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Buffer standards for the physiological pH of *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) from (278.15 to 328.15) K

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Abstract

This paper reports the results for the pH of six buffer solutions free of chloride ion with compositions: (a) BES (0.03 mol·kg⁻¹) + NaBES (0.09 mol·kg⁻¹); (b) BES (0.02 mol·kg⁻¹) + NaBES (0.04 mol·kg⁻¹); (c) BES (0.04 mol·kg⁻¹) + NaBES (0.08 mol·kg⁻¹); (d) BES (0.04 mol·kg⁻¹) + NaBES (0.04 mol·kg⁻¹) (e) BES (0.05 mol·kg⁻¹) + NaBES (0.05 mol·kg⁻¹); and (f) (0.06 mol·kg⁻¹) + NaBES (0.06 mol·kg⁻¹). The remaining eight buffer solutions (g) to (n) have saline media of the ionic strength $I = 0.16$ mol·kg⁻¹, matching closely to that of the physiological sample. Conventional p_{aH} values, designated as pH(s), for all six buffer solutions (a) – (f) without the chloride ion and eight buffer solutions with the chloride ion (g) – (n) at $I = 0.16$ mol·kg⁻¹ from (278.15 K to 328.15) K have been calculated. The operational pH values for five buffer solutions at $T = 298.15$ K and $T = 310.15$ K 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 secondary standards for the physiological pH range 7.5 to 8.5.

Keywords

Dissociation Constant; Ionic Strength; Buffer; Emf

1. Introduction

In biomedical, biological, and clinical laboratories, knowledge of the pH of blood and physiological fluids is of importance. Previously, we have reported the pK_2 values of *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) [1] at temperatures from (278.15 K to 328.15) K, including $T = 310.15$ K. This zwitterionic buffer system has been recommended by Good and coworkers [2, 3] for use as a physiological buffer. The structure of the BES 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

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Bureau of Standards (NBS) by using 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, $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$. The disadvantages [5] are as follows: (i) phosphates act as inhibitors to enzymatic processes; (ii) phosphate precipitates occur with some polyvalent cations, such as Mg^{2+} and Ca^{2+} , present in the blood; and (iii) the temperature coefficient of the phosphate buffer is $-0.0028 \text{ pH unit/K}$ as compared to that of whole blood (-0.015 pH unit/K) and plasma (0.01 pH unit/K) [5].

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 (TAPS) [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 NaTRICINEate. 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 BES.

We now propose to investigate BES buffer, the structure shown in figure 1, in order to provide very accurate and reproducible pH values for six NaCl-free buffer solutions with the compositions:

- (a) BES ($0.02 \text{ mol}\cdot\text{kg}^{-1}$) + NaBES ($0.04 \text{ mol}\cdot\text{kg}^{-1}$); (b) BES ($0.03 \text{ mol}\cdot\text{kg}^{-1}$) + NaBES ($0.09 \text{ mol}\cdot\text{kg}^{-1}$); (c) BES ($0.04 \text{ mol}\cdot\text{kg}^{-1}$) + NaBES ($0.08 \text{ mol}\cdot\text{kg}^{-1}$); (d) BES ($0.04 \text{ mol}\cdot\text{kg}^{-1}$) + NaBES ($0.04 \text{ mol}\cdot\text{kg}^{-1}$); (e) BES ($0.05 \text{ mol}\cdot\text{kg}^{-1}$) + NaBES ($0.05 \text{ mol}\cdot\text{kg}^{-1}$); (f) BES ($0.06 \text{ mol}\cdot\text{kg}^{-1}$) + NaBES ($0.06 \text{ mol}\cdot\text{kg}^{-1}$)

In the range of physiological application at $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$, the composition of eight buffer solutions with NaCl are given below:

- (g) BES ($0.04 \text{ mol}\cdot\text{kg}^{-1}$) + NaBES ($0.08 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.08 \text{ mol}\cdot\text{kg}^{-1}$), $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$
- (h) BES ($0.03 \text{ mol}\cdot\text{kg}^{-1}$) + NaBES ($0.06 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.10 \text{ mol}\cdot\text{kg}^{-1}$), $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$
- (i) BES ($0.02 \text{ mol}\cdot\text{kg}^{-1}$) + NaBES ($0.04 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.12 \text{ mol}\cdot\text{kg}^{-1}$), $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$
- (j) BES ($0.03 \text{ mol}\cdot\text{kg}^{-1}$) + NaBES ($0.09 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.07 \text{ mol}\cdot\text{kg}^{-1}$), $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$
- (k) BES ($0.04 \text{ mol}\cdot\text{kg}^{-1}$) + NaBES ($0.04 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.12 \text{ mol}\cdot\text{kg}^{-1}$), $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$
- (l) BES ($0.05 \text{ mol}\cdot\text{kg}^{-1}$) + NaBES ($0.05 \text{ mol}\cdot\text{kg}^{-1}$) + NaCl ($0.11 \text{ mol}\cdot\text{kg}^{-1}$), $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$

- (m) BES (0.06 mol·kg⁻¹) + NaBES (0.06 mol·kg⁻¹) + NaCl (0.10 mol·kg⁻¹), $I = 0.16 \text{ mol·kg}^{-1}$
- (n) BES (0.08 mol·kg⁻¹) + NaBES (0.08 mol·kg⁻¹) + NaCl (0.08 mol·kg⁻¹), $I = 0.16 \text{ mol·kg}^{-1}$

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

2. Materials and Methods

A commercial sample of BES (Research Organics) was recrystallized twice from a 0.7 mass fraction of ethanol and was dried at room temperature under vacuum in a desiccator. The sample was powdered and then stored in a desiccator over Drierite (CaSO₄) until used. The product was assayed at a mass fraction of 99.98 (standard deviation 0.1%) when titrated with the same standard solution of sodium hydroxide (NaOH) that was subsequently used to prepare the buffer solutions. The titration was carried out under carbon dioxide-free conditions, either to a calculated end point of pH equal to 9.44 (0.05 M solution). In the present study, the analyses of the unpurified and purified BES averaged mass fractions of 99.71 and 99.98 pure, respectively. All mass measurements were made with a mass factor uncertainty of 0.02, substance BES, NaCl (ACS reagent grade dried at $T = 383.15 \text{ K}$), a standard solution of NaOH to prepare NaBESate, 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, 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:

where m_1 , m_2 and m_3 indicate molalities of the respective species, and 101.325 kPa is the pressure of hydrogen in SI units. 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.

For cell (C), the phosphate salts were NIST standard reference materials with the composition KH₂PO₄ (0.008695 mol·kg⁻¹) + Na₂HPO₄ (0.03043 mol·kg⁻¹) and its solutions are recommended for pH measurements in physiological solutions.

For the liquid junction potential measurements using cells (B) and (C), the diffusion (mixing) between the saturated KCl solution and the sample solution was minimized by siphoning the solution at the interface with one drop per three seconds with maximum stability.

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

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

where $E_{SCE}^{\circ} = -0.2415 \text{ V}$, $k = 0.059156$, and $\text{pH} = 7.415$ (standard physiological phosphate buffer solution) at $T = 298.15 \text{ K}$; $E_{SCE}^{\circ} = -0.2335$, $k = 0.061538$, and $\text{pH} = 7.395$ at $T = 310.15 \text{ K}$. We have attempted to calculate values of the liquid junction potential for five buffer solutions out of fourteen buffer solutions. The difference in δE_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 $\text{pH}(x)$:

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

where the subscript “x” refers to the unknown buffer BES + NaBESate; s is the reference standard solution (NIST physiological phosphate buffer) of known pH and $\delta E_j = E_{j(s)} - E_{j(x)}$. Equation (2) takes the form:

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

where $\delta E_j = 0$ if the values of $E_{j(s)}$ and $E_{j(x)}$ are the same when liquid junction potential measurements are carried out by matching the ionic strength ($I = 0.16 \text{ mol} \cdot \text{kg}^{-1}$) of the standards and the unknown solution x. But δE_j is not equal to zero since buffer compositions of the solutions are different.

3. Methods and Results

The cell potential data for cell (A) containing six buffer solutions without the presence of chloride ion, and eight buffer solutions in which NaCl had been added to make $I = 0.16 \text{ mol} \cdot \text{kg}^{-1}$, have been corrected to a hydrogen pressure of 101.325 kPa. The values of the cell potential at $T = 298.15 \text{ K}$ are the average of two readings (at the beginning and in the middle of the run). Duplicate cells usually gave readings on the average within $\pm 0.02 \text{ mV}$ in the temperature range (278.15 to 328.15) K. All these results are listed in tables 1 and 2, respectively.

3.1 The pH of BES Buffer

Conventional pH values have been evaluated by the method of Bates *et al.* [11,14–17] for six buffer solutions without NaCl and eight buffer solutions in the presence of NaCl.

In order to calculate pH values for six buffer solutions without NaCl, calculations of the values of the acidity function $p(a_{\text{H}^+/\text{Cl}})^{\circ}$ in the absence of Cl^- , and $p(a_{\text{H}^+/\text{Cl}})$ for all eight buffer solutions in the presence of Cl^- were made in the temperature range (278.15 to

328.15) K, from the cell potential (E) listed in tables 1 and 2, the molality of the chloride ion, and E° , and the standard potential of the silver-silver chloride electrode 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 six buffer solutions without the presence of NaCl listed in table 3, were calculated using equation (5). The acidity function, $p(a_H \gamma_{Cl})$ for eight buffers with NaCl are listed in table 4 in the temperature range (278.15 to 328.15) K. 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 solution without the presence of the chloride ion were calculated by the following expression:

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

where the single-ion activity coefficient, γ_{Cl}° , cannot be measured experimentally. A nonthermodynamic convention [4,9] for the estimation of γ_{Cl}° has been adopted for the calculation of pa_H by equation (5). The pH values obtained 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 $\log_{10} \gamma_{Cl}^\circ$ for all of the buffer-chloride solutions were made by using the following equation:

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

where I is the ionic strength of the buffer solution, A and B are the Debye-Hückel constants [6–7], C is an adjustable parameter, Ba° was taken to be $1.38 \text{ kg}^{1/2} \cdot \text{mol}^{-1/2}$ at all temperatures [9]. In the Bates-Guggenheim convention [4], the value of Ba° was assigned to be $1.5 \text{ kg}^{1/2} \cdot \text{mol}^{-1/2}$ and $C = 0$ for ionic strength $I \leq 0.1 \text{ mol} \cdot \text{kg}^{-1}$. The following equation is used for the calculation of the parameter C [8–9]:

$$C = C_{298.15} + 6.2 \times 10^{-4}(T - 298.15) - 8.7 \times 10^{-6}(T - 298.15)^2 \quad (7)$$

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

The values of pa_H are listed in table 5 for six buffer solutions of BES without NaCl. These are calculated using equations (4) to (7) and are expressed as a function of temperature.

For BES ($0.02 \text{ mol} \cdot \text{kg}^{-1}$) + NaBES ($0.04 \text{ mol} \cdot \text{kg}^{-1}$):

$$p a_{\text{H}} = 7.336 - 1.457 \times 10^{-2}(T - 298.15) + 4.510 \times 10^{-5}(T - 298.15)^2 \quad (8)$$

For BES (0.03 mol·kg⁻¹) + NaBES (0.09 mol·kg⁻¹):

$$p a_{\text{H}} = 7.526 - 1.476 \times 10^{-2}(T - 298.15) + 4.900 \times 10^{-5}(T - 298.15)^2 \quad (9)$$

For BES (0.04 mol·kg⁻¹) + NaBES (0.08 mol·kg⁻¹):

$$p a_{\text{H}} = 7.345 - 1.369 \times 10^{-2}(T - 298.15) + 2.620 \times 10^{-5}(T - 298.15)^2 \quad (10)$$

For BES (0.04 mol·kg⁻¹) + NaBES (0.04 mol·kg⁻¹):

$$p a_{\text{H}} = 6.990 - 1.466 \times 10^{-2}(T - 298.15) + 5.310 \times 10^{-5}(T - 298.15)^2 \quad (11)$$

For BES (0.05 mol·kg⁻¹) + NaBES (0.05 mol·kg⁻¹):

$$p a_{\text{H}} = 7.032 - 1.448 \times 10^{-2}(T - 298.15) + 4.150 \times 10^{-5}(T - 298.15)^2 \quad (12)$$

For BES (0.06 mol·kg⁻¹) + NaBES (0.06 mol·kg⁻¹):

$$p a_{\text{H}} = 6.995 - 1.459 \times 10^{-2}(T - 298.15) + 4.120 \times 10^{-5}(T - 298.15)^2 \quad (13)$$

where 278.15 K ≤ T ≤ 328.15 K. The standard deviations of regression for the $p a_{\text{H}}$ of these buffer solutions are 0.0015, 0.0021, 0.0022, 0.0019, 0.0018, and 0.0023, respectively.

For the eight buffer solutions containing NaCl with an ionic strength $I = 0.16$ mol·kg⁻¹, the values of $p a_{\text{H}}$ listed in table 6 are expressed by the equations:

For BES (0.04 mol·kg⁻¹) + NaBES (0.08 mol·kg⁻¹) + NaCl (0.08 mol·kg⁻¹):

$$p a_{\text{H}} = 7.347 - 1.501 \times 10^{-2}(T - 298.15) + 1.900 \times 10^{-5}(T - 298.15)^2 \quad (14)$$

For BES (0.03 mol·kg⁻¹) + NaBES (0.06 mol·kg⁻¹) + NaCl (0.10 mol·kg⁻¹):

$$p a_{\text{H}} = 7.391 - 1.484 \times 10^{-2}(T - 298.15) + 3.330 \times 10^{-5}(T - 298.15)^2 \quad (15)$$

For BES (0.02 mol·kg⁻¹) + NaBES (0.04 mol·kg⁻¹) + NaCl (0.12 mol·kg⁻¹):

$$p a_{\text{H}} = 7.435 - 1.485 \times 10^{-2}(T - 298.15) + 4.370 \times 10^{-5}(T - 298.15)^2 \quad (16)$$

For BES (0.03 mol·kg⁻¹) + NaBES (0.09 mol·kg⁻¹) + NaCl (0.07 mol·kg⁻¹):

$$p a_{\text{H}} = 7.319 - 1.506 \times 10^{-2}(T - 298.15) + 2.960 \times 10^{-5}(T - 298.15)^2 \quad (17)$$

For BES (0.04 mol·kg⁻¹) + NaBES (0.04 mol·kg⁻¹) + NaCl (0.12 mol·kg⁻¹):

$$p a_{\text{H}} = 7.054 - 1.485 \times 10^{-2}(T - 298.15) + 4.860 \times 10^{-5}(T - 298.15)^2 \quad (18)$$

For BES (0.05 mol·kg⁻¹) + NaBES (0.05 mol·kg⁻¹) + NaCl (0.11 mol·kg⁻¹):

$$p a_{\text{H}} = 7.391 - 1.480 \times 10^{-2}(T - 298.15) + 3.330 \times 10^{-5}(T - 298.15)^2 \quad (19)$$

For BES (0.06 mol·kg⁻¹) + NaBES (0.06 mol·kg⁻¹) + NaCl (0.10 mol·kg⁻¹):

$$p a_{\text{H}} = 7.435 - 1.480 \times 10^{-2}(T - 298.15) + 4.370 \times 10^{-5}(T - 298.15)^2 \quad (20)$$

For BES (0.08 mol·kg⁻¹) + NaBES (0.08 mol·kg⁻¹) + NaCl (0.08 mol·kg⁻¹):

$$p a_{\text{H}} = 7.319 - 1.510 \times 10^{-2}(T - 298.15) + 2.960 \times 10^{-5}(T - 298.15)^2 \quad (21)$$

where T is the temperature in K. The standard deviations for regression of the “observed” results from equations (14) to (21) are 0.0021, 0.0018, 0.0013, 0.0020, 0.0020, 0.0018, 0.0013, and 0.0020, respectively.

The values of the cell potential of the cells (B) and (C) at temperatures (298.15 and 310.15) K are given in table 7. By means of the flowing junction cell, the values of E_j listed in table 8 were estimated by using equation (1). As evident from the pH data of table 9 at temperatures (298.15 and 310.15) K, there is a wide variation in pH values (as high as ± 0.04 pH) obtained with and without inclusion of the liquid junction correction. The

experimental method for accurately determining the single-ion activity coefficient, $\log_{10} \gamma_{\text{Cl}}^\circ$ is not known. Covington and Ferra [19], used ionic interaction theory of Pitzer for the calculation 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. A separate publication from this laboratory, including the calculation of the $p a_{\text{H}}$ values of these buffer solutions is in progress by using Pitzer treatment as a convention for $\log_{10} \gamma_{\text{Cl}}$ for an ionic strength $I = 0.16$ mol·kg⁻¹ at (298.15 and 310.15) K. The uncertainty for the calculation of $\log_{10} \gamma_{\text{Cl}}$ lies in the pH unit of ± 0.001 . For example, $m_1 = 0.03$ mol·kg⁻¹, $m_2 = 0.06$ mol·kg⁻¹, $m_3 = 0.10$ mol·kg⁻¹ at 298.15 K, the calculated pH value is 7.392 and the value obtained from the δE_j correction is 7.391. These values are accurate to within ± 0.001 . The total uncertainties were estimated by combining the various sources of error: (i) assumption for the calculation of the $\log_{10} \gamma_{\text{Cl}}^\circ$ (± 0.004 pH unit); (ii) extrapolation to $p(a_{\text{H}}\gamma_{\text{Cl}})^\circ$ at $m_{\text{Cl}^-} = 0$ (within ± 0.001 pH unit); (iii) liquid junction potential measurement using the flowing junction cell; (iv) error in the experimental emf measurement (± 0.02 mV); and (v) standard potential of the Ag-AgCl electrode. The operational pH values at $T = 298.15$ K and $T = 310.15$ K for five buffer solutions having the compositions (with and without the presence of NaCl) are recommended as secondary standards for the measurement of pH in the biological region.

Highlights

- i. This research involves the determination of pH values of a biological buffer.
- ii. These values are used as standards for physiological applications with pH 6–8.
- iii. Biomedical scientists use this data for biomedicine and clinical diagnosis.

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FIGURE 1.
Structure of *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES)

TABLE 1
Electromotive force E (V) of Cell A: Pt(s); H₂(g, $p^\circ = 101.325$ kPa) | BES (m_1), NaBES (m_2), NaCl (m_3) | AgCl(s), Ag(s)

$m/\text{mol}\cdot\text{kg}^{-1}$			T/K											
m_1	m_2	m_3	278.15 K	283.15 K	288.15 K	293.15 K	298.15 K	303.15 K	308.15 K	310.15 K	313.15 K	318.15 K	323.15 K	328.15 K
0.02	0.04	0.005	0.78762	0.79026	0.79271	0.79493	0.79730	0.79933	0.80119	0.80199	0.80305	0.80469	0.80637	0.80790
0.02	0.04	0.010	0.77126	0.77360	0.77571	0.77758	0.77961	0.78129	0.78283	0.78347	0.78436	0.78572	0.78746	0.78806
0.02	0.04	0.015	0.76161	0.76376	0.76567	0.76735	0.76920	0.77067	0.77199	0.77258	0.77335	0.77454	0.77634	0.77735
0.02	0.04	0.020	0.75497	0.75698	0.75875	0.76025	0.76193	0.76324	0.76443	0.76491	0.76560	0.76667	0.76873	0.76956
0.03	0.09	0.005	0.79945	0.80230	0.80482	0.80728	0.80954	0.81191	0.81393	0.81492	0.81624	0.81804	0.81958	0.82133
0.03	0.09	0.010	0.78277	0.78530	0.78754	0.78962	0.79142	0.79367	0.79550	0.79630	0.79741	0.79893	0.80024	0.80164
0.03	0.09	0.015	0.77290	0.77522	0.77730	0.77898	0.78041	0.78274	0.78458	0.78526	0.78625	0.78758	0.78878	0.78983
0.03	0.09	0.020	0.76580	0.76789	0.76986	0.77137	0.77261	0.77494	0.77668	0.77729	0.77812	0.77935	0.78046	0.78141
0.04	0.08	0.005	0.78883	0.79209	0.79487	0.79738	0.80005	0.80225	0.80411	0.80496	0.80608	0.80777	0.80962	0.81100
0.04	0.08	0.010	0.77342	0.77641	0.77852	0.78087	0.78290	0.78509	0.78651	0.78721	0.78817	0.78959	0.79097	0.79219
0.04	0.08	0.015	0.76505	0.76750	0.76948	0.77145	0.77320	0.77538	0.77655	0.77706	0.77790	0.77917	0.78038	0.78136
0.04	0.08	0.020	0.75925	0.76169	0.76327	0.76498	0.76660	0.76864	0.76956	0.76991	0.77070	0.77183	0.77285	0.77373
0.04	0.04	0.005	0.76998	0.77202	0.77433	0.77627	0.77816	0.77999	0.78162	0.78211	0.78315	0.78455	0.78585	0.78690
0.04	0.04	0.010	0.75484	0.75632	0.75864	0.76034	0.76196	0.76347	0.76485	0.76523	0.76604	0.76721	0.76823	0.76906
0.04	0.04	0.015	0.74638	0.74769	0.74990	0.75151	0.75299	0.75426	0.75558	0.75584	0.75652	0.75753	0.75839	0.75910
0.04	0.04	0.020	0.74091	0.74168	0.74429	0.74587	0.74704	0.74831	0.74940	0.74984	0.75043	0.75132	0.75207	0.75207
0.05	0.05	0.005	0.77150	0.77406	0.77651	0.77821	0.78018	0.78210	0.78370	0.78434	0.78543	0.78666	0.78793	0.78906
0.05	0.05	0.010	0.75580	0.75796	0.75982	0.76146	0.76303	0.76475	0.76605	0.76649	0.76729	0.76833	0.76930	0.77012
0.05	0.05	0.015	0.74688	0.74884	0.75047	0.75194	0.75299	0.75486	0.75586	0.75653	0.75702	0.75808	0.75883	0.75952
0.05	0.05	0.020	0.74073	0.74246	0.74396	0.74530	0.74621	0.74794	0.74892	0.74945	0.75008	0.75085	0.75141	0.75203
0.06	0.06	0.005	0.77088	0.77311	0.77526	0.77750	0.77925	0.78094	0.78243	0.78317	0.78433	0.78565	0.78674	0.78788
0.06	0.06	0.010	0.75566	0.75774	0.75957	0.76160	0.76293	0.76435	0.76552	0.76616	0.76725	0.76854	0.76925	0.77017
0.06	0.06	0.015	0.74737	0.74922	0.75092	0.75287	0.75382	0.75520	0.75597	0.75667	0.75778	0.75907	0.75968	0.76051
0.06	0.06	0.020	0.74208	0.74386	0.74543	0.74734	0.74795	0.74937	0.74967	0.75057	0.75176	0.75287	0.75365	0.75444
$E^\circ_{\text{Ag}^+/\text{AgCl}}$			0.23416	0.23147	0.22863	0.22562	0.22244	0.21913	0.21572	0.21429	0.21214	0.20840	0.20455	0.20064

TABLE 2
Electromotive force E (V) of Cell A (in volts): Pt(s); H₂ (g, $p^\circ = 101.325$ kPa) | BES (m_1), NaBES (m_2), NaCl (m_3) | AgCl (s), Ag (s)

$m/\text{mol}\cdot\text{kg}^{-1}$			T/K														
m_1	m_2	m_3	278.15 K	283.15 K	288.15 K	293.15 K	298.15 K	303.15 K	308.15 K	310.15 K	313.15 K	318.15 K	323.15 K	328.15 K			
0.04	0.08	0.08	0.72403	0.72578	0.72744	0.72850	0.72946	0.73038	0.73078	0.73069	0.73093	0.73113	0.73097	0.73090			
0.03	0.06	0.10	0.72137	0.72273	0.72410	0.72537	0.72636	0.72710	0.72781	0.72782	0.72796	0.72825	0.72837	0.72856			
0.02	0.04	0.12	0.71959	0.72102	0.72229	0.72347	0.72432	0.72484	0.72560	0.72580	0.72608	0.72645	0.72669	0.72682			
0.03	0.09	0.07	0.72593	0.72770	0.72917	0.73026	0.73123	0.73192	0.73266	0.73289	0.73287	0.73316	0.73309	0.73345			
0.04	0.04	0.12	0.69873	0.69967	0.70044	0.70112	0.70167	0.70206	0.70237	0.70244	0.70247	0.70246	0.70246	0.70219			
0.05	0.05	0.11	0.70134	0.70236	0.70352	0.70438	0.70507	0.70504	0.70580	0.70588	0.70619	0.70592	0.70588	0.70576			
0.06	0.06	0.10	0.70481	0.70591	0.70683	0.70771	0.70849	0.70837	0.70922	0.70932	0.70960	0.70947	0.70949	0.70940			
0.08	0.08	0.08	0.71143	0.71270	0.71380	0.71479	0.71574	0.71593	0.71645	0.71659	0.71676	0.71691	0.71709	0.71708			

TABLE 3
 $p(\alpha_{H^+})^\circ$ of (BES + NaBES) buffer solutions from (278.15 to 328.15) K, computed using equation (5)

$m/\text{mol}\cdot\text{kg}^{-1}$							
T/K	$0.02\text{ }m\text{ BES}$ $+ 0.04\text{ }m\text{ NaBES}$ $I = 0.04\text{ }m$	$0.03\text{ }m\text{ BES}$ $+ 0.09\text{ }m\text{ NaBES}$ $I = 0.09\text{ }m$	$0.04\text{ }m\text{ BES}$ $+ 0.08\text{ }m\text{ NaBES}$ $I = 0.08\text{ }m$	$0.04\text{ }m\text{ BES}$ $+ 0.04\text{ }m\text{ NaBES}$ $I = 0.04\text{ }m$	$0.05\text{ }m\text{ BES}$ $+ 0.05\text{ }m\text{ NaBES}$ $I = 0.05\text{ }m$	$0.06\text{ }m\text{ BES}$ $+ 0.06\text{ }m\text{ NaBES}$ $I = 0.06\text{ }m$	
278.15	7.724	7.945	7.727	7.383	7.421	7.396	
283.15	7.642	7.864	7.658	7.300	7.344	7.313	
288.15	7.563	7.781	7.586	7.219	7.270	7.233	
293.15	7.485	7.705	7.515	7.140	7.188	7.159	
298.15	7.415	7.632	7.450	7.068	7.119	7.087	
303.15	7.344	7.559	7.380	6.999	7.048	7.013	
308.15	7.274	7.486	7.310	6.930	6.978	6.947	
310.15	7.249	7.463	7.287	6.900	6.950	6.919	
313.15	7.210	7.426	7.248	6.864	6.913	6.882	
318.15	7.145	7.361	7.184	6.801	6.847	6.817	
323.15	7.081	7.295	7.127	6.741	6.786	6.750	
328.15	7.022	7.237	7.065	6.678	6.724	6.688	

TABLE 4

$p(a_{H^+})$ of (BES + NaBES) buffer solutions from (278.15 to 328.15) K, computed using equation (4)

T/K	$m/\text{mol}\cdot\text{kg}^{-1}$									
	0.04 <i>m</i> BES + 0.08 <i>m</i> NaBES + 0.08 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.03 <i>m</i> BES + 0.06 <i>m</i> NaBES + 0.10 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.02 <i>m</i> BES + 0.04 <i>m</i> NaBES + 0.12 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.03 <i>m</i> BES + 0.09 <i>m</i> NaBES + 0.07 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.04 <i>m</i> BES + 0.04 <i>m</i> NaBES + 0.12 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.05 <i>m</i> BES + 0.05 <i>m</i> NaBES + 0.11 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.06 <i>m</i> BES + 0.06 <i>m</i> NaBES + 0.10 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.08 <i>m</i> BES + 0.08 <i>m</i> NaBES + 0.08 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>		
278.15	7.779	7.828	7.875	7.756	7.497	7.507	7.528	7.551		
283.15	7.702	7.744	7.793	7.678	7.413	7.423	7.445	7.469		
288.15	7.628	7.666	7.714	7.600	7.331	7.348	7.364	7.389		
293.15	7.549	7.592	7.638	7.521	7.254	7.272	7.288	7.313		
298.15	7.474	7.518	7.563	7.446	7.180	7.200	7.216	7.242		
303.15	7.403	7.445	7.487	7.370	7.108	7.120	7.134	7.162		
308.15	7.327	7.375	7.418	7.300	7.039	7.057	7.071	7.093		
310.15	7.295	7.345	7.391	7.272	7.012	7.030	7.044	7.066		
313.15	7.253	7.302	7.351	7.226	6.971	6.993	7.006	7.025		
318.15	7.184	7.235	7.286	7.158	6.906	6.923	6.938	6.959		
323.15	7.113	7.170	7.223	7.088	6.845	6.860	6.875	6.897		
328.15	7.047	7.108	7.161	7.028	6.782	6.799	6.814	6.835		

TABLE 5
pH of (BES + NaBES) buffer solutions from (278.15 to 328.15) K, computed using equations (4) – (7)

$m/\text{mol}\cdot\text{kg}^{-1}$							
T/K		$0.02\ m\ \text{BES}$ $+ 0.04\ m\ \text{NaBES}$ $I = 0.04\ m$	$0.03\ m\ \text{BES}$ $+ 0.09\ m\ \text{NaBES}$ $I = 0.09\ m$	$0.04\ m\ \text{BES}$ $+ 0.08\ m\ \text{NaBES}$ $I = 0.08\ m$	$0.04\ m\ \text{BES}$ $+ 0.04\ m\ \text{NaBES}$ $I = 0.04\ m$	$0.05\ m\ \text{BES}$ $+ 0.05\ m\ \text{NaBES}$ $I = 0.05\ m$	$0.06\ m\ \text{BES}$ $+ 0.06\ m\ \text{NaBES}$ $I = 0.06\ m$
278.15	7.647	7.841	7.627	7.306	7.337	7.307	
283.15	7.565	7.760	7.558	7.223	7.260	7.223	
288.15	7.485	7.676	7.485	7.141	7.185	7.142	
293.15	7.407	7.601	7.415	7.062	7.103	7.069	
298.15	7.337	7.526	7.349	6.990	7.033	6.996	
303.15	7.265	7.453	7.278	6.919	6.961	6.921	
308.15	7.195	7.380	7.208	6.851	6.891	6.854	
310.15	7.169	7.356	7.185	6.820	6.863	6.826	
313.15	7.129	7.319	7.145	6.784	6.826	6.789	
318.15	7.064	7.253	7.080	6.720	6.759	6.723	
323.15	6.999	7.186	7.022	6.659	6.698	6.655	
328.15	6.939	7.127	6.959	6.596	6.635	6.593	

TABLE 6
pH of (BES + NaBES) buffer solutions from (278.15 to 328.15) K, computed using equations (4) – (7)

T/K	m/mol·kg ⁻¹							
	0.04 m BES + 0.08 m NaBES + 0.08 m NaCl I = 0.16 m	0.03 m BES + 0.06 m NaBES + 0.10 m NaCl I = 0.16 m	0.02 m BES + 0.04 m NaBES + 0.12 m NaCl I = 0.16 m	0.03 m BES + 0.09 m NaBES + 0.07 m NaCl I = 0.16 m	0.04 m BES + 0.04 m NaBES + 0.12 m NaCl I = 0.16 m	0.05 m BES + 0.05 m NaBES + 0.11 m NaCl I = 0.16 m	0.06 m BES + 0.06 m NaBES + 0.10 m NaCl I = 0.16 m	0.08 m BES + 0.08 m NaBES + 0.08 m NaCl I = 0.16 m
278.15	7.654	7.703	7.750	7.630	7.372	7.381	7.403	7.426
283.15	7.576	7.619	7.667	7.552	7.288	7.298	7.319	7.343
288.15	7.502	7.540	7.588	7.474	7.206	7.222	7.238	7.263
293.15	7.424	7.467	7.513	7.396	7.129	7.147	7.163	7.188
298.15	7.347	7.392	7.436	7.319	7.054	7.073	7.090	7.115
303.15	7.276	7.318	7.360	7.243	6.981	6.993	7.007	7.035
308.15	7.199	7.248	7.291	7.172	6.911	6.929	6.944	6.965
310.15	7.167	7.217	7.263	7.144	6.884	6.902	6.916	6.938
313.15	7.124	7.173	7.222	7.098	6.842	6.864	6.878	6.896
318.15	7.055	7.106	7.156	7.029	6.776	6.793	6.808	6.829
323.15	6.983	7.039	7.092	6.958	6.714	6.730	6.745	6.766
328.15	6.916	6.977	7.029	6.897	6.651	6.668	6.683	6.704

TABLE 7

Emf of Cell B for BES buffer

$m/\text{mol}\cdot\text{kg}^{-1}$				
m_1	m_2	m_3		
			$T = 298.15 \text{ K}$	$T = 310.15 \text{ K}$
			E/V	
0.03	0.09	0.00	0.68890	0.68858
0.02	0.04	0.12	0.68186	0.68112
0.03	0.06	0.10	0.67934	0.67837
0.04	0.08	0.08	0.67659	0.67522
0.08	0.08	0.08	0.66293	0.66118
Emf of Cell C ^a				
Cell C			E/V	
			$T = 298.15 \text{ K}$	$T = 310.15 \text{ K}$
0.008695 m KH_2PO_4 + 0.0304 m Na_2HPO_4			0.68275	0.69147

^aPublished data [8–10] for physiological phosphate buffer solutions

TABLE 8

Values of the liquid junction potentials (V) for BES buffer at (298.15 and 310.15) K

System	E_j^a/mV	
$m/\text{mol}\cdot\text{kg}^{-1}$	$T = 298.15$	$T = 310.15 \text{ K}$
Physiological phosphate (0.008695 <i>m</i> KH ₂ PO ₄ + 0.03043 <i>m</i> NaCl)	2.6	2.9
0.03 <i>m</i> BES + 0.09 <i>m</i> NaBES + 0.00 <i>m</i> NaCl	2.2	2.4
0.02 <i>m</i> BES + 0.04 <i>m</i> NaBES + 0.12 <i>m</i> NaCl	0.5	0.7
0.03 <i>m</i> BES + 0.06 <i>m</i> NaBES + 0.10 <i>m</i> NaCl	0.6	0.8
0.04 <i>m</i> BES + 0.08 <i>m</i> NaBES + 0.08 <i>m</i> NaCl	0.5	0.7
0.08 <i>m</i> BES + 0.08 <i>m</i> NaBES + 0.08 <i>m</i> NaCl	0.5	0.7

^a $E_j = E + E_{SCE}^\circ - k \text{ pH}$ where the emf (*E*) from table 7, *k* = Nernst slope with values 0.059156 at *T* = 298.15 K, and 0.061538 at *T* = 310.15 K; the pH values of the primary standard phosphate buffer are 7.415 and 7.395 at (298.15 and 310.15) K, respectively; for experimental buffer solution, the pH is substituted as *paH* taken from tables 5 and 6; E_{SCE}° = electrode potential of the saturated calomel electrode = −0.2415 and −0.2335 at (298.15 and 310.15) K [14,15], respectively.

TABLE 9

Values of pH at (298.15 and 310.15) K for BES buffer solutions

Cell B $m/\text{mol}\cdot\text{kg}^{-1}$			$T = 298.15$			$T = 310.15 \text{ K}$		
m_1	m_2	m_3	Ionic Strength, I					
			Without ^a δE_j corr	With ^b δE_j corr	Calc ^c	Without ^a δE_j corr	With ^b δE_j corr	Calc ^c
0.03	0.09	0.00	0.09	7.519	7.526	7.348	7.356	7.356
0.02	0.04	0.12	0.16	7.400	7.435	7.227	7.262	7.263
0.03	0.06	0.10	0.16	7.357	7.391	7.182	7.216	7.217
0.04	0.08	0.08	0.16	7.311	7.346	7.131	7.166	7.167
0.08	0.08	0.08	0.16	7.080	7.115	6.903	6.938	6.938

^a Values obtained from equation (3) and data of table 7

^b Obtained from equation (2) and table 8

^c Obtained from tables 5 and 6