Open Journal of Physical Chemistry, **2011, 1, 77-84**
doi:10.4236/ojpc.2011.13011 Published Online November 2011 (http://www.SciRP.org/journal/ojpc)

Calculation of the pH of Buffer Solution of 2-[*N*-Morpholino]ethanesulfonic Acid (MES) from 5˚C to 55˚C

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Received June 22, 2011; *revised August* 7, 2011; *accepted September* 15, 2011

Abstract

This paper reports the results for the pH of three buffer solutions free of chloride ion. The remaining six buffer solutions have saline media of the ionic strength I = 0.16 mol·kg^{-1}, matching closely to that of the physiological sample. Conventional pa_H values for the three buffer solutions without the chloride ion and six buffer solutions with the chloride ion at I = 0.16 mol·kg^{-1} from 5˚C to 55˚C have been calculated. The operational pH values for five buffer solutions at 5˚C and 55˚C have been determined based on the difference in the values of the liquid junction potentials between the blood phosphate standard and the experimental buffer solutions. Five of these buffers are recommended as standards for the physiological pH range 7.5 to 8.5.

Keywords: Buffers, MES, BICINE, Liquid Junction, Ionic Strength, Emf, pH

1. Introduction

The buffer substances recommended by Good *et al.* [1-3] have proven very useful for the measurement of the pH of blood and the control of pH in a region close to that of physiological solution. In biomedical, biological, and clinical laboratories, knowledge of the pH of blood and physiological fluids is of great importance. Previously, we have reported the pK_2 values of 2-[*N*-morpholino] ethanesulfonic acid (MES) [1] at temperatures from 5˚C to 55˚C, including 37˚C. This zwitterionic buffer system has been recommended by Good and coworkers [2,3] for use as a physiological buffer. The structure of MES is as follows:

Standardization for calibrating electrodes of the pH meter assembly at a point close to the pH of blood (that is, 7.407) can be obtained within the framework of the former National Bureau of Standards (NBS) by using a physiological phosphate pH buffer as a primary standard [4]. The phosphate buffer has been widely used as a physiological pH standard, but it is not an ideal primary pH standard buffer for physiological use at an ionic strength of I = 0.16 mol·kg^{-1}. The disadvantages are as follows: 1) phosphates act as inhibitors to enzymatic processes; 2) phosphate precipitates occur with some polyvalent cations, such as Mg^{2+} and Ca^{2+}, present in the blood; and 3) the temperature coefficient of the phosphate buffer is –0.0028 pH unit/K as compared to that of whole blood (–0.015 pH unit/K) and plasma (0.01 pH unit/K) [5].

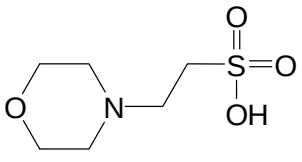

Figure 1. 2-[*N*-morpholino]ethanesulfonic acid (MES).

Good and his associates [2,3] provided 25 primarily new biological buffers which are mostly compatible with common physiological media. They outlined suitable criteria for the evaluation of these materials. Roy *et al.* [6] have published the pK_2 and pH values of the biological buffer [bis(2-hydroxyethyl)amino]acetic acid (BICINE), and the values of pH for the zwitterionic buffer *N*-[tris (hydroxymethyl)methyl-3-amino]propanesulfonic acid (T-APS) [7]. Both of these buffers have been recommended as pH standards in the range of physiological application. Feng and coworkers [8] have published the values of pK_2 and pH of the zwitterionic buffer *N*-(2-hydroxyethyl) piperazine-*N*-2-ethanesulfonic acid (HEPES). The HEPES buffer has been certified by the National Institute of

Standards and Technology (NIST) as a primary reference standard [8]. The values of pK_2 and pH for 3-(*N*-morpholino)propanesulfonic acid (MOPS) [9] and 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid (MOPSO) [10] have been reported. The pH of these solutions closely matches that of the common clinical media. In 1973, Bates *et al*. [11] recommended pH standard for a buffer solution of 0.06 *m* TRICINE + 0.02 *m* Na-TRICINEate. Goldberg *et al*. [12] reported the results of the thermodynamic quantities of about 68 physiological buffers. The comprehensive review article indicated that no results of pH are available in the literature for MES.

We now propose to investigate MES in order to provide very accurate and reproducible pH values in the range of physiological application.

2. Materials and Methodology

MES was obtained from Research Organics (Cleveland, OH). The details of the purification by further crystallization as well as the determination of the assay have been reported in an earlier paper [1]. In the present study, the analyses of the unpurified and purified MES were 99.71% and 99.88% pure, respectively. All mass measurements were made with a mass factor uncertainty of 0.02% including the substance MES, NaCl (ACS reagent grade dried at 110˚C), a standard solution of NaOH to prepare NaMES, and finally calculated amounts of CO_2-free doubly distilled water. Air buoyancy corrections were applied for all masses used.

The following buffer compositions on the molality scale are given:

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

(b) MES (0.04 mol·kg^{-1}) + NaMES (0.08 mol·kg^{-1}), I = 0.08 mol·kg^{-1}

(c) MES (0.08 mol·kg^{-1}) + NaMES (0.08 mol·kg^{-1}), I = 0.08 mol·kg^{-1}

(d) MES (0.04 mol·kg^{-1}) + NaMES (0.04 mol·kg^{-1}) + NaCl (0.12 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

(e) MES (0.05 mol·kg^{-1}) + NaMES (0.05 mol·kg^{-1}) + NaCl (0.11 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

(f) MES (0.06 mol·kg^{-1}) + NaMES (0.06 mol·kg^{-1}) + NaCl (0.10 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

(g) MES (0.08 mol·kg^{-1}) + NaMES (0.08 mol·kg^{-1}) + NaCl (0.08 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

(h) MES (0.04 mol·kg^{-1}) + NaMES (0.08 mol·kg^{-1}) + NaCl (0.08 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

(i) MES (0.03 mol·kg^{-1}) + NaMES (0.06 mol·kg^{-1}) + NaCl (0.10 mol·kg^{-1}), I = 0.16 mol·kg^{-1}

The preparation of the hydrogen electrodes and the silver-silver chloride electrodes of the thermal electrolytic type [13], the design of the all-glass cells, the purification of the hydrogen gas, preparation of the solutions, control of temperature, and use of digital voltmeter have been reported previously [1,9]. A correction for the residual liquid-junction potential is required if accurate pH values are to be achieved. Thus the cells studied were the following:

$$\text{Pt(s), H}_2\text{(g, 1 atm)} \,|\, \text{MES}\,(m_1) + \text{NaMES}\,(m_2) + \text{NaCl}\,(m_3) \,|\, \text{AgCl(s), Ag(s)} \quad \text{(A)}$$

where m_1, m_2 and m_3 indicate molalities of the respective species, and the pressure of hydrogen in SI units is 1 atm = 101.325 kPa. 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 of the calomel electrode shown with a double vertical line.

$$\text{Pt(s), H}_2\text{(g, 1 atm)} \,|\, \text{MES}(m_1) + \text{NaMES}(m_2) + \text{NaCl}(m_3) \,||\, \text{KCl(satd)} \,|\, \text{Hg}_2\text{Cl}_2\text{(s), Hg(l)} \quad \text{(B)}$$

where the abbreviations (s), (l) and (g) denote solid, liquid, and gaseous state, respectively.

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.

$$\text{Pt(s), H}_2\text{ (g, 1 atm)} \,|\, \text{phosphate buffer} \,||\, \text{KCl(satd)} \,|\, \text{Hg}_2\text{Cl}_2\text{(s), Hg(l)} \quad \text{(C)}$$

The values of the liquid junction potential, E_j, for the physiological phosphate solutions and other experimental buffer solutions of MES from cell (B) were obtained [8, 9] using the following equation [9]:

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

where E is the emf value in volt dependent on the buffer compositions, E°_{SCE} = –0.2415 V, k = 0.059156 V, and pH = 7.415 (physiological phosphate buffer solution) at 25˚C; E°_{SCE} = –0.2335, k = 0.061538 V, and pH = 7.395 at 37˚C. We have attempted to calculate values of the liquid junction potential for five buffer solutions out of nine buffer solutions. The difference in E_j between the phosphate standard and each experimental buffer solution is an important factor when different standards are selected to obtain the values of the operational pH for an unknown medium. This error can be estimated by the operational definition of pH, indicated as pH(x):

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

where E_x is the emf value of the unknown buffer MES + NaMESate; E_s is the emf of the reference solution (NIST physiological phosphate buffer) of known pH and δE_j =

$E_{j(s)} - E_{j(x)}$. If $\delta E_j = 0$, then Equation (2) takes the form:

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

3. Results

The cell potential data for cell (A) containing three buffer solutions without the presence of the chloride ion, and six buffer solutions in which NaCl has 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 potential at 25˚C are the average of two or three readings (at the beginning, in the middle, and sometimes at the end of the temperature run). Duplicate cells usually gave readings on the average within (0.02 ± 0.01) mV in the temperature range 5˚C to 55˚C. All these results are listed in **Tables 1** and **2**, respectively.

Conventional pa_H values have been evaluated by the method of Bates *et al.* [11,14-16] for three buffer solutions without NaCl and six buffer solutions in the presence of NaCl. The complete buffer compositions (a)-(i) are listed in the introduction section.

In order to calculate pa_H values for three buffer solutions without NaCl, calculations of the values of the acidity function $p(a_H\gamma_{Cl})^\circ$ in the absence of Cl^-, and $p(a_H\gamma_{Cl})$ for the six buffer solutions in the presence of Cl^- were made in the temperature range 5˚C to 55˚C, 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 are listed at the bottom of **Table 1.** The expression for the acidity function [11,13] is given by:

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

where k is the Nernst slope.

Values of the acidity function $p(a_H\gamma_{Cl})$ were derived at each temperature for each buffer solution and were plotted as a function of m_{Cl^-}, straight lines of small slopes were obtained. The values of the intercepts, $p(a_H\gamma_{Cl})^\circ$, for three buffer solutions without the presence of NaCl listed above from (a) to (c), were calculated using Equation (5) and are given in **Table 3**. The acidity function, $p(a_H\gamma_{Cl})$ for six buffers (d)-(i) listed above are entered in **Table 4** from 5˚C to 55˚C. The uncertainty (mean deviation) introduced in this type of graphical extrapolation is usually less than 0.002 from the lines drawn. Conventional pa_H values for the three solutions without the presence of the chloride ion were calculated by the following expression:

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

where the single-ion activity coefficient, γ°_{Cl}, cannot be measured experimentally. A non-thermodynamic convention [4,9] for the estimation of γ°_{Cl} has been adopted for the calculation of pa_H by Equation (5). The pH values ob-

Table 1. Electromotive force of cell A: Pt(s); H_2(g, 1 atm)|MES(m_1), NaMES(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
0.04	0.04	0.005	0.71532	0.71843	0.72137	0.72413	0.72667	0.72931	0.73170	0.73266	0.73392	0.73589	0.73790	0.73968
0.04	0.04	0.010	0.69926	0.70209	0.70443	0.70698	0.70929	0.71186	0.71395	0.71477	0.71592	0.71764	0.71925	0.72075
0.04	0.04	0.015	0.68993	0.69260	0.69456	0.69696	0.69911	0.70177	0.70366	0.70442	0.70549	0.70708	0.70848	0.70981
0.04	0.04	0.020	0.68340	0.68593	0.68754	0.68981	0.69200	0.69468	0.69647	0.69717	0.69820	0.69973	0.70091	0.70208
0.04	0.08	0.005	0.73248	0.73596	0.73927	0.74225	0.74521	0.74798	0.75081	0.75171	0.75333	0.75569	0.75806	0.76029
0.04	0.08	0.010	0.71658	0.71974	0.72271	0.72543	0.72804	0.73065	0.73313	0.73396	0.73535	0.73746	0.73952	0.74140
0.04	0.08	0.015	0.70747	0.71041	0.71308	0.71558	0.71796	0.72069	0.72290	0.72365	0.72498	0.72682	0.72873	0.73034
0.04	0.08	0.020	0.70106	0.70385	0.70647	0.70891	0.71110	0.71376	0.71602	0.71663	0.71779	0.71965	0.72135	0.72289
0.08	0.08	0.005	0.72001	0.72301	0.72585	0.72866	0.73149	0.73401	0.73632	0.73728	0.73860	0.74062	0.74282	0.74472
0.08	0.08	0.010	0.70308	0.70584	0.70833	0.71090	0.71343	0.71562	0.71770	0.71856	0.71968	0.72140	0.72327	0.72487
0.08	0.08	0.015	0.69295	0.69555	0.69791	0.70023	0.70262	0.70471	0.70658	0.70740	0.70840	0.70998	0.71165	0.71303
0.08	0.08	0.020	0.68552	0.68808	0.69026	0.69252	0.69474	0.69671	0.69849	0.69929	0.70017	0.70168	0.70317	0.70443
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)|MES(m_1), NaMES(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
0.04	0.04	0.12	0.64471	0.64654	0.64819	0.64973	0.65107	0.65228	0.65337	0.65358	0.65401	0.65462	0.65509	0.65578
0.05	0.05	0.11	0.64639	0.64820	0.64991	0.65142	0.65285	0.65412	0.65526	0.65546	0.65592	0.65659	0.65707	0.65780
0.06	0.06	0.10	0.64805	0.64991	0.65160	0.65317	0.65465	0.65571	0.65680	0.65717	0.65768	0.65847	0.65911	0.65973
0.08	0.08	0.08	0.65259	0.65443	0.65626	0.65779	0.65941	0.66068	0.66189	0.66229	0.66283	0.66375	0.66443	0.66515
0.04	0.08	0.08	0.66820	0.67035	0.67243	0.67429	0.67588	0.67768	0.67918	0.67974	0.68048	0.68175	0.68290	0.68386
0.03	0.06	0.10	0.65992	0.66183	0.66371	0.66541	0.66717	0.66864	0.67005	0.67064	0.67127	0.67242	0.67335	0.67418

[a]Units of m, mol·kg^{-1}.

Table 3. p($a_H\gamma_{Cl}$)° of (MES + NaMES) buffer solutions from 5˚C to 55˚C, computed using Equation (5)[a].

t (˚C)	0.04 *m* MES + 0.04 *m* NaMES *I* = 0.08 *m*	0.04 *m* MES + 0.08 *m* NaMES *I* = 0.08 *m*	0.08 *m* MES + 0.08 *m* NaMES *I* = 0.08 *m*
5	6.411	6.718	6.511
10	6.360	6.669	6.456
15	6.315	6.622	6.403
20	6.267	6.573	6.355
25	6.218	6.529	6.311
30	6.173	6.481	6.265
35	6.131	6.440	6.220
37	6.115	6.422	6.203
40	6.089	6.400	6.178
45	6.047	6.359	6.136
50	6.010	6.323	6.100
55	5.971	6.286	6.062

[a]Units of *m*, mol·kg^{-1}.

Table 4. p($a_H\gamma_{Cl}$) of (MES + NaMES) buffer solutions from 5˚C to 55˚C, computed using Equation (4)[a].

t (˚C)	0.04 *m* MES + 0.04 *m* NaMES + 0.12 *m* NaCl *I* = 0.16 *m*	0.05 *m* MES + 0.05 *m* NaMES 0.11 *m* NaCl *I* = 0.16 *m*	0.06 *m* MES + 0.06 *m* NaMES + 0.10 *m* NaCl *I* = 0.16 *m*	0.08 *m* MES + 0.08 *m* NaMES + 0.08 *m* NaCl *I* = 0.16 *m*	0.04 *m* MES + 0.08 *m* NaMES + 0.08 *m* NaCl *I* = 0.16 *m*	0.03 *m* MES + 0.06 *m* NaMES + 0.10 *m* NaCl *I* = 0.16 *m*
5	6.518	6.511	6.499	6.485	6.768	6.715
10	6.467	6.459	6.448	6.432	6.715	6.660
15	6.418	6.410	6.398	6.383	6.666	6.610
20	6.371	6.362	6.351	6.333	6.617	6.561
25	6.325	6.317	6.306	6.290	6.568	6.518
30	6.280	6.273	6.258	6.244	6.527	6.473
35	6.237	6.230	6.214	6.200	6.483	6.431
37	6.218	6.210	6.197	6.183	6.467	6.416
40	6.191	6.184	6.171	6.157	6.441	6.389
45	6.148	6.141	6.130	6.116	6.402	6.351
50	6.106	6.099	6.089	6.076	6.364	6.312
55	6.070	6.063	6.051	6.037	6.325	6.273

[a]Units of *m*, mol·kg^{-1}.

Table 5. pa_H of (MES + NaMES) buffer solutions from 5˚C to 55˚C, computed using Equations (4)-(6)[a].

t (˚C)	0.04 *m* MES + 0.04 *m* NaMES *I* = 0.08 *m*	0.04 *m* MES + 0.08 *m* NaMES *I* = 0.08 *m*	0.08 *m* MES + 0.08 *m* NaMES *I* = 0.08 *m*
5	6.333	6.619	6.411
10	6.283	6.570	6.356
15	6.236	6.522	6.303
20	6.189	6.473	6.255
25	6.139	6.427	6.210
30	6.094	6.379	6.164
35	6.051	6.337	6.118
37	6.035	6.319	6.101
40	6.008	6.297	6.075
45	5.965	6.255	6.032
50	5.928	6.218	5.995
55	5.888	6.180	5.956

[a]Units of *m*, mol·kg^{-1}.

tained from the liquid junction cell are referred by the "operational" pH, whereas the "conventional" pH calculated from Equation (5) is designated as pa_H.

The convention is reasonable but is not subject to any proof. The Equation (6) of a "pH convention" [4], based on an extended Debye-Hückel equation, has been widely used. In the assignment of pa_H values and in the establishment of NIST pH standard [8,10,14-18], the calculation of $\log_{10}\gamma^{\circ}_{Cl}$ for all of the buffer-chloride solutions were made by using the following equation:

$$\log_{10}\gamma^{\circ}_{Cl} = -\frac{A\sqrt{I}}{1+Ba^{\circ}\sqrt{I}} + CI \qquad (6)$$

where I is the ionic strength of the buffer solution, A and

B are the Debye-Hückel constants [6-7,13], hydrolysis of the buffer species is negligible, C is an adjustable parameter, Ba° was taken to be 1.38 $kg^{½}·mol^{-½}$ at all temperatures [9]. In the Bates-Guggenheim convention [4], the value of $Ba°$ was assigned to be 1.5 $kg^{½}·mol^{-½}$ and C = 0 for ionic strength $I \le 0.1$ $mol·kg^{-1}$. The following equation is used for the calculation of the parameter C [8,9]:

$$C = C_{25} + 6.2\times10^{-4}(T-25) - 8.7\times10^{-6}(T-25)^2 \quad (7)$$

where C_{25} = 0.032 $mol·kg^{-1}$ at 25˚C and T is the absolute temperature [8].

The values of pa_H are listed in **Table 5** for three buffer solutions of MES without NaCl. These are calculated using Equations (4)-(7) and are expressed as a function of temperature.

For MES (0.04 $mol·kg^{-1}$) + NaMES (0.04 $mol·kg^{-1}$)

$$pa_H = 6.140 - 9.17\times10^{-3}(T-25) + 2.55\times10^{-5}(T-25)^2 \quad (8)$$

For MES (0.04 $mol·kg^{-1}$) + NaMES (0.08 $mol·kg^{-1}$)

$$pa_H = 6.426 - 9.11\times10^{-3}(T-25) + 3.04\times10^{-5}(T-25)^2 \quad (9)$$

For MES (0.08 $mol·kg^{-1}$) + NaMES (0.08 $mol·kg^{-1}$)

$$pa_H = 6.208 - 9.39\times10^{-3}(T-25) + 3.29\times10^{-5}(T-25)^2 \quad (10)$$

where 25˚C ≤ T ≤ 55˚C. The standard deviations of regression for the pa_H of the three chloride-free buffer solutions are 0.0014, 0.0013 and 0.0017, respectively.

For the six buffer solutions containing NaCl at an indicated ionic strength, I = 0.16 $mol·kg^{-1}$, the values of pa_H listed in **Table 6** are expressed by the equations:

For MES (0.04 $mol·kg^{-1}$) + NaMES (0.04 $mol·kg^{-1}$) + NaCl (0.12 $mol·kg^{-1}$)

$$pa_H = 6.198 - 9.30\times10^{-3}(T-25) + 1.89\times10^{-5}(T-25)^2 \quad (11)$$

For MES (0.05 $mol·kg^{-1}$) + NaMES (0.05 $mol·kg^{-1}$) + NaCl (0.11 $mol·kg^{-1}$)

$$pa_H = 6.191 - 9.28\times10^{-3}(T-25) + 1.93\times10^{-5}(T-25)^2 \quad (12)$$

For MES (0.06 $mol·kg^{-1}$) + NaMES (0.06 $mol·kg^{-1}$) + NaCl (0.10 $mol·kg^{-1}$)

$$pa_H = 6.178 - 9.33\times10^{-3}(T-25) + 2.39\times10^{-5}(T-25)^2 \quad (13)$$

For MES (0.08 $mol·kg^{-1}$) + NaMES (0.08 $mol·kg^{-1}$) + NaCl (0.08 $mol·kg^{-1}$)

$$pa_H = 6.162 - 9.28\times10^{-3}(T-25) + 2.46\times10^{-5}(T-25)^2 \quad (14)$$

For MES (0.04 $mol·kg^{-1}$) + NaMES (0.08 $mol·kg^{-1}$) + NaCl (0.08 $mol·kg^{-1}$)

$$pa_H = 6.444 - 9.24\times10^{-3}(T-25) + 3.06\times10^{-5}(T-25)^2 \quad (15)$$

For MES (0.03 $mol·kg^{-1}$) + NaMES (0.06 $mol·kg^{-1}$) + NaCl (0.10 $mol·kg^{-1}$)

$$pa_H = 6.391 - 9.17\times10^{-3}(T-25) + 3.04\times10^{-5}(T-25)^2 \quad (16)$$

where T is the temperature in ˚C. The standard deviations for regression of the "observed" results from Equations (11) to (16) are 0.0014, 0.0015, 0.0009, 0.0009, 0.0012 and 0.0015, respectively.

4. Discussion

The MES is a zwitterionic buffer material. It is like a neutral molecule and hence makes no contribution to the ionic strength. The values of K_2 of MES lie between 10^{-6} and 10^{-8} and hence are useful in the preparation of buffer solutions for pH control in the physiological interest. Similar recommendations were made for two other buffer systems, HEPES [19] and HEPPS [20], which are useful for pH measurements in the clinical laboratory.

The cell potential data of the cells (B) and (C) at 25˚C and 37˚C are given in **Table 7**. By means of the flowing

Table 6. pa_H of (MES + NaMES) buffer solutions from 5˚C to 55˚C, computed using Equations (4)-(6)[a].

t (˚C)	0.04 *m* MES + 0.04 *m* NaMES + 0.12 *m* NaCl *I* = 0.16 *m*	0.05 *m* MES + 0.05 *m* NaMES + 0.11 *m* NaCl *I* = 0.16 *m*	0.06 *m* MES + 0.06 *m* NaMES + 0.10 *m* NaCl *I* = 0.16 *m*	0.08 *m* MES + 0.08 *m* NaMES + 0.08 *m* NaCl *I* = 0.16 *m*	0.04 *m* MES + 0.08 *m* NaMES + 0.08 *m* NaCl *I* = 0.16 *m*	0.03 *m* MES + 0.06 *m* NaMES + 0.10 *m* NaCl *I* = 0.16 *m*
5	6.393	6.385	6.374	6.359	6.642	6.589
10	6.342	6.333	6.323	6.306	6.590	6.535
15	6.292	6.284	6.272	6.257	6.540	6.484
20	6.246	6.237	6.226	6.208	6.492	6.436
25	6.198	6.191	6.180	6.163	6.442	6.391
30	6.153	6.146	6.131	6.117	6.400	6.346
35	6.109	6.103	6.086	6.073	6.356	6.303
37	6.090	6.083	6.069	6.055	6.339	6.286
40	6.062	6.055	6.042	6.028	6.312	6.261
45	6.019	6.012	6.000	5.987	6.272	6.221
50	5.976	5.969	5.959	5.945	6.233	6.181
55	5.938	5.931	5.920	5.906	6.193	6.142

[a]Units of m, $mol·kg^{-1}$.

Table 7. Emf of cell B for MES buffer.

m_1	m_2	m_3	E/V	
			25˚C	37˚C
0.04	0.08	0.00	0.62387	0.62472
0.03	0.06	0.10	0.62004	0.62088
0.04	0.04	0.12	0.60851	0.60883
0.04	0.08	0.08	0.62306	0.62415
0.08	0.08	0.08	0.60655	0.60679
Emf of Cell C[a]				
Cell C			E/V	
			25˚C	37˚C
0.008695 *m* KH_2PO_4 + 0.03043 *m* Na_2HPO_4			0.68275	0.69147

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

junction cell, the values of E_j listed in **Table 8** were estimated by using Equation (1). As evident from the pH data at 25˚C and 37˚C from **Table 9**, there is a wide variation in pH (as high as ±0.04 pH units). There is no known experimental method for accurately determining the single-ion activity coefficient, $\log_{10}\gamma_{Cl}^{\circ}$. Partanen and Minkkinen [19], as well as Covington and Ferra [20], used the Pitzer theory approach for the estimation of the single ion activity coefficient at ionic strengths higher than 0.1 mol·kg^{-1} in the calculation of the pH standards of the phosphate buffer solutions. In separate publications from this laboratory, the pa_H values of eight different buffer solutions will be reported by using Pitzer formalism for an ionic strength I = 0.16 mol·kg^{-1} at 25˚C and 37˚C. The calculation of $\log_{10}\gamma_{Cl}$ leads to uncertainty (±0.001 pH unit) in the pa_H values. A second source is the error in the liquid junction potential measurement. However, the calculated pH values and the values obtained from the E_j corrections are in very good agreement (within ±0.003). The total uncertainties were estimated by combining the various sources of error: 1) assumption for the calculation of the $\log_{10}\gamma_{Cl}^{\circ}$ (±0.004 pH unit); 2) extrapolation to $p(a_H\gamma_{Cl})^{\circ}$ at m_{Cl^-} = 0 (within ±0.001 pH unit); 3) liquid junction potential measurement using

Table 8. Values of the liquid junction potentials for MES buffer at 25˚C 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
0.04 *m* MES + 0.08 *m* NaMES + 0.00 *m* NaCl	2.2	2.4
0.03 *m* MES + 0.06 *m* NaMES + 0.10 *m* NaCl	0.5	0.6
0.04 *m* MES + 0.04 *m* NaMES + 0.12 *m* NaCl	0.4	0.6
0.04 *m* MES + 0.08 *m* NaMES + 0.08 *m* NaCl	0.5	0.6
0.08 *m* MES + 0.08 *m* NaMES + 0.08 *m* NaCl	0.5	0.7

[a]$E_j = E + E_{SCE}^{\circ} - k^{\circ}$ pH from Equation (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; 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·kg^{-1}.

Table 9. Values of pH at 25˚C and 37˚C for MES buffer solutions.

Cell B			Ionic	25˚C			37˚C		
m_1	m_2	m_3	Strength, 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]
0.04	0.08	0.00	0.08	6.420	6.426	6.427	6.310	6.318	6.319
0.03	0.06	0.10	0.16	6.355	6.390	6.391	6.248	6.285	6.286
0.04	0.04	0.12	0.16	6.160	6.197	6.198	6.052	6.089	6.090
0.04	0.08	0.08	0.16	6.406	6.441	6.442	6.301	6.338	6.339
0.08	0.08	0.08	0.16	6.127	6.162	6.163	6.019	5.054	6.055

[a]Values obtained from Equation (3) and data of Table 7; [b]Obtained from Equation (2) and E_j data in Table 8; [c]Obtained from Tables 5 and 6.

the flowing junction cell; 4) error in the experimental emf measurement (±0.02 mV); and 5) standard potential of the Ag-AgCl electrode (±0.03 mV). The overall uncertainty is about ±0.009 pH unit.

5. Conclusions

All emf data are stable, reliable, and accurate. The MES buffer solutions are considered as standards of assigned pa_H and will be useful when buffer solutions of known conventional pa_H are required. From Table 9, uncertainty in pH values obtained with and without liquid junction is ±0.001 pH. Thus the operational pH values at 25˚C and 37˚C (Table 9) for one buffer solution with NaCl and four buffer solutions matching the ionic strength of blood serum are recommended as secondary pH standards for the measurement of the pH of blood and other physiological fluids.

6. Acknowledgements

The authors are grateful for the 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 David Jones and Morghan Olson for their dedication 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.

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