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Buffer Standards for pH Measurement of *N*-(2-Hydroxyethyl) piperazine-*N'*-2-ethanesulfonic Acid (HEPES) for $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$ from 5 to 55°C

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Abstract

The values of the second dissociation constant, pK_2 of *N*-(2-hydroxyethyl) piperazine-*N'*-2-ethanesulfonic acid (HEPES) have been reported at 12 temperatures over the temperature range 5 to 55°C, including 37°C. This paper reports the results for the p_{aH} of eight isotonic saline buffer solutions with an $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$ including compositions: (a) HEPES ($0.01 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.01 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.15 \text{ mol}\cdot\text{kg}^{-1}$); (b) HEPES ($0.02 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.02 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.14 \text{ mol}\cdot\text{kg}^{-1}$); (c) HEPES ($0.03 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.03 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.13 \text{ mol}\cdot\text{kg}^{-1}$); (d) HEPES ($0.04 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.04 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.12 \text{ mol}\cdot\text{kg}^{-1}$); (e) HEPES ($0.05 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.05 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.11 \text{ mol}\cdot\text{kg}^{-1}$); (f) HEPES ($0.06 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.06 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.10 \text{ mol}\cdot\text{kg}^{-1}$); (g) HEPES ($0.07 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.07 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.09 \text{ mol}\cdot\text{kg}^{-1}$); and (h) HEPES ($0.08 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.08 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.08 \text{ mol}\cdot\text{kg}^{-1}$). Conventional p_{aH} values, for all eight buffer solutions from 5 to 55°C have been calculated. The operational pH values with liquid junction corrections, at 25 and 37°C have been determined based on the NBS/NIST standard between the physiological phosphate standard and four buffer solutions. These are recommended as pH standards for physiological fluids in the range of pH 7.3 to 7.5 at $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$.

Keywords

Buffers; HEPES; Ionic strength; Emf; Zwitterions; pH; Acidity function

1 Introduction

An isotonic strength at $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$ was chosen for all eight buffer solutions in order to maintain the isotonic strength consistency with the NBS/ NIST physiological pH standards. For measurements of the pH of blood and biological specimens, it is necessary to know accurate values of pK_2 since appropriate buffer systems include that of HEPES. The values of pK_2 and pH are interdependent. Very recently, Feng et al. [1] reported the values of *N*-(2-hydroxyethyl) piperazine-*N'*-2-ethanesulfonic acid (HEPES) at temperatures from 5 to 55°C including 37°C. This zwitterionic buffer system has been recommended by Good and coworkers [2,3] for use as a buffer of biochemical importance. The structure is as follows:

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***N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid HEPES**

Measurements of pH of clinical media at a point close to the pH of blood (that is, 7.3 to 7.5) can be obtained within the framework of the former NBS/NIST by using physiological phosphate pH buffer as a primary standard [4]. The pH of this physiological phosphate buffer standard is 7.415 at 25°C and 7.395 at 37°C, and has been widely accepted and used as an integral part of clinical diagnosis.

Nevertheless, there are some difficulties regarding the use of the phosphate buffer for reasons as outlined: (i) phosphates interact with biological media and precipitate with Mg^{+2} and Ca^{+2} present in blood, and (ii) the temperature coefficient of the phosphate buffer is -0.0028 pH unit/°C compared to that of whole blood (-0.015 pH unit/°C) [5].

Good and his associates [2,3] provided about 25 primarily new hydrogen ion buffers which are mostly compatible with common biological media. Roy et al. [6] recently investigated the zwitterionic compound, AMPSO. Wu and coworkers [7] have published the values of $\text{p}K_2$ and pH of the zwitterionic buffer 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid (MOPSO). The MOPSO buffers have been certified by the National Institute of Standards and Technology (NIST) as primary reference standard. Roy et al. [8] published results for $\text{p}K_2$ and pH for 3-(*N*-morpholino)propanesulfonic acid (MOPS); 4-*N*-(morpholino)butanesulfonic acid (MOBS) [9]; and Bis-[(2-hydroxyethyl)amino]acetic acid (BICINE) [10]. The pH of these solutions nearly matches that of the common biological media. For the physiological range of pH 7.2 to 8.5, Bates et al. [11] suggested the use of tris(hydroxymethyl)methylglycine (TRICINE) as a buffer standard. For 0.06 m TRICINE + 0.02 m sodium TRICINEate buffer solution, as the pH at 37°C is 7.407, which matches the pH of blood. Goldberg et al. [12], in their recent review article, compiled the thermodynamic quantities of important biological buffers.

In order to provide reproducible pH values for physiological pH standards, we have investigated the aqueous buffer system HEPES + NaHEPES + NaCl at $I = 0.16$ mol•kg⁻¹ and the eight compositions are given in the abstract.

The detailed procedure for the preparation of these buffer solutions for HEPES is described in the experimental section.

2 Experimental

HEPES was obtained from Sigma Chemical Co. The assay showed that the HEPES was (99.94 ± 0.03) % pure when the HEPES solution was titrated under carbon dioxide-free conditions (nitrogen bubbling) with a standard solution of NaOH to a theoretical equivalent point of pH. All buffer solutions were prepared by weighing the buffer substance HEPES, NaCl (ACS reagent grade), a standard solution of NaOH to prepare NaHEPES, and finally calculated

amounts of CO₂-free doubly distilled water. Air buoyancy corrections were applied for all masses used.

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, and preparation of the solutions have been described previously [8,10]. Details about the control of temperature (within $\pm 0.005^\circ\text{C}$) using a digital platinum resistance thermometer (Guildline Model 9540), a digital voltmeter (Hewlett-Packard 2000 multimeter), and other experimental procedures, were published [8,10].

A correction for the liquid-junction potential is required if accurate pH values are to be achieved. The cells studied were the following:

where m_1 , m_2 , and m_3 indicate molalities of the respective species, and 1 atm = 101.325 kPa in SI units. The 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 more dense saturated KCl solution shown with a double vertical line.

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 (C), the phosphate salts were NIST standard reference materials with the composition $[\text{KH}_2\text{PO}_4 (0.008695 \text{ mol}\cdot\text{kg}^{-1}) + \text{Na}_2\text{HPO}_4 (0.03043 \text{ mol}\cdot\text{kg}^{-1})]$ and its solutions are widely used for pH measurements in physiological solutions.

The values of the standard electrode potential, E_{SCE}° , of the saturated calomel electrode were taken as: -0.2415 V , and -0.2335 V at 25 and 37°C , respectively [13–15]. The values of the liquid junction potential, E_j , for the physiological phosphate solutions and other buffer solutions of HEPES from cell (B) were obtained [1,7–8] using the following equation [8]:

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

where $k = 0.059156$ obtained from the equation $k = \frac{RT_\kappa \ln_{10}}{F}$, as $F = 96,487 \frac{\text{C}}{\text{mol}}$ and $R = 8.31433 \frac{\text{J}}{\text{mol}\cdot\text{K}}$ and $\text{pH} = 7.415$ (physiological phosphate buffer solution) at 25°C ; $k = 0.061538$ and $\text{pH} = 7.395$ at 37°C . We have attempted to calculate a value of the liquid junction potential for each buffer solution. The difference in E_j between the accepted phosphate standard and the buffer solution is important when different standards are selected to obtain the values of the operational pH for an unknown medium.

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

where x refers to the unknown buffer HEPES + NaHEPES; s is the reference solution (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:

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

3 Methods and Results

Accurate values of the second dissociation constants, pK_2 , and related thermodynamic quantities have been reported by Feng et al. [1]. The emf data for cell (A) containing eight buffer solutions at $I = 0.16 \text{ mol} \cdot \text{kg}^{-1}$, have been corrected to a hydrogen pressure of 1 atm. The values of the emf at 25°C are the average of at least two readings (at the beginning and the middle). The silver-silver chloride electrodes were used throughout the experiments in order to determine pH values of buffer solutions. Duplicate cells usually gave readings on the average within $0.02 \pm 0.01 \text{ mV}$ in the temperature range 5–55°C. All these results are listed in Table 1, except at 25°C where average values are reported.

3.1 The pH of HEPES Buffer

The conventional pH values have been evaluated by the method of Bates et al. [4,11] for eight standard buffer solutions, the compositions of which are mentioned in the abstract. In order to calculate $\text{pH}(s)$ values for all eight buffer solutions, calculations of the values of the acidity function $p(a_H\gamma_{Cl})$ were made in the temperature range 5 to 55°C, from the emf (E) listed in Table 1, the molality of the chloride ion, and E° , the standard potential of the silver-silver chloride electrode. The commonly used equation [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.

The acidity function, $p(a_H\gamma_{Cl})$ for eight buffers (a) – (h) listed above are entered in Table 2 and Table 3 from 5 to 55°C, respectively. The Conventional pH values determined from the emf of cells with liquid junction for the solution without the presence of the chloride ion were determined by the equation:

$$p a_H = p(a_H\gamma_{Cl}) + \log_{10} \gamma_{Cl}, \quad (5)$$

where the single-ion activity coefficient, γ_{Cl} , cannot be measured experimentally. The estimation of γ_{Cl} for the calculation of pH by Eq. (5) has been outlined before [8]. 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 .

The convention is not subject to any proof and the calculation of $\log_{10} \gamma_{Cl}$ needed in Eq. (5) was based on an extended Debye-Hückel equation in Eq. (6). In the assignment of pH values

for the NIST standard [1,7,15–19], Eq. (6) has been used. The calculation of $\log_{10} \gamma_{Cl}$ for all of the buffer-chloride solutions were made by using the following equation:

$$\log_{10} \gamma_{Cl} = -\frac{A\sqrt{I}}{1+Ba^{\circ}\sqrt{I}} + CI, \quad (6)$$

where I is the ionic strength of the buffer solution, A and B are the Debye-Hückel constants, hydrolysis of the buffer species is negligible, C is an adjustable parameter, Ba° was assumed to be $1.38 [8] \text{ kg}^{1/2}\cdot\text{mol}^{-1/2}$ for all the experimental temperatures, corresponding to an (ion-size parameter) a° of $4.2 \text{ \AA}$ [8]. The empirical equation for the calculation of the parameter C [1, 8] is given below:

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

where $C_{25} = 0.032 [6] \text{ kg}\cdot\text{mol}^{-1}$ at 25°C and t is the Celsius temperature.

For eight buffer solutions containing NaCl at an isotonic saline media of the ionic strength $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$, the values of $\text{p}a_{\text{H}}$ listed in Tables 4 are expressed by the equations:

For HEPES ($0.01 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.01 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.15 \text{ mol}\cdot\text{kg}^{-1}$):

$$\text{p}a_{\text{H}} = 7.5167 - 1.2653 \times 10^{-2}(t - 25) + 2.00 \times 10^{-5}(t - 25)^2 \quad (8)$$

For HEPES ($0.02 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.02 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.14 \text{ mol}\cdot\text{kg}^{-1}$):

$$\text{p}a_{\text{H}} = 7.478 - 1.2467 \times 10^{-2}(t - 25) + 2.70 \times 10^{-5}(t - 25)^2 \quad (9)$$

For HEPES ($0.03 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.03 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.13 \text{ mol}\cdot\text{kg}^{-1}$):

$$\text{p}a_{\text{H}} = 7.502 - 1.2442 \times 10^{-2}(t - 25) + 2.44 \times 10^{-5}(t - 25)^2 \quad (10)$$

For HEPES ($0.04 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.04 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.12 \text{ mol}\cdot\text{kg}^{-1}$):

$$\text{p}a_{\text{H}} = 7.517 - 1.2526 \times 10^{-2}(t - 25) + 2.30 \times 10^{-5}(t - 25)^2 \quad (11)$$

For HEPES ($0.05 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.05 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.11 \text{ mol}\cdot\text{kg}^{-1}$):

$$\text{p}a_{\text{H}} = 7.515 - 1.2486 \times 10^{-2}(t - 25) + 2.83 \times 10^{-5}(t - 25)^2 \quad (12)$$

For HEPES ($0.06 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.06 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.10 \text{ mol}\cdot\text{kg}^{-1}$):

$$\text{p}a_{\text{H}} = 7.522 - 1.3194 \times 10^{-2}(t - 25) + 4.18 \times 10^{-5}(t - 25)^2 \quad (13)$$

For HEPES ($0.07 \text{ mol}\cdot\text{kg}^{-1}$) + NaHEPES ($0.07 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.09 \text{ mol}\cdot\text{kg}^{-1}$):

$$\text{p}a_{\text{H}} = 7.508 - 1.267 \times 10^{-2}(t - 25) + 2.78 \times 10^{-5}(t - 25)^2 \quad (14)$$

For HEPES (0.08 mol•kg⁻¹) + NaHEPES (0.08 mol•kg⁻¹) + NaCl (0.08 mol•kg⁻¹):

$$p_{a_H} = 7.507 - 1.2464 \times 10^{-2}(t - 25) + 2.51 \times 10^{-5}(t - 25)^2 \quad (15)$$

where t is the temperature in °C. The standard deviations for regression of the “observed” results from Eqs. (8)–Eqs. (15) are 0.0005, 0.0002, 0.0004, 0.0007, 0.0003, 0.0005, 0.0006, and 0.0004, respectively.

The pH values at 25 and 37°C were evaluated from cells with liquid junctions (cells B and C) by means of the flowing junction cell. The emf values of the cells (B) and (C) at 25 and 37°C are given in Table 5. Also, the values of E_j from Table 6 were obtained by using Eqs. (1) and Eqs. (2). It is clear from the data of Table 7, the pH values vary as much as ± 0.04 pH units. There is no known method for accurately determining the single-ion activity coefficient, $\log_{10} \gamma_{Cl}$, which is the major source of error. The liquid junction potential measurement is another source of uncertainty. However, the agreement is excellent (within ± 0.001) between the calculated p_{a_H} values and the values obtained from the residual liquid junction correction. The total uncertainty for the pH values was evaluated by combining the various uncertainties due to the: (i) assumption for the calculation of the $\log_{10} \gamma_{Cl}$ (± 0.003 pH unit), (ii) liquid junction potential measurement using the flow junction cell, and (iii) error in the experimental emf measurement (± 0.02 mV). Thus the overall estimated uncertainty is ± 0.013 pH units. The discrepancy between the conventional p_{a_H} values from cell (A) with those obtained from operational pH values using cell (B) is nearly removed by matching the ionic strength of the standard and buffer solutions under study. The operational pH values at 25 and 37°C (Table 7) for four buffer solutions are recommended as primary pH standards for isotonic saline media of $I = 0.16$ mol•kg⁻¹.

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Table 1

Electromotive force of the Cell A (in volts): Pt(s); H₂ (g, 1 atm) | HEPES (*m*₁), NaHEPES (*m*₂), NaCl (*m*₃) | AgCl(s), Ag(s)

<i>m</i> ₁ ^a	<i>m</i> ₂ ^a	<i>m</i> 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
0.01	0.01	0.15	0.71576	0.71800	0.72004	0.72188	0.72327	0.72467	0.72588	0.72624	0.72676	0.72762	0.72818	0.72876
0.02	0.02	0.14	0.71525	0.71748	0.71943	0.72118	0.72279	0.72422	0.72546	0.72592	0.72654	0.72751	0.72824	0.72887
0.03	0.03	0.13	0.71831	0.72055	0.72261	0.72440	0.72609	0.72757	0.72892	0.72942	0.73008	0.73104	0.73164	0.73246
0.04	0.04	0.12	0.72120	0.72343	0.72546	0.72720	0.72908	0.73065	0.73200	0.73242	0.73307	0.73410	0.73467	0.73545
0.05	0.05	0.11	0.72311	0.72546	0.72762	0.72939	0.73115	0.73272	0.73413	0.73460	0.73538	0.73647	0.73737	0.73811
0.06	0.06	0.10	0.72680	0.72911	0.73078	0.73248	0.73406	0.73545	0.73674	0.73721	0.73787	0.73898	0.73991	0.74070
0.07	0.07	0.09	0.72779	0.73008	0.73226	0.73413	0.73596	0.73761	0.73898	0.73954	0.73998	0.74133	0.74212	0.74307
0.08	0.08	0.08	0.73032	0.73264	0.73499	0.73695	0.73892	0.74062	0.74210	0.74266	0.74344	0.74463	0.74569	0.74643

^aUnits of *m*, mol•kg^{−1}

Table 2

$p(a_{\text{H}^+}\gamma_{\text{Cl}^-})$ of (HEPES + NaHEPES) buffer solutions from 5 to 55°C, computed using Eq.(4)^a

$t(^{\circ}\text{C})$	0.01 <i>m</i> HEPES + 0.01 <i>m</i> NaHEPES + 0.15 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.02 <i>m</i> HEPES + 0.02 <i>m</i> NaHEPES + 0.14 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.03 <i>m</i> HEPES + 0.03 <i>m</i> NaHEPES + 0.13 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.04 <i>m</i> HEPES + 0.04 <i>m</i> NaHEPES + 0.12 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>
5	7.903	7.863	7.887	7.904
10	7.836	7.797	7.819	7.836
15	7.771	7.731	7.754	7.769
20	7.708	7.666	7.689	7.703
25	7.642	7.604	7.628	7.643
30	7.581	7.543	7.567	7.583
35	7.520	7.483	7.508	7.523
37	7.495	7.460	7.485	7.499
40	7.459	7.425	7.450	7.463
45	7.401	7.370	7.393	7.407
50	7.343	7.314	7.335	7.347
55	7.287	7.259	7.282	7.293

^aUnits of *m*, mol·kg⁻¹

Table 3

$p(a_{\text{H}}\gamma_{\text{Cl}})$ of (HEPES + NaHEPES) buffer solutions from 5 to 55°C, computed using Eq. (4)^a

$t(^{\circ}\text{C})$	0.05 <i>m</i> HEPES + 0.05 <i>m</i> NaHEPES + 0.11 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.06 <i>m</i> HEPES + 0.06 <i>m</i> NaHEPES + 0.10 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.07 <i>m</i> HEPES + 0.07 <i>m</i> NaHEPES + 0.09 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.08 <i>m</i> HEPES + 0.08 <i>m</i> NaHEPES + 0.08 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>
5	7.901	7.926	7.899	7.893
10	7.834	7.858	7.829	7.824
15	7.769	7.783	7.763	7.760
20	7.702	7.714	7.697	7.694
25	7.641	7.649	7.635	7.634
30	7.580	7.584	7.574	7.573
35	7.520	7.522	7.512	7.512
37	7.496	7.498	7.490	7.789
40	7.463	7.461	7.449	7.454
45	7.407	7.405	7.397	7.398
50	7.351	7.350	7.338	7.343
55	7.296	7.295	7.285	7.286

^aUnits of *m*, mol•kg⁻¹

Table 4

$p\alpha_H$ values for HEPES (m_1) + NaHEPES (m_2) + NaCl (m_3) buffer solutions from 5 to 55°C from Eq. (5)^a

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
0.01	0.01	0.15	7.777	7.711	7.645	7.583	7.516	7.454	7.392	7.367	7.330	7.272	7.212	7.156
0.02	0.02	0.14	7.738	7.671	7.605	7.541	7.478	7.416	7.355	7.332	7.297	7.240	7.183	7.128
0.03	0.03	0.13	7.761	7.694	7.628	7.564	7.501	7.440	7.380	7.357	7.321	7.264	7.204	7.151
0.04	0.04	0.12	7.779	7.710	7.643	7.578	7.517	7.456	7.395	7.371	7.335	7.278	7.217	7.162
0.05	0.05	0.11	7.776	7.709	7.643	7.577	7.514	7.453	7.393	7.369	7.334	7.277	7.221	7.165
0.06	0.06	0.10	7.801	7.732	7.657	7.589	7.522	7.457	7.394	7.370	7.333	7.276	7.219	7.163
0.07	0.07	0.09	7.773	7.704	7.637	7.572	7.508	7.447	7.385	7.362	7.321	7.267	7.208	7.154
0.08	0.08	0.08	7.768	7.698	7.634	7.569	7.507	7.446	7.385	7.361	7.326	7.268	7.213	7.154

^aUnits of m , mol•kg⁻¹

Table 5

Emf of Cell B for HEPES buffer

m_1	m_2	m_3	E, V	
			25°C	37°C
0.08	0.08	0.08	0.68604	0.68525
0.06	0.06	0.10	0.68693	0.68765
0.04	0.04	0.12	0.68654	0.68769
0.02	0.02	0.14	0.68422	0.68713

Emf of Cell C ^a				
Cell C			E, V	
0.008695 <i>m</i> KH ₂ PO ₄			25°C	37°C
+ 0.03043 <i>m</i> Na ₂ HPO ₄			0.68275	0.69147

^aCorrected to a hydrogen pressure of 1 atm, for physiological phosphate buffer solutions (primary reference standard buffer) at 25 and 37°C

Table 6

Values of the liquid junction potentials for HEPES buffer at 25 and 37°C, from Eq. (1)

System	E_j^a , mV	
	25°C	37°C
Physiological phosphate (0.008695 <i>m</i> KH ₂ PO ₄ + 0.03043 <i>m</i> NaCl)	2.6	2.9
0.08 <i>m</i> HEPES + 0.08 <i>m</i> NaHEPES + 0.08 <i>m</i> NaCl	0.5	0.7
0.06 <i>m</i> HEPES + 0.06 <i>m</i> NaHEPES + 0.10 <i>m</i> NaCl	0.5	0.7
0.04 <i>m</i> HEPES + 0.04 <i>m</i> NaHEPES + 0.12 <i>m</i> NaCl	0.4	0.6
0.02 <i>m</i> HEPES + 0.02 <i>m</i> NaHEPES + 0.14 <i>m</i> NaCl	0.4	0.6

$^a E_j = E + E_{SCE}^\circ k$ pH from Eq. (1), E is the emf from Table 5, 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 and 37°C; E_{SCE}° = electrode potential of the saturated calomel electrode = -0.2415 V and -0.2335 V at 25 and 37°C, respectively.

Table 7

Values of pH at 25 and 37°C for HEPES buffer solutions using data for E_j corrections from Table 6

Cell B		25°C			37°C		
$m_1 = m_2$	m_3	Ionic strength, I	Without ^a E_j corr	With ^b E_j corr	Calc ^c E_j corr	Without ^a E_j corr	With ^b E_j corr
0.08	0.08	0.16	7.471	7.506	7.507	7.324	7.360
0.06	0.10	0.16	7.486	7.521	7.522	7.334	7.370
0.04	0.12	0.16	7.479	7.516	7.517	7.333	7.370
0.02	0.14	0.16	7.439	7.478	7.478	7.294	7.331
							7.332

^a pH = 7.415 + [(E - 0.68275)/(0.059156)] at 25°C, and pH = 7.395 + [(E - 0.69144)/(0.061538)] at 37°C; the emf values (Table 5) are 0.68275 volt and 0.69144 volt at 25 and 37°C for the physiological phosphate buffer standard solution.

^b Values obtained from Eq. (2) and E_j data of Table 6

^c Obtained from Table 4