

# Buffer Standards for Physiological pH of the Buffer *N*-(2-Acetamido)-2-aminoethanesulfonic Acid from 5°C to 55°C

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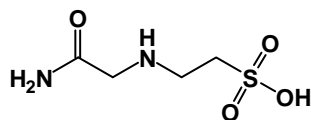
## Abstract

Electromotive force (emf) measurements of the Cell Pt(s), H<sub>2</sub>(g)|ACES(*m*<sub>1</sub>) + NaACES(*m*<sub>2</sub>) + NaCl (*m*<sub>3</sub>)|AgCl, Ag(s) have been carried out from 5°C to 55°C. The agreement of pH values between two calculated (extended Debye-Hückel and liquid junction correction) is very good. Two buffer solutions without the chloride ion and seven buffer solutions with NaCl, at an ionic strength (*I* = 0.16 mol·kg<sup>-1</sup>) similar to that of physiological fluids, have been studied. The pH values for these buffer solutions have been evaluated in the temperature range of 5°C to 55°C using the extended Debye-Hückel equation of the Bates-Guggenheim convention. Values of the residual liquid junction potential ( $\delta E_j$ ) between the ACES solutions and the saturated KCl calomel electrode solution have been estimated at 25°C and 37°C from the previously determined *E<sub>j</sub>* values using the flowing junction cell to determine the operational pH values at 25°C and 37°C. These ACES buffer solutions are recommended as secondary standard reference solutions for pH measurements in the range of physiological application at *I* = 0.16 mol·kg<sup>-1</sup>.

**Keywords:** Buffer, Emf, pH, Zwitterionic

## 1. Introduction

The zwitterionic amino acid buffer solutions recommended by Good *et al.* [1,2] have been studied in the authors' laboratory for the purpose of pH measurement control in the physiological range of pH. Concerning the current investigation, the authors' goal is to provide pH values for the ACES, which is depicted by the following structure:



*N*-(2-acetamido)-2-aminoethanesulfonic acid  
ACES

This phosphate buffer [3,4] is comprised of KH<sub>2</sub>PO<sub>4</sub> (0.008695 mol·kg<sup>-1</sup>) and Na<sub>2</sub>HPO<sub>4</sub> (0.03043 mol·kg<sup>-1</sup>). This buffer solution is widely used as the primary reference standard for routine laboratory measurements. Some of the limitations have been previously reported [5-8].

The zwitterionic buffer compound ACES may form complexes with cations such as Mg<sup>+2</sup> and Ca<sup>+2</sup>. At a high NaCl concentration, the complex formation is minimized.

Wu *et al.* [7] have studied MOPSO using two point pH calibration measurements. Roy *et al.* [9] studied *pK*<sub>2</sub> and pH values of 3-(*N*-morpholino)propanesulfonic acid (MOPS) from 5°C to 55°C.

The following two solutions of ACES were studied without the presence of the chloride ion: (a) ACES (0.02 mol·kg<sup>-1</sup>) + NaACES (0.06 mol·kg<sup>-1</sup>); (b) ACES (0.04 mol·kg<sup>-1</sup>) + NaACES (0.08 mol·kg<sup>-1</sup>).

The following seven solutions were also studied but contain the chloride ion yielding an ionic strength close to that of blood plasma (*I* = 0.16 mol·kg<sup>-1</sup>): (c) ACES (0.08 mol·kg<sup>-1</sup>) + NaACES (0.08 mol·kg<sup>-1</sup>) + NaCl (0.08 mol·kg<sup>-1</sup>); (d) ACES (0.06 mol·kg<sup>-1</sup>) + NaACES (0.06 mol·kg<sup>-1</sup>) + NaCl (0.10 mol·kg<sup>-1</sup>); (e) ACES (0.04 mol·kg<sup>-1</sup>) + NaACES (0.04 mol·kg<sup>-1</sup>) + NaCl (0.12 mol·kg<sup>-1</sup>); (f) ACES (0.03 mol·kg<sup>-1</sup>) + NaACES (0.03 mol·kg<sup>-1</sup>) + NaCl (0.13 mol·kg<sup>-1</sup>); (g) ACES (0.03

mol·kg<sup>-1</sup>) + NaACES (0.09 mol·kg<sup>-1</sup>) + NaCl (0.07 mol·kg<sup>-1</sup>); (h) ACES (0.02 mol·kg<sup>-1</sup>) + NaACES (0.08 mol·kg<sup>-1</sup>) + NaCl (0.08 mol·kg<sup>-1</sup>); (i) ACES (0.02 mol·kg<sup>-1</sup>) + NaACES (0.10 mol·kg<sup>-1</sup>) + NaCl (0.06 mol·kg<sup>-1</sup>).

## 2. Experimental Section

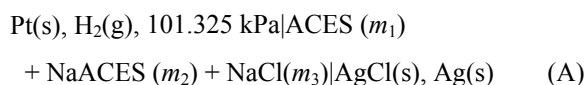
The ACES was obtained from Research Organics (Cleveland, OH) and was crystallized further. The method and the assay have been reported elsewhere [8,9]. The analyses averaged 99.97% with a standard deviation of 0.05%. Buffer were prepared from the weighed amount of the ACES solid, ACS reagent grade NaCl, a standard NaOH solution (for preparation of NaACES), and CO<sub>2</sub>-free doubly distilled water. Buoyancy corrections were made for all masses.

The cell design, method of preparation of hydrogen electrodes, hydrogen gas purification, silver-silver chloride electrodes, solution preparation, temperature measurements, and emf techniques have been described [8,10, 11].

## 3. Methods and Results

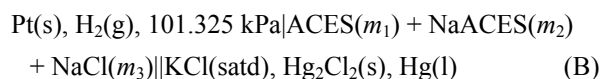
The emf values required for the pH(s) calculations are given in Table 1 for cell A. Cell A contains two solutions without the chloride ion and seven solutions with Cl<sup>-</sup>. The cell voltage values have been corrected to a hydrogen pressure of 1 atm. At 25°C, the emf values are averaged from readings taken twice at the beginning and the middle. The uncertainty lies within 0.02 mV on the average in.

**pH of the ACES Buffer.** The Bates *et al.* [7,8,11-13] method has been used to evaluate the conventional standard pH values for solutions (a) to (i). For accurate calculations of pH for nine buffer solutions, the following cell A is used:



where  $m_1$ ,  $m_2$  and  $m_3$  denote the molalities of the respective species at 1 atm = 101.325 kPa. Cell A is known as the Harned-type cell.

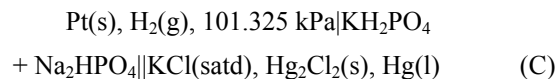
Cell B, the flowing junction cell, was used for the evaluation of the liquid junction potential:



where the abbreviations “s” “l” and “g” imply the solid, liquid, and gaseous states, respectively.

For cell C, a primary standard phosphate buffer solution, NBS/NIST certified, was used. The diagram of cell

C is shown below:



The  $E_{SCE}^\circ$  values of the saturated calomel electrode were taken as -0.2415 and -0.2335 at 25°C and 37°C, respectively [14]. The residual liquid junction potential  $\delta E_j$  values of the buffer solution of ACES were calculated from cells B and C using the following equation:

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

where  $k = 0.059156$  and  $\text{pH} = 7.415$  at 25°C and  $k = 0.061538$  and  $\text{pH} = 7.395$  at 37°C. The pH values are that of the standard phosphate buffer solution and buffer solution of ACES obtained using the extended Debye-Hückel equation. The operational definition of pH, denoted as pH(x), can be calculated by use of the subsequent equation

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

where “x” refers to the unknown buffer, “s” is the NBS/NIST standard phosphate buffer solution of known pH, and  $\delta E_j = E_{j(s)} - E_{j(x)}$ .

To calculate the pH(s) values for the buffer solutions under investigation, calculations were made to determine acidity function,  $p(a_H\gamma_{Cl})$ , in the temperature range of 25°C and 37°C. These calculations were made using emf values listed in **Table 1**, the molality of the chloride ion, and the standard electrode potential of the silver-silver chloride electrode ( $E^\circ$ ). The values of  $p(a_H\gamma_{Cl})$  were obtained from the equation below:

$$p(a_H\gamma_{Cl}) = \frac{E - E^\circ}{k} + \log m_{Cl} \quad (3)$$

where “k” is the Nernst slope.

The values of  $p(a_H\gamma_{Cl})$  were plotted against the molality of the chloride ion. A linear line was obtained. The intercept is the  $p(a_H\gamma_{Cl})^\circ$  value at  $m_{Cl^-} = 0$ . The  $p(a_H\gamma_{Cl})$  values for the seven buffer solutions containing Cl<sup>-</sup> are entered in **Tables 2** and **3** from 5°C to 55°C.

Conventional pH(s) values for solutions without liquid junction and absence a chloride ion were determined using the equation:

$$pH(s) = p(a_H\gamma_{Cl})^\circ + \log \gamma_{Cl} \quad (4)$$

where the single-ion activity coefficient,  $\gamma_{Cl}^\circ$ , can be estimated based on corrections. A previous publication outlines the method used for obtaining this figure [9]. The pH values obtained from the liquid junction cell are indicated by pH whereas the “conventional” pH calculated from Equations (5) and (6) are designated as pH(s). The Bates-Guggenheim convention [15,16], is expressed by the equation:

**Table 1. Cell Potential of Cell A (in Volts): Pt(s); H<sub>2</sub>(g), 101.35 kPa|ACES(m<sub>1</sub>), NaACES(m<sub>2</sub>), NaCl(m<sub>3</sub>)|AgCl(s), Ag(s).**

<i>m</i> <sub>1</sub>	<i>m</i> <sub>2</sub>	<i>m</i> <sub>3</sub>	<i>T</i> /°C											
			5	10	15	20	25	30	35	37	40	45	50	55
0.02	0.06	0.005	0.79035	0.79189	0.79332	0.79440	0.79541	0.79654	0.79731	0.79779	0.79807	0.79866	0.79921	0.79961
0.02	0.06	0.010	0.7736	0.77487	0.77591	0.77671	0.77739	0.77819	0.77868	0.77898	0.77914	0.77941	0.77968	0.77980
0.02	0.06	0.015	0.76380	0.76467	0.76539	0.76607	0.76655	0.76717	0.76745	0.76763	0.76773	0.76794	0.76790	0.76781
0.02	0.06	0.020	0.75659	0.75741	0.75803	0.75848	0.75880	0.75921	0.75943	0.75949	0.75957	0.75956	0.75948	0.75941
0.04	0.08	0.005	0.77395	0.77491	0.77581	0.77641	0.77666	0.77766	0.77828	0.77844	0.77855	0.77884	0.77917	0.77911
0.04	0.08	0.010	0.75930	0.75990	0.76029	0.76038	0.76004	0.76140	0.76166	0.76190	0.76266	0.76262	0.76292	0.76266
0.04	0.08	0.015	0.75154	0.75176	0.75188	0.75143	0.75064	0.75253	0.75276	0.75301	0.75411	0.75418	0.75449	0.75428
0.04	0.08	0.020	0.74645	0.74651	0.74629	0.74557	0.74427	0.74672	0.74680	0.74714	0.74884	0.74891	0.74955	0.74911
0.08	0.08	0.080	0.69577	0.69532	0.69475	0.69403	0.69331	0.69244	0.69146	0.69104	0.69035	0.68924	0.68815	0.68797
0.06	0.06	0.100	0.69083	0.69030	0.68972	0.68869	0.68805	0.68710	0.68600	0.68558	0.68487	0.68373	0.68257	0.68193
0.04	0.04	0.120	0.68684	0.69629	0.68565	0.68498	0.68379	0.68281	0.68160	0.68126	0.68052	0.67922	0.67805	0.67702
0.03	0.03	0.130	0.68510	0.68453	0.68391	0.68322	0.68195	0.68093	0.67968	0.67935	0.67860	0.67726	0.67601	0.67486
0.03	0.09	0.070	0.72884	0.72906	0.72911	0.72905	0.72980	0.72878	0.72846	0.72827	0.72792	0.72743	0.72674	0.72590
0.02	0.08	0.080	0.73082	0.73107	0.73119	0.73119	0.73109	0.73086	0.73068	0.73047	0.73020	0.72970	0.72912	0.72814
0.02	0.10	0.060	0.75064	0.75128	0.75170	0.75202	0.75210	0.75213	0.75174	0.75196	0.75184	0.75169	0.75128	0.75082

<sup>a</sup>*m* = 1 mol·kg<sup>-1</sup>.**Table 2. p(a<sub>H</sub>γ<sub>Cl</sub>)° of ACES + NaACES buffer solutions from 5°C to 55°C obtained by extrapolation for chloride-free solutions, p(a<sub>H</sub>γ<sub>Cl</sub>) of ACES + NaACES buffer solutions from 5°C to 55°C, computed using Equations (4)-(6)<sup>a</sup>.**

<i>T</i> (°C)	0.02 <i>m</i> ACES + 0.06 <i>m</i> NaACES + 0.00 <i>m</i> NaCl <i>I</i> = 0.06 <i>m</i>	0.04 <i>m</i> ACES + 0.08 <i>m</i> NaACES + 0.00 <i>m</i> NaCl <i>I</i> = 0.08 <i>m</i>	0.08 <i>m</i> ACES + 0.08 <i>m</i> NaACES + 0.08 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.06 <i>m</i> ACES + 0.06 <i>m</i> NaACES + 0.10 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.04 <i>m</i> ACES + 0.04 <i>m</i> NaACES + 0.12 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.03 <i>m</i> ACES + 0.03 <i>m</i> NaACES + 0.13 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>
	5	7.781	7.446	7.267	7.275	7.281
10	7.679	7.341	7.159	7.167	7.175	7.178
15	7.582	7.241	7.056	7.065	7.073	7.077
20	7.484	7.145	6.956	6.966	6.977	6.981
25	7.391	7.050	6.863	6.871	6.878	6.882
30	7.306	6.956	6.772	6.780	6.788	6.791
35	7.218	6.871	6.684	6.692	6.699	6.702
37	7.189	6.836	6.650	6.659	6.668	6.671
40	7.136	6.780	6.600	6.608	6.617	6.621
45	7.056	6.694	6.520	6.530	6.538	6.541
50	6.980	6.145	6.446	6.455	6.464	6.467
55	6.904	6.537	6.388	6.392	6.396	6.397

**Table 3. p(a<sub>H</sub>γ<sub>Cl</sub>)° of ACES + NaACES buffer solutions from 5°C to 55°C, computed using Equations (4)-(6)<sup>a</sup>.**

<i>T</i> (°C)	0.03 <i>m</i> ACES + 0.09 <i>m</i> NaACES + 0.07 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.02 <i>m</i> ACES + 0.08 <i>m</i> NaACES + 0.08 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.02 <i>m</i> ACES + 0.10 <i>m</i> NaACES + 0.06 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>
	5	7.809	7.902
10	7.702	7.796	8.030
15	7.599	7.693	7.926
20	7.500	7.595	7.828
25	7.406	7.501	7.731
30	7.318	7.411	7.639
35	7.231	7.325	7.544
37	7.197	7.291	7.515
40	7.146	7.241	7.434
45	7.067	7.161	7.384
50	6.989	7.084	7.305
55	6.912	7.005	7.228

<sup>a</sup>*m* = 1 mol·kg<sup>-1</sup>.

$$\log \gamma_{Cl}^{\circ} = -\frac{A\sqrt{I}}{1+1.5\sqrt{I}} \quad (5)$$

Equation (5) is assumed to be valid for concentrations  $I \leq 0.1 \text{ mol}\cdot\text{kg}^{-1}$ . For  $I > 0.1 \text{ mol}\cdot\text{kg}^{-1}$ , a more logical choice may be need.

Hence, an extended version of the Debye-Hückel, equation of the Bates-Guggenheim convention, has been selected to calculate  $\log \gamma_{Cl}^{\circ}$  for all buffer solutions containing  $Cl^{-}$ . This equation is shown below:

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

where “ $T$ ” is the ionic strength of the buffer solution, “ $A$ ” and “ $B$ ” are slope parameters, and “ $C$ ” is an adjustable parameter. The empirical equation for the calculation of the adjustable parameter “ $C$ ” is given below [6,8]:

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

where  $C_{25} = 0.032 \text{ kg}\cdot\text{mol}^{-1}$  at  $25^{\circ}\text{C}$  and  $T$  is the absolute temperature in Celsius.

The values of pH(s) for two buffer solutions without

NaCl are listed in **Table 4**. These values are expressed as a function of temperature.

$$\begin{aligned} \text{a. pH(s)} &= 6.734 + (1.90 \cdot 10^{-2})(T - 25) \\ &\quad - (8.60 \cdot 10^{-5})(T - 25)^2 \end{aligned} \quad (8)$$

$$\begin{aligned} \text{b. pH(s)} &= 6.949 + (1.88 \cdot 10^{-2})(T - 25) \\ &\quad - (5.01 \cdot 10^{-5})(T - 25)^2 \end{aligned} \quad (9)$$

The observed standard deviations of regression from are 0.003 and 0.002, respectively.

For the seven buffer solutions containing  $Cl^{-}$ , with an isotonic saline media ionic strength of  $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$ , the pH(s) values were also calculated using Equations (4) to (7). The acidity function data listed in **Tables 2** and **3** were used to generate the pH(s) data. These values of pH(s) are entered in **Tables 4** and **5** and are expressed by use of the following equations:

$$\begin{aligned} \text{a. pH(s)} &= 6.734 + (1.90 \cdot 10^{-2})(T - 25) \\ &\quad - (8.60 \cdot 10^{-5})(T - 25)^2 \end{aligned} \quad (10)$$

$$\begin{aligned} \text{b. pH(s)} &= 6.743 + (1.90 \cdot 10^{-2})(T - 25) \\ &\quad - (8.30 \cdot 10^{-5})(T - 25)^2 \end{aligned} \quad (11)$$

**Table 4. pH(s) for ACES + NaACES buffer solutions from 5°C to 55°C computed using Equations (4)-(7)<sup>a</sup> pH(s) for for ACES + NaACES buffer solutions from 5°C to 55°C, computed using Equations (4)-(6)<sup>a</sup>.**

$T(^{\circ}\text{C})$	0.02 m ACES + 0.06 m NaACES + 0.00 m NaCl $I = 0.06 \text{ m}$	0.04 m ACES + 0.08 m NaACES + 0.00 m NaCl $I = 0.08 \text{ m}$	0.08 m ACES + 0.08 m NaACES + 0.08 m NaCl $I = 0.16 \text{ m}$	0.06 m ACES + 0.06 m NaACES + 0.10 m NaCl $I = 0.16 \text{ m}$	0.04 m ACES + 0.04 m NaACES + 0.12 m NaCl $I = 0.16 \text{ m}$	0.03 m ACES + 0.03 m NaACES + 0.13 m NaCl $I = 0.16 \text{ m}$
5	7.691	7.346	7.142	7.149	7.156	7.159
10	7.589	7.241	7.034	7.041	7.049	7.053
15	7.491	7.141	6.930	6.939	6.947	6.591
20	7.394	7.045	6.831	6.841	6.852	6.856
25	7.300	6.949	6.736	6.744	6.751	6.755
30	7.214	6.854	6.645	6.653	6.661	6.664
35	7.125	6.768	6.556	6.564	6.571	6.575
37	7.096	6.733	6.522	6.531	6.540	6.543
40	7.043	6.677	6.471	6.480	6.489	6.493
45	6.962	6.589	6.391	6.401	6.408	6.412
50	6.886	6.510	6.315	6.325	6.337	6.337
55	6.809	6.431	6.256	6.261	6.264	6.266

<sup>a</sup> $m = 1 \text{ mol}\cdot\text{kg}^{-1}$ .

**Table 5. pH(s) for ACES + NaACES buffer solutions from 5°C to 55°C, computed using Equations (4)-(6)<sup>a</sup>.**

$T(^{\circ}\text{C})$	0.03 m ACES + 0.09 m NaACES + 0.07 m NaCl $I = 0.16 \text{ m}$	0.02 m ACES + 0.08 m NaACES + 0.08 m NaCl $I = 0.16 \text{ m}$	0.02 m ACES + 0.10 m NaACES + 0.06 m NaCl $I = 0.16 \text{ m}$
5	7.683	7.777	8.011
10	7.577	7.670	7.905
15	7.473	7.568	7.801
20	7.375	7.470	7.703
25	7.280	7.375	7.605
30	7.191	7.284	7.512
35	7.104	7.198	7.417
37	7.069	7.163	7.387
40	7.018	7.113	7.335
45	6.938	7.032	7.255
50	6.859	6.954	7.174
55	6.781	6.873	7.096

<sup>a</sup> $m = 1 \text{ mol}\cdot\text{kg}^{-1}$ .

**Table 6. Emf of Cell B and pH values with  $\delta E_j$  correction at 25°C and 37°C for ACES buffer.**

$m_1$	$m_2$	$m_3$	$I$	$E/V$		$\delta E_j^b/mV$		Without <sup>c</sup>	With <sup>d</sup>	Extended <sup>e</sup>	Without <sup>c</sup>	With <sup>d</sup>	Extended <sup>e</sup>
				25°C	37°C	25°C	37°C	$\delta E_j$ corr	$\delta E_j$ corr	D-H eqn.	$\delta E_j$ corr	$\delta E_j$ corr	D-H eqn.
				25°C	37°C	25°C	37°C	25°C	37°C				
0.02	0.06	0.00	0.06	0.67559	0.67263	0.3	0.4	7.294	7.299	7.300	7.089	7.095	7.096
0.02	0.08	0.08	0.16	0.67825	0.67498	2.1	2.2	7.338	7.374	7.375	7.127	7.162	7.163
0.03	0.09	0.07	0.16	0.67263	0.66917	2.1	2.2	7.244	7.279	7.280	7.033	7.068	7.069
0.02	0.01	0.06	0.16	0.69186	0.68874	2.1	2.2	7.569	7.604	7.605	7.351	7.386	7.387
Emf of Cell C <sup>a</sup>													
0.008695 <i>m</i> KH <sub>2</sub> PO <sub>4</sub> + 0.03043 <i>m</i> Na <sub>2</sub> HPO <sub>4</sub>				0.68275	0.69147	2.6	2.9						

<sup>a</sup>Corrected to a hydrogen pressure of 101.325 kPa for physiological phosphate buffer solutions (primary reference standard buffer) at 25°C and 37°C; <sup>b</sup> $\delta E_j = E + E_{SCE}^o - k^o$  pH from Equation (1); the pH of the primary standard phosphate buffer solution is 7.415 and 7.395 at 25°C and 37°C, respectively;  $E_{SCE}^o$  = electrode potential of the saturated calomel electrode = -0.2415 and -0.2335 at 25°C and 37°C [14], respectively; units of *m*, mol·kg<sup>-1</sup>; <sup>c</sup>Values obtained from Equation (2) where  $\delta E_j = 0$  and Table 6; <sup>d</sup>Obtained from Equation (2) and cell potential data from Table 6; <sup>e</sup>Obtained from extended Debye-Hückel (DH) equation of the Bates-Guggenheim convention.

$$\text{c. pH}(s) = 6.752 + (1.90 \cdot 10^{-2})(T - 25) - (7.80 \cdot 10^{-5})(T - 25)^2 \quad (12)$$

$$\text{d. pH}(s) = 6.756 + (1.90 \cdot 10^{-2})(T - 25) - (7.66 \cdot 10^{-5})(T - 25)^2 \quad (13)$$

$$\text{e. pH}(s) = 7.282 + (1.86 \cdot 10^{-2})(T - 25) - (6.68 \cdot 10^{-5})(T - 25)^2 \quad (14)$$

$$\text{f. pH}(s) = 7.376 + (1.86 \cdot 10^{-2})(T - 25) - (6.57 \cdot 10^{-5})(T - 25)^2 \quad (15)$$

$$\text{g. pH}(s) = 7.604 + (1.90 \cdot 10^{-2})(T - 25) - (6.90 \cdot 10^{-5})(T - 25)^2 \quad (16)$$

The observed standard deviations of regression from Equations (10)-(17) are 0.003, 0.002, 0.002, 0.002, 0.002, 0.002 and 0.002, respectively.

#### 4. Discussion and Conclusions

The operational pH values at 25°C and 37°C were evaluated from cells with a liquid junction (cells B and C) by means of the flowing junction cell [6,8]. The emf values of cells B and C at 25°C and 37°C are given in Table 6 for four buffer solutions of ACES. The values of  $\delta E_j$  are also listed in Table 6. The estimated uncertainties are due to 1) the calculation of  $\log \gamma_{Cl}$ , 2) extrapolation of the acidity function, 3) emf measurements, and 4) the estimation of  $\delta E_j$ . From Table 6, the pH values of four buffer solutions lie in the range of 7.1 to 7.6. Thus, these buffer solutions are recommended as useful standards for pH measurements in the clinical laboratory. The overall uncertainty of pH is within 0.01 in the experimental temperature range. Work is in progress for the calculation of pH of the buffer solution using the specific ionic interaction theory of Pitzer [17,18] for the calculation of  $\log \gamma_{Cl}$ . The main application of these pH data is to establish a unified pH scale applicable to a wide range of ionic str-

engths for practical pH measurements.

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