ$ at $m_{Cl^-} = 0$. The values of E, E°, and m_{Cl^-} can be obtained from Tables **1** and **2**. From the intercept of the plot, these values of $p(a_H\gamma_{Cl})^\circ$ for six buffer solutions with an accuracy of ± 0.001 are listed in Table **3**. The values of $p(a_H\gamma_{Cl})$ from 5 to 55 °C for eight isotonic saline buffer solutions (I = 0.16) containing NaCl are entered in Table **4**. The values of pH(s) for six Cl^- free buffer solutions were estimated from Eq. 5 shown below:

$$\text{pH(s)} = p(a_H\gamma_{Cl})^\circ + \log_{10} \gamma^\circ_{Cl} \tag{5}$$

The extended Debye-Hückel equation of the Bates-Guggenheim convention [4, 9] was used for the calculation of the single ion activity coefficient, $\log_{10} \gamma^\circ_{Cl}$, necessary in the assignment of pH values for standards of reference buffer solutions [8, 10, 14-18]. The Eq. 6 given below was used for the estimation of $\log_{10} \gamma^\circ_{Cl}$:

$$\log_{10} \gamma^\circ_{Cl} = -\frac{A\sqrt{I}}{1 + Ba^\circ\sqrt{I}} + CI \tag{6}$$

in which I, A, B, and C have the usual physical significance [1, 6-7, 13].

The zwitterionic form of HEPBS was assumed to make no contribution to the ionic strength (I). Since pH values of HEPBS buffer solutions are close to 7, hydrolysis of HEPBS and Na-HEPBS are ignored. The adjustable parameter C was estimated from the quadratic equation of the form shown below in Eq. 7:

$$C = C_{25} + 6.2 \times 10^{-4}(t - 25) - 8.7 \times 10^{-6}(t - 25)^2 \tag{7}$$

where C_{25} = 0.032 kg·mol^{-1} at 25 °C and t is the temperature in degrees C [8].

By using Eqs. 4-7, the values of pa_H for six Cl^- free buffer solutions are given in Table **5**. The quadratic equation for pa_H as a function of temperature is expressed by the following equations for the following compositions:

HEPBS (0.02) + NaHEPBS (0.04):

$$pa_H = 8.522 - 1.460\times10^{-2}(t - 25) - 1.100\times10^{-5}(t - 25)^2 \tag{8}$$

HEPBS (0.04) + NaHEPBS (0.08):

$$pa_H = 8.499 - 1.439\times10^{-2}(t - 25) + 3.640\times10^{-6}(t - 25)^2 \tag{9}$$

HEPBS (0.08) + NaHEPBS (0.08):

$$pa_H = 8.213 - 1.474\times10^{-2}(t - 25) + 1.310\times10^{-5}(t - 25)^2 \tag{10}$$

HEPBS (0.04) + NaHEPBS (0.04):

$$pa_H = 8.209 - 1.374\times10^{-2}(t - 25) + 4.570\times10^{-5}(t - 25)^2 \tag{11}$$

HEPBS (0.05) + NaHEPBS (0.05):

Table 1. Electromotive Force of Cell A: Pt(s); H_2 (g, 1 atm) | HEPBS (m_1), NaHEPBS (m_2), NaCl (m_3) | AgCl(s), Ag(s)

m_1[a]	m_2[a]	m_3[a]	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	37 °C	40 °C	45 °C	50 °C	55 °C
0.02	0.04	0.005	0.85160	0.85591	0.85998	0.86381	0.86733	0.87067	0.87350	0.87459	0.87611	0.87862	0.88077	0.88247
0.02	0.04	0.010	0.83484	0.83886	0.84265	0.84619	0.84939	0.85259	0.85530	0.85636	0.85781	0.86031	0.86235	0.86389
0.02	0.04	0.015	0.82511	0.82896	0.83257	0.83594	0.83896	0.84200	0.84464	0.84575	0.84722	0.84970	0.85181	0.85325
0.02	0.04	0.020	0.81804	0.82181	0.82529	0.82853	0.83143	0.83457	0.83707	0.83833	0.83980	0.84246	0.84470	0.84613
0.04	0.08	0.005	0.85210	0.85645	0.86042	0.86409	0.86762	0.87115	0.87403	0.87514	0.87683	0.87919	0.88127	0.88320
0.04	0.08	0.010	0.83598	0.83991	0.84363	0.84709	0.85043	0.85354	0.85625	0.85715	0.85872	0.86075	0.86262	0.86418
0.04	0.08	0.015	0.82668	0.83046	0.83403	0.83732	0.84040	0.84326	0.84601	0.84697	0.84838	0.85024	0.85176	0.85312
0.04	0.08	0.020	0.82018	0.82382	0.82725	0.83041	0.83353	0.83629	0.83885	0.83972	0.84108	0.84281	0.84428	0.84547
0.08	0.08	0.005	0.83687	0.84074	0.84436	0.84768	0.85071	0.85390	0.85651	0.85737	0.85854	0.86044	0.86196	0.86324
0.08	0.08	0.010	0.82048	0.82413	0.82756	0.83069	0.83361	0.83642	0.83853	0.83946	0.84042	0.84168	0.84246	0.84290
0.08	0.08	0.015	0.81118	0.81461	0.81779	0.82076	0.82364	0.82619	0.82843	0.82900	0.82979	0.83050	0.83068	0.83047
0.08	0.08	0.020	0.80465	0.80796	0.81106	0.81390	0.81672	0.81911	0.82131	0.82183	0.82261	0.82275	0.82250	0.82152
0.04	0.04	0.005	0.83487	0.83889	0.84249	0.84586	0.84922	0.85237	0.85544	0.85648	0.85826	0.86099	0.86336	0.86585
0.04	0.04	0.010	0.81874	0.82246	0.82582	0.82894	0.83199	0.83481	0.83753	0.83833	0.83986	0.84229	0.84422	0.84602
0.04	0.04	0.015	0.80932	0.81287	0.81611	0.81911	0.82187	0.82459	0.82713	0.82767	0.82919	0.83140	0.83285	0.83427
0.04	0.04	0.020	0.80276	0.80622	0.80939	0.81232	0.81502	0.81751	0.81980	0.82038	0.82159	0.82360	0.82502	0.82575
0.05	0.05	0.005	0.83493	0.83884	0.84245	0.84593	0.84935	0.85241	0.85534	0.85625	0.85782	0.86036	0.86258	0.86462
0.05	0.05	0.010	0.81873	0.82238	0.82569	0.82885	0.83199	0.83470	0.83733	0.83808	0.83949	0.84169	0.84350	0.84507
0.05	0.05	0.015	0.80938	0.81289	0.81607	0.81906	0.82200	0.82455	0.82696	0.82752	0.82889	0.83087	0.83240	0.83360
0.05	0.05	0.020	0.80274	0.80616	0.80921	0.81209	0.81489	0.81728	0.81954	0.82000	0.82132	0.82312	0.82444	0.82532
0.06	0.06	0.005	0.83527	0.84004	0.84380	0.84718	0.85034	0.85348	0.85628	0.85719	0.85879	0.86122	0.86330	0.86465
0.06	0.06	0.010	0.81894	0.82355	0.82700	0.83008	0.83295	0.83582	0.83838	0.83911	0.84063	0.84277	0.84458	0.84482
0.06	0.06	0.015	0.80932	0.81395	0.81713	0.82004	0.82274	0.82544	0.82787	0.82851	0.83002	0.83200	0.83366	0.83306
0.06	0.06	0.020	0.80276	0.80718	0.81029	0.81307	0.81567	0.81823	0.82060	0.82121	0.82274	0.82464	0.82618	0.82483
Values of $E°$			0.23416	0.23147	0.22863	0.22562	0.22244	0.21913	0.21572	0.21429	0.21214	0.20840	0.20455	0.20064

[a] Units of m, mol·kg^{-1}

Table 2. Emf of the Cell A (in volts): Pt(s); H_2(g, 1 atm) | HEPBS (m_1), NaHEPBS (m_2), NaCl (m_3) | AgCl(s), Ag(s)

m_1[a]	m_2[a]	m_3[a]	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	37 °C	40 °C	45 °C	50 °C	55 °C
0.02	0.04	0.12	0.77892	0.78184	0.78460	0.78709	0.78926	0.79137	0.79323	0.79380	0.79476	0.79618	0.79742	0.79812
0.03	0.06	0.10	0.78342	0.78640	0.78918	0.79172	0.79409	0.79621	0.79816	0.79881	0.79989	0.80144	0.80285	0.80388
0.04	0.08	0.08	0.78930	0.79200	0.79482	0.79748	0.79987	0.80208	0.80411	0.80482	0.80595	0.80757	0.80918	0.81049
0.08	0.08	0.08	0.79260	0.79533	0.79817	0.80089	0.80335	0.80561	0.80770	0.80844	0.80959	0.81136	0.81307	0.81443
0.04	0.04	0.12	0.76270	0.76535	0.76773	0.76987	0.77179	0.77357	0.77516	0.77563	0.77649	0.77764	0.77880	0.77945
0.05	0.05	0.11	0.76491	0.76757	0.77000	0.77217	0.77416	0.77592	0.77756	0.77804	0.77881	0.78014	0.78127	0.78202
0.06	0.06	0.10	0.76735	0.77003	0.77244	0.77469	0.77675	0.77851	0.78020	0.78063	0.78155	0.78287	0.78401	0.78487
0.08	0.08	0.08	0.77311	0.77590	0.77838	0.78068	0.78279	0.78467	0.78642	0.78697	0.78804	0.78944	0.79061	0.79163

[a] Units of *m*, mol·kg^{-1}

Table 3. $\mathrm{p}(a_{\mathrm{H}}\gamma_{\mathrm{Cl}})^{\circ}$ **of (HEPBS + NaHEPBS) Buffer Solutions from 5 to 55 °C, Computed Using Eq. 5**[a]

t (°C)	0.02 *m* HEPBS + 0.04 *m* NaHEPBS	0.04 *m* HEPBS + 0.08 *m* NaHEPBS	0.08 *m* HEPBS + 0.08 *m* NaHEPBS	0.04 *m* HEPBS + 0.04 *m* NaHEPBS	0.05 *m* HEPBS + 0.05 *m* NaHEPBS	0.06 *m* HEPBS + 0.06 *m* NaHEPBS
	I = 0.04 *m*	*I* = 0.08 *m*	*I* = 0.08 *m*	*I* = 0.04 *m*	*I* = 0.05 *m*	*I* = 0.06 *m*
5	8.888	8.888	8.613	8.578	8.579	8.587
10	8.815	8.816	8.537	8.505	8.503	8.526
15	8.743	8.742	8.462	8.429	8.428	8.454
20	8.672	8.669	8.388	8.355	8.357	8.381
25	8.601	8.597	8.312	8.287	8.290	8.309
30	8.530	8.532	8.246	8.220	8.221	8.241
35	8.456	8.458	8.170	8.156	8.155	8.171
37	8.425	8.429	8.142	8.130	8.127	8.141
40	8.380	8.388	8.100	8.094	8.086	8.100
45	8.307	8.317	8.028	8.034	8.023	8.034
50	8.233	8.246	7.957	7.973	7.959	7.966
55	8.157	8.175	7.889	7.921	7.898	7.900

[a]Units of m, mol·kg^{-1}

Table 4. $\mathrm{p}(a_{\mathrm{H}}\gamma_{\mathrm{Cl}})$ **of (HEPBS + NaHEPBS) Buffer Solutions From 5 to 55 °C, Computed using Eq. 4**[a]

t (°C)	0.02 *m* HEPBS + 0.04 *m* NaHEPBS + 0.12 *m* NaCl	0.03 *m* HEPBS + 0.06 *m* NaHEPBS + 0.10 *m* NaCl	0.04 *m* HEPBS + 0.08 *m* NaHEPBS + 0.08 *m* NaCl	0.08 *m* HEPBS + 0.08 *m* NaHEPBS + 0.08 *m* NaCl	0.04 *m* HEPBS + 0.04 *m* NaHEPBS + 0.12 *m* NaCl	0.05 *m* HEPBS + 0.05 *m* NaHEPBS + 0.11 *m* NaCl	0.06 *m* HEPBS + 0.06 *m* NaHEPBS + 0.10 *m* NaCl	0.08 *m* HEPBS + 0.08 *m* NaHEPBS + 0.08 *m* NaCl
	I = 0.16 *m*	*I* = 0.16 *m*	*I* = 0.16 *m*	*I* = 0.16 *m*	*I* = 0.16 *m*	*I* = 0.16 *m*	*I* = 0.16 *m*	*I* = 0.16 *m*
5	8.950	8.952	8.962	8.964	8.656	8.658	8.661	8.669
10	8.875	8.877	8.880	8.881	8.582	8.584	8.586	8.594
15	8.804	8.804	8.806	8.807	8.509	8.510	8.512	8.519
20	8.732	8.733	8.735	8.735	8.436	8.438	8.440	8.446
25	8.661	8.663	8.664	8.665	8.366	8.368	8.370	8.375
30	8.593	8.594	8.595	8.595	8.297	8.298	8.300	8.305
35	8.525	8.526	8.526	8.527	8.229	8.230	8.232	8.237
37	8.496	8.498	8.499	8.500	8.201	8.202	8.203	8.209
40	8.456	8.459	8.460	8.461	8.162	8.162	8.164	8.172
45	8.390	8.395	8.195	8.397	8.097	8.099	8.100	8.108
50	8.326	8.331	8.333	8.336	8.035	8.036	8.037	8.043
55	8.256	8.265	8.269	8.272	7.969	7.971	7.973	7.980

[a]Units of *m*, mol·kg^{-1}

Table 5. Values of pa_H of (HEPBS + NaHEPBS) Buffer Solutions from 5 to 55 °C, Computed Using Eqs. 4-6[a]

t (°C)	0.02 *m* HEPBS + 0.04 *m* NaHEPBS *I* = 0.04 *m*	0.04 *m* HEPBS + 0.08 *m* NaHEPBS *I* = 0.08 *m*	0.08 *m* HEPBS + 0.08 *m* NaHEPBS *I* = 0.08 *m*	0.04 *m* HEPBS + 0.04 *m* NaHEPBS *I* = 0.04 *m*	0.05 *m* HEPBS + 0.05 *m* NaHEPBS *I* = 0.05 *m*	0.06 *m* HEPBS + 0.06 *m* NaHEPBS *I* = 0.06 *m*
5	8.811	8.789	8.513	8.501	8.495	8.510
10	8.737	8.716	8.437	8.427	8.419	8.436
15	8.665	8.642	8.362	8.351	8.344	8.364
20	8.595	8.569	8.288	8.277	8.272	8.291
25	8.523	8.496	8.210	8.208	8.204	8.217
30	8.451	8.430	8.144	8.141	8.135	8.149
35	8.376	8.355	8.067	8.077	8.068	8.078
37	8.345	8.326	8.039	8.050	8.040	8.048
40	8.299	8.285	7.992	8.014	7.999	8.007
45	8.226	8.213	7.924	7.953	7.935	7.940
50	8.151	8.141	7.853	7.891	7.871	7.871
55	8.074	8.069	7.784	7.838	7.808	7.805

[a]Units of *m*, mol·kg^{-1}

Table 6. Values of pa_H for HEPBS (m_1) + NaHEPBS (m_2) + NaCl (m_3) Buffer Solutions from 5 to 55 °C, Computed Using Eqs. 4-6[a]

t (°C)	0.02 *m* HEPBS + 0.04 *m* NaHEPBS + 0.12 *m* NaCl *I* = 0.16 *m*	0.03 *m* HEPBS + 0.06 *m* NaHEPBS + 0.10 *m* NaCl *I* = 0.16 *m*	0.04 *m* HEPBS + 0.08 *m* NaHEPBS + 0.08 *m* NaCl *I* = 0.16 *m*	0.08 *m* HEPBS + 0.08 *m* NaHEPBS + 0.08 *m* NaCl *I*= 0.16 *m*	0.04 *m* HEPBS + 0.04 *m* NaHEPBS + 0.12 *m* NaCl *I* = 0.16 *m*	0.05 *m* HEPBS + 0.05 *m* NaHEPBS + 0.11 *m* NaCl *I* = 0.16 *m*	0.06 *m* HEPBS + 0.06 *m* NaHEPBS + 0.10 *m* NaCl *I* = 0.16 *m*	0.08 *m* HEPBS + 0.08 *m* NaHEPBS + 0.08 *m* NaCl *I*= 0.16 *m*
5	8.825	8.827	8.836	8.838	8.531	8.533	8.536	8.543
10	8.750	8.752	8.755	8.756	8.456	8.458	8.461	8.468
15	8.678	8.679	8.680	8.681	8.383	8.385	8.386	8.393
20	8.607	8.608	8.610	8.610	8.311	8.313	8.315	8.321
25	8.534	8.537	8.538	8.538	8.239	8.241	8.244	8.249
30	8.466	8.467	8.468	8.468	8.170	8.171	8.173	8.178
35	8.397	8.398	8.399	8.400	8.101	8.103	8.105	8.109
37	8.368	8.370	8.371	8.372	8.073	8.074	8.075	8.081
40	8.328	8.331	8.332	8.332	8.034	8.033	8.036	8.043
45	8.261	8.265	8.265	8.268	7.968	7.969	7.971	7.978
50	8.195	8.201	8.203	8.205	7.905	7.906	7.907	7.913
55	8.124	8.134	8.138	8.141	7.838	7.839	7.842	7.849

[a]Units of *m*, mol·kg^{-1}

$$\mathrm{p}a_H = 8.203 - 1.397\times10^{-2}(t - 25) + 2.650\times10^{-5}(t - 25)^2 \qquad (12)$$

HEPBS (0.06) + NaHEPBS (0.06):

$$\mathrm{p}a_H = 8.218 - 1.428\times10^{-2}(t - 25) + 1.580\times10^{-5}(t - 25)^2 \qquad (13)$$

where 5 °C $\le t \le$ 55 °C. The uncertainties in the values of pa_H for six HEPBS buffer solutions are 0.0013, 0.0017, 0.0020, 0.0014, 0.0012, and 0.0010, respectively.

For eight isotonic HEPBS solutions (I = 0.16) containing NaCl, the pa_H data entered in Table **6** are given below:

HEPBS (0.02) + NaHEPBS (0.04) + NaCl (0.12):

$$pa_H = 8.536 - 1.409\times10^{-2}(t - 25) + 1.490\times10^{-5}(t - 25)^2 \quad (14)$$

HEPBS (0.03) + NaHEPBS (0.06) + NaCl (0.10):

$$pa_H = 8.537 - 1.404\times10^{-2}(t - 25) + 2.170\times10^{-5}(t - 25)^2 \quad (15)$$

HEPBS (0.04) + NaHEPBS (0.08) + NaCl (0.08):

$$pa_H = 8.537 - 1.420\times10^{-2}(t - 25) + 3.110\times10^{-5}(t - 25)^2 \quad (16)$$

HEPBS (0.03) + NaHEPBS (0.09) + NaCl (0.07):

$$pa_H = 8.538 - 1.420\times10^{-2}(t - 25) + 3.380\times10^{-5}(t - 25)^2 \quad (17)$$

HEPBS (0.04) + NaHEPBS (0.04) + NaCl (0.12):

$$pa_H = 5.240 - 1.408\times10^{-2}(t - 25) + 2.410\times10^{-5}(t - 25)^2 \quad (18)$$

HEPBS (0.05) + NaHEPBS (0.05) + NaCl (0.11):

$$pa_H = 8.241 - 1.411\times10^{-2}(t - 25) + 2.490\times10^{-5}(t - 25)^2 \quad (19)$$

HEPBS (0.06) + NaHEPBS (0.06) + NaCl (0.10):

$$pa_H = 8.245 - 1.413\times10^{-2}(t - 25) + 2.590\times10^{-5}(t - 25)^2 \quad (20)$$

HEPBS (0.08) + NaHEPBS (0.08) + NaCl (0.08):

$$pa_H = 8.249 - 1.416\times10^{-2}(t - 25) + 2.840\times10^{-5}(t - 25)^2 \quad (21)$$

The uncertainties obtained by the method of least squares from Eqs. 14 to 21 are 0.0014, 0.0009, 0.0015, 0.0017, 0.0010, 0.0010, 0.0010, and 0.0009, respectively.

4. CONCLUSIONS

The emf values listed in Tables **1** and **2**, of the cell (A) are stable, reproducible and reliable within ± 0.02 mV. The standard deviations of the values of $p(a_H\gamma_{Cl})°$, given in Table **3**, for six chloride free buffer solutions and $p(a_H\gamma_{Cl})$ for eight buffer solutions with chloride, listed in Table **4**, lie within ± 0.002 pH. The pa_H values, shown in Tables **5** and **6**, for all experimental buffer solutions at 25 and 37 °C are within the physiological range of pH 8.53 to 8.04, respectively.

Table 7. Emf of Cell B for HEPBS Buffer

m_1	m_2	m_3	E/V	
			25 °C	37 °C
0.08	0.08	0.00	0.72932	0.73066
0.04	0.04	0.12	0.72942	0.73085
0.05	0.05	0.11	0.72948	0.73092
0.06	0.06	0.10	0.72966	0.73110
0.08	0.08	0.08	0.73001	0.73135
Emf of Cell C[a]				
Cell C			E/V	
			25 °C	37 °C
0.008695 m KH_2PO_4 + 0.0304 m Na_2HPO_4			0.68275	0.69147

[a] Published data [7, 19] for physiological phosphate buffer solutions; units of m, $mol\cdot kg^{-1}$

Table 8. Values of the Liquid Junction Potentials, E_j, for HEPBS Buffer at 25 and 37 °C

System	E_j^a/mV	
	25 °C	37 °C
Physiological phosphate (0.008695 m KH_2PO_4 + 0.03043 m NaCl)	2.6	2.9
0.08 m HEPBS + 0.08 m NaHEPBS + 0.00 m NaCl	2.2	2.5
0.04 m HEPBS + 0.04 m NaHEPBS + 0.12 m NaCl	0.5	0.6
0.05 m HEPBS + 0.05 m NaHEPBS + 0.11 m NaCl	0.5	0.6
0.06 m HEPBS + 0.06 m NaHEPBS + 0.10 m NaCl	0.5	0.7
0.08 m HEPBS + 0.08 m NaHEPBS + 0.08 m NaCl	0.5	0.6

[a] $E_j = E + E^{\circ}_{SCE} - k$ pH from Eq. 1 is the Emf from Table 7, k = Nernst slope with values 0.059156 at 25 °C, and 0.061538 at 37 °C; the pH of the primary reference standard phosphate buffer is 7.415 and 7.395 at 25 °C and 37 °C, respectively; E°_{SCE} = electrode potential of the saturated calomel electrode = -0.2415 and -0.2335 at 25 °C and 37 °C [14, 15], respectively; units of m, $mol\cdot kg^{-1}$.

Table 9. Values of pH at 25 and 37 °C for HEPBS Buffer Solutions

Cell B			Ionic	25 °C			37 °C		
m_1	m_2	m_3	Strength, I	Without^a	With^b		Without^a	With^b	
				E_j corr	E_j corr.	Calc^c	E_j corr	E_j corr	Calc^c
0.08	0.08	0.00	0.08	8.203	8.210	8.210	8.032	8.038	8.039
0.04	0.04	0.12	0.16	8.204	8.239	8.239	8.035	8.072	8.073
0.05	0.05	0.11	0.16	8.205	8.240	8.241	8.036	8.073	8.074
0.06	0.06	0.10	0.16	8.208	8.243	8.244	8.039	8.075	8.075
0.08	0.08	0.08	0.16	8.214	8.249	8.249	8.044	8.080	8.081

^{a}Values obtained from Eq. 3 and data in Table **7**
^{b}Obtained from Eq. 2 and E_j values in Table **8**
^{c}Obtained from Tables **5** and **6**.

The emf values at 25 and 37 °C, are obtained from Harned cells (B) and (C). The values of E_j listed in Table **8** are obtained from Eq. 1. Table **9** lists the pH values of five buffers at 25 and 37 °C with and without the liquid junction correction. The pH values with E_j correction [18] are in good agreement with that of the calculated value. The use of Bates-Guggenheim convention is inapplicable at an ionic strength I = 0.16. Hence IUPAC and NIST [17] recommend the use of Pitzer formalism [21] at this concentration for the estimation of the single ion activity coefficient, γ_{Cl}. Partanen and coworker [19], and Covington and Ferra [20], applied Pitzer theory for the calculation of γ_{Cl} for NIST blood phosphate buffers in the assignment of pH values. The uncertainties in the pa_H values are generally due to E_j estimation, the estimation of γ°_{Cl}, and the extrapolation to $p(a_H\gamma_{Cl})^{\circ}$. The overall uncertainty is about ±0.006 pH unit for buffer solutions without Cl^- and ±0.008 for solutions with Cl^-. Low liquid junction potential values, E_j, listed in Table **8** indicate HEPBS to be included as secondary standard for pH measurement near pH 7-8.

ACKNOWLEDGEMENTS

The authors acknowledge funding from the National Institutes of Health (AREA), under the grant 2-R15 GM 066866-03 and the diversity supplemental grant 3-R15 GM 066866-03 S1. The authors would also like to thank Robert Chaney and Gregory Galloway for their dedicated and hard work. The content of this paper is the sole responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the National Institutes of General Medical Sciences.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

REFERENCES

[1] Roy, R. N.; Grant, J. G.; Roy, L.N.; 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] butanesulfonic acid and *N*-[2-hydroxyethyl]piperazine-*N*'-4-butanesulfonic acid from 5 to 55 °C. *J. Solut. Chem.*, **2002**, *31*, 861-872.

[2] 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.

[3] 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.

[4] Bower, V. E., Paabo, M., Bates, R. G. A standard for the measurement of the pH of blood and other physiological media. *J. Res. Natl. Bur. Stand*., **1961**, *65A*, 267-270.

[5] Durst, R. A., Staples, B. R. Tris/Tris HC1: Standard buffer for use in the physiological pH range. *Clin. Chem*., **1972**, *18*, 206-208.

[6] Roy, L. N.; Roy, R. N.; Denton, C. E.; LeNoue, S. R.; Roy, C. N.; Ashkenazi, S.; Williams, T. B.; Church, D. R.; Fuge, M. S.; Sreepada, K. S. Second dissociation constant of bis-[(2-hydroxyethyl) amino]acetic acid (BICINE) and pH of its buffer solutions from 5 - 55° C. *J. Solut. Chem*., **2006**, *35*, 605-624.

[7] Roy, R. N.; Roy, L. N.; Henson, I.; Stegner, J.; Dinga, J.; Summers, C.; Suhrheinrich, G.; Veliz, J. Buffer standards for the physiological pH of *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine (TRICINE) from 5-55 °C. *J. Chem. Thermodyn*., **2012**, *52*, 11-15.

[8] 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.

[9] 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. Solut. Chem*., **1998**, *27,* 73-87.

[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] 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.

[12] 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.

[13] Bates, R. G. *Determination of pH*, chapters 4 and 10. 2nd ed.; Wiley: New York, **1972**.

[14] Bates, R. G. Revised standard values for pH measurements from 0 to 95 °C. *J. Res. Natl. Bur. Stand*., **1962**, *66A*, 179-184.

[15] Bates, R. G., Guggenheim, E. A. Report on the standardization of pH and related terminology. *Pure Appl. Chem*., **1960**, *1*, 163-168.

[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] 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.

[18] Wu, Y. C., Feng, D., Koch, W. F. Evaluation of liquid junction potentials and determination of pH values of strong acids at moderate ionic strengths. *J. Solut. Chem*., **1989**, *18*, 641-649.

[19] Partanen, J. I., Minkkinen, P. O. Redetermination of the second dissociation constant of phosphoric acid and calculation of the pH values of the pH standards based on solutions of dihydrogen and hydrogen phosphate ions at 298.15 K. *Acta Chem. Scand*., **1996**, *50*, 1081-1086.

[20] Covington, A. K., Ferra, M. I. A. A Pitzer mixed electrolyte solution theory approach to assignment of pH to standard buffer solutions. *J. Solution Chem*., **1994**, *23*, 1-10.

[21] Pitzer, K. S., Mayorga, G. J. Thermodynamics of electrolytes. II. Activity and osmotic coefficients for strong electrolytes with one or both ions univalent. *J. Phys. Chem*., **1973**, *77*, 2300-2310.

Received: May 15, 2012 Revised: June 25, 2012 Accepted: June 26, 2012