

# Buffer standards for the physiological pH of the zwitterionic buffer 3-[*N*-tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid (TAPSO) from (278.15 to 328.15) K

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## ABSTRACT

The second dissociation constant,  $pK_2$ , and related thermodynamic quantities for TAPSO have been previously determined and reported from the temperatures (278.15 to 328.15) K. In the current study there are five buffer solutions without NaCl and five buffer solutions with NaCl present which yield an ionic strength ( $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$ ) similar to that of blood plasma. These buffer solutions have been evaluated in the temperature range of (278.15 to 328.15) K using the extended Debye-Hückel equation, due to the limitations of the Bates-Guggenheim convention such that it is only valid when  $I \leq 0.1 \text{ mol}\cdot\text{kg}^{-1}$ . The liquid junction potential ( $E_j$ ) values between the TAPSO solution and the saturated KCl calomel electrode solution have been estimated at (298.15 and 310.15) K using a flowing junction cell measurement. The previously mentioned  $E_j$  values have been used in determining the operational pH values at (298.15 and 310.15) K. These TAPSO buffer solutions are recommended as reference solutions for pH measurements in saline media with an ionic strength of  $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$ .

**Keywords:** Zwitterionic; Buffer; pH; Liquid Junction; Physiological

## 1. INTRODUCTION

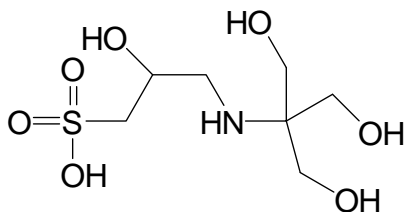
Good and his associates [1,2] have suggested several useful zwitterionic amino acid buffer solutions used in measuring the pH of blood and having the pH constrained so it closely reflects that of the physiological range (pH = 7 - 9). Very recently, the authors have reported the pH

values of 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid (MOPSO) [3] from (278.15 to 328.15) K, including 310.15 K. The zwitterionic buffer 3-[*N,N*-bis(2-hydroxymethyl)amino]-2-hydroxypropanesulfonic acid (DIPSO) [4] is also recommended for physiological use at (278.15 to 328.15) K. Regarding the current experiment the goal of the authors is to provide staunch pH values for the ampholyte 3-[*N*-tris(hydroxymethyl)-methylamino]-2-hydroxypropanesulfonic acid (TAPSO) depicted by the following structure:

This zwitterionic buffer, among others suggested by Bates and coworkers [5,10], may be used as biological buffers for biological media. The currently used NBS certified physiological phosphate primary standard buffer has recorded pH values of 7.415 and 7.395 at (298.15 and 310.15) K, respectively [5]. This phosphate buffer is comprised of  $\text{KH}_2\text{PO}_4$  ( $0.008695 \text{ mol}\cdot\text{kg}^{-1}$ ) and  $\text{Na}_2\text{HPO}_4$  ( $0.03043 \text{ mol}\cdot\text{kg}^{-1}$ ). Though it has been accepted by the NBS, problems still occur when using this particular phosphate buffer.

Some of the disadvantages concerning the phosphate buffer are: 1) phosphates precipitate some polyvalent cations in the blood, such as the constituents  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ ; 2) the phosphate may also inhibit or unnecessarily activate enzymatic processes such as some metabolic cycles; and 3) the phosphate buffer's temperature coefficient ( $-0.0028 \text{ pH unit}\cdot\text{K}^{-1}$ ) does not accurately approximate that of whole blood ( $-0.015 \text{ pH unit}\cdot\text{K}^{-1}$ ) [6]. The zwitterionic buffer compound TAPSO is not expected to yield such adverse effects, but the potential for complex formation with cations such as  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  does still exist. The likeliness of this occurring has been minimized with a high NaCl: buffer concentration ratio for an isotonic saline solution of  $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$ .

Good and coworkers established a group of hydrogen-ion buffers suitable for use within the physiological pH range [1,2]. They also cited published works from other



**Figure 1.** 3-[*N*-tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid (TAPSO).

researchers for structurally related zwitterionic buffer compounds and their substituents for comparison of their pH and  $pK_2$  values. Wu and associates [7] have published pH and  $pK_2$  values for *N*-(2-hydroxyethyl)piperazine-*N*-2-ethanesulfonic acid (HEPES), another zwitterionic buffer. Wu *et al.* [8] have also studied MOPSO using two point pH calibration measurements. Roy *et al.* [9] studied  $pK_2$  and pH values of 3-(*N*-morpholino)propanesulfonic acid (MOPS) and reported the results in the temperature range of (278.15 to 328.15) K, including 310.15 K. The previously mentioned buffer solutions have yielded a pH similar to common biological media.

The following compositions were examined with solutions (a) to (e) lacking the NaCl and solutions (f) to (j) containing NaCl resulting in an ionic strength of  $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$ :

- (a) TAPSO ( $0.04 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaTAPSO ( $0.02 \text{ mol}\cdot\text{kg}^{-1}$ )
- (b) TAPSO ( $0.06 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaTAPSO ( $0.03 \text{ mol}\cdot\text{kg}^{-1}$ )
- (c) TAPSO ( $0.05 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaTAPSO ( $0.05 \text{ mol}\cdot\text{kg}^{-1}$ )
- (d) TAPSO ( $0.06 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaTAPSO ( $0.06 \text{ mol}\cdot\text{kg}^{-1}$ )
- (e) TAPSO ( $0.08 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaTAPSO ( $0.08 \text{ mol}\cdot\text{kg}^{-1}$ )
- (f) TAPSO ( $0.02 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaTAPSO ( $0.04 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaCl ( $0.12 \text{ mol}\cdot\text{kg}^{-1}$ )
- (g) TAPSO ( $0.03 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaTAPSO ( $0.06 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaCl ( $0.10 \text{ mol}\cdot\text{kg}^{-1}$ )
- (h) TAPSO ( $0.04 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaTAPSO ( $0.02 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaCl ( $0.14 \text{ mol}\cdot\text{kg}^{-1}$ )
- (i) TAPSO ( $0.06 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaTAPSO ( $0.03 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaCl ( $0.13 \text{ mol}\cdot\text{kg}^{-1}$ )
- (j) TAPSO ( $0.04 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaTAPSO ( $0.04 \text{ mol}\cdot\text{kg}^{-1}$ ) + NaCl ( $0.12 \text{ mol}\cdot\text{kg}^{-1}$ )

The detailed procedure for preparation of the aforementioned TAPSO buffer solutions is described in the Experimental Section below.

## 2. EXPERIMENTAL

TAPSO was obtained from Research Organics (Cleveland, Ohio). Further crystallization was used for purification. This technique and the assay have been previously reported [8]. It was concluded from the assay that the TAPSO used was 99.99% pure. Buffer solutions (a) to (j) were prepared by massing the TAPSO buffer, ACS reagent grade NaCl, a standard NaOH solution (which al-

lowed the NaTAPSO to be prepared), and finally carefully calculated amounts of  $\text{CO}_2$ -free doubly distilled water. Buoyancy corrections were applied to all masses used during the preparation of the solutions.

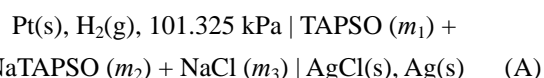
In previous publications, the cell design and preparation procedures for the chloroplatinic acid, hydrogen electrodes, purification using hydrogen gas, silver-silver chloride electrodes (of the thermal electrolytic type), and solution preparation have been described [9,12] Details about the equipment (including model numbers) and experimental techniques have also been previously reported [12].

## 3. METHODS AND RESULTS

The cell voltage values for the  $p_{aH}$  calculations are given in **Tables 1** and **2** for cell A. Cell A contains five solutions lacking their  $\text{Cl}^-$  component and five solutions with  $\text{Cl}^-$  to give them an ionic strength of  $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$ . The cell voltage values have been corrected to a hydrogen pressure of 101.325 kPa. At (298.15) K, cell voltage values are averaged from readings taken once at each portion of the reading (the beginning, middle, and end). Duplicate cells gave readings of  $\pm 0.04 \text{ mV}$  on average in the temperature range under study.

### 3.1. pH of the TAPSO Buffer

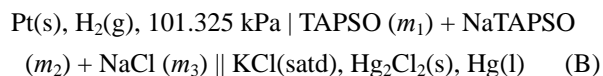
The Bates *et al.* [10,11,16-18] method has been used to evaluate the conventional standard pH values for solutions (a) to (j) described in the Introduction. For accurate calculations of the ten buffer solutions, the following cell A was used for the collection of cell potential data:



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

### 3.2. Evaluation of Liquid Junction Potential

Cell B, the flowing junction cell, was used to evaluate the liquid junction potential at the contact point between the buffer solution and the heavier, saturated KCl solution shown with a double vertical line as follows:



where the abbreviations “s,” “l,” and “g” indicate the solid, liquid, and gaseous states, respectively. In routine laboratory measurements, a glass electrode commonly replaces the hydrogen electrode. For cell B, the values of the standard electrode potential, denoted as  $E_{SCE}^\circ$ , of the saturated calomel electrode were recorded as  $-0.2415 \text{ V}$

**Table 1.** Cell potential of cell A (in volts): Pt(s); H<sub>2</sub>(g), 101.325 kPa | TAPSO (*m*<sub>1</sub>), NaTAPSO (*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>	T/K											
mol·kg <sup>-1</sup>			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	0.02	0.005	0.79300	0.79326	0.79335	0.79328	0.79308	0.79289	0.79252	0.79233	0.79214	0.79150	0.79086	0.79027
0.04	0.02	0.010	0.77688	0.77688	0.77666	0.77628	0.77580	0.77529	0.77462	0.77431	0.77393	0.77303	0.77210	0.77120
0.04	0.02	0.015	0.76749	0.76726	0.76685	0.76632	0.76566	0.76499	0.76416	0.76378	0.76328	0.76221	0.76113	0.76004
0.04	0.02	0.020	0.76103	0.76069	0.76019	0.75957	0.75876	0.75797	0.75702	0.75661	0.75602	0.75482	0.75363	0.75244
0.06	0.03	0.005	0.78725	0.78726	0.78703	0.78682	0.78651	0.78601	0.78568	0.78588	0.78512	0.78463	0.78410	0.78315
0.06	0.03	0.010	0.77285	0.77256	0.77202	0.77169	0.77112	0.77008	0.76940	0.76991	0.76895	0.76803	0.76712	0.76555
0.06	0.03	0.015	0.76510	0.76468	0.76429	0.76370	0.76295	0.76192	0.76096	0.76175	0.76034	0.75947	0.75822	0.75604
0.06	0.03	0.020	0.76033	0.75994	0.75937	0.75870	0.75787	0.75657	0.75539	0.75684	0.75497	0.75383	0.75237	0.74966
0.05	0.05	0.005	0.80442	0.80501	0.80548	0.80590	0.80592	0.80600	0.80599	0.80597	0.80573	0.80578	0.80545	0.80499
0.05	0.05	0.010	0.78848	0.78870	0.78891	0.78898	0.78872	0.78852	0.78821	0.78806	0.78765	0.78740	0.78679	0.78605
0.05	0.05	0.015	0.77916	0.77916	0.77919	0.77907	0.77865	0.77831	0.77780	0.77756	0.77707	0.77664	0.77588	0.77495
0.05	0.05	0.020	0.77281	0.77262	0.77257	0.77231	0.77174	0.77129	0.77067	0.77041	0.76983	0.76929	0.76841	0.76738
0.06	0.06	0.005	0.80675	0.80721	0.80727	0.80740	0.80757	0.80768	0.80796	0.80780	0.80800	0.80803	0.80834	0.80868
0.06	0.06	0.010	0.79064	0.79085	0.79060	0.79046	0.79033	0.79021	0.79018	0.78985	0.78991	0.78962	0.78964	0.78968
0.06	0.06	0.015	0.78124	0.78123	0.78089	0.78055	0.78029	0.78002	0.77977	0.77939	0.77935	0.77885	0.77877	0.77861
0.06	0.06	0.020	0.77465	0.77457	0.77408	0.77361	0.77328	0.77293	0.77259	0.77208	0.77201	0.77134	0.77112	0.77087
0.08	0.08	0.005	0.80454	0.80532	0.80594	0.80619	0.80627	0.80619	0.80616	0.80589	0.80591	0.80537	0.80488	0.80420
0.08	0.08	0.010	0.78849	0.78897	0.78926	0.78919	0.78901	0.78866	0.78843	0.78797	0.78780	0.78696	0.78616	0.78519
0.08	0.08	0.015	0.78067	0.78102	0.78121	0.78097	0.78064	0.78015	0.77978	0.77924	0.77905	0.77803	0.77710	0.77599
0.08	0.08	0.020	0.77269	0.77288	0.77288	0.77261	0.77209	0.77144	0.77085	0.77034	0.77003	0.76889	0.76782	0.76658

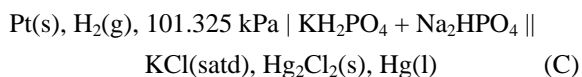
**Table 2.** Cell potential of cell A (in volts): Pt(s); H<sub>2</sub>(g), 101.325 kPa | TAPSO (*m*<sub>1</sub>), NaTAPSO (*m*<sub>2</sub>), NaCl (*m*<sub>3</sub>) | AgCl(s), Ag(s).

<i>m</i> <sub>1</sub>	<i>m</i> <sub>2</sub>	<i>m</i> <sub>3</sub>	T/K											
mol·kg <sup>-1</sup>			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	0.12	0.75257	0.75197	0.75126	0.75044	0.74949	0.74843	0.74722	0.74676	0.74601	0.74465	0.74313	0.74157
0.03	0.06	0.10	0.75278	0.75239	0.75184	0.75091	0.75017	0.74912	0.74824	0.74785	0.74689	0.74576	0.74428	0.74273
0.04	0.02	0.14	0.71657	0.71536	0.71421	0.71291	0.71104	0.70929	0.70743	0.70656	0.70533	0.70336	0.70121	0.69912
0.06	0.03	0.13	0.71759	0.71639	0.71530	0.71381	0.71221	0.71053	0.70870	0.70802	0.70687	0.70466	0.70263	0.70061
0.04	0.04	0.12	0.73711	0.73637	0.73554	0.73443	0.73318	0.73192	0.73058	0.73003	0.72912	0.72746	0.72577	0.72400

and -0.2335 V at (298.15 and 310.15) K, respectively. These values are periodically confirmed through experimentation.

### 3.3. Application in Physiological Media

For cell C, the phosphate salts were NBS standard reference materials whose compositions were mentioned in the Introduction. Its solutions are recommended for pH measurements in physiological media. Cell C's cell diagram is as follows:



### 3.4. Application of Liquid Junction Corrections

The  $E_{SCE}^\circ$  values of the saturated calomel electrode were recorded as -0.2415 and -0.2335 at (298.15 and 310.15) K, respectively [8,9]. The  $E_j$  values were also obtained by use of the flowing junction cell [9]. The  $E_j$  values of the standard buffer solution, as well as others in cell B, were calculated using the following equation:

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

where  $k = 0.059156$  and  $\text{pH} = 7.415$  at (298.15) K and  $k = 0.061538$  and  $\text{pH} = 7.395$  at (310.15) K. The pH inputs are that of the standard phosphate buffer solution. The

operational definition of pH, denoted as pH(x), can be calculated by use of the subsequent equation:

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

where the subscript “x” refers to the unknown buffer (in this case, a solution of TAPSO + NaTAPSO), “s” is the NBS standard buffer solution of a known pH, and  $\delta E_j = E_{j(s)} - E_{j(x)}$ . The  $\delta E_j$  value is of interest rather than the actual  $E_j$  value in mV.

To calculate the  $\text{p}a_{\text{H}}$  values for the buffer solutions under investigation, the acidity function was calculated, denoted as  $\text{p}(a_{\text{H}}\gamma_{\text{Cl}})$ , in the temperature range of (278.15 to 328.15) K. These calculations were made using the cell voltage ( $E$ ) values listed in **Tables 1** and **2**, the molality of the chloride ion, and the standard electrode potential of the silver-silver chloride electrode ( $E^\circ$ ). The tool used to calculate  $\text{p}(a_{\text{H}}\gamma_{\text{Cl}})$ , the Nernst equation [11, 12], is shown below:

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

where “ $k$ ” is the Nernst slope.

### 3.5. Plotting of Results

When plotting  $\text{p}(a_{\text{H}}\gamma_{\text{Cl}})$  against the molality of the chloride ion, linear regression analysis is used to determine the y-intercept to give a  $\text{p}(a_{\text{H}}\gamma_{\text{Cl}})^\circ$  value at  $m_{\text{Cl}} = 0$ . These  $\text{p}(a_{\text{H}}\gamma_{\text{Cl}})^\circ$  values for the five chloride-free buffer solutions are listed in **Table 3**. The mean deviation from this linear regression technique was approximately 0.002 from the lines generated. The  $\text{p}(a_{\text{H}}\gamma_{\text{Cl}})$  values for the buffer solutions containing  $\text{Cl}^-$  are listed in **Table 4** from (278.15 to 328.15) K.

### 3.6. Calculation of pH Results

Conventional  $\text{p}a_{\text{H}}$  values for solutions without a liquid junction and a chloride ion were determined using the following equation:

$$\text{p}a_{\text{H}} = \text{p}(a_{\text{H}}\gamma_{\text{Cl}})^\circ + \log \gamma_{\text{Cl}}^\circ \quad (4)$$

where the single-ion activity coefficient,  $\gamma_{\text{Cl}}^\circ$ , cannot be experimentally measured. A previous publication describes 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 **Eq. 5**, is denoted as  $\text{p}a_{\text{H}}$ . The “pH convention,” commonly known as the Bates-Guggenheim convention [17] is expressed by the use of the successive equation:

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

The International Union of Pure and Applied Chemis-

try has recommended this convention. **Eq. 5** is assumed to be true for concentrations  $I \leq 0.1 \text{ mol}\cdot\text{kg}^{-1}$ . For  $I > 0.1 \text{ mol}\cdot\text{kg}^{-1}$ , there is no widely accepted convention. A more logical choice for solutions with  $I > 0.01 \text{ mol}\cdot\text{kg}^{-1}$  may need to include a linear-dependent “ $CT$ ” term from **Eq. 6** with the ion size parameter as a function of temperature.

Due to this need for alteration, a pH convention [7,9] based on an extended version of the Debye-Hückel equation [7] has been selected as a more logical approach to calculate  $\log \gamma_{\text{Cl}}^\circ$  when  $(0.1 < I \leq 1.0) \text{ mol}\cdot\text{kg}^{-1}$  for all of the buffer solutions containing  $\text{Cl}^-$ . This equation is shown below:

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

where “ $T$ ” is the ionic strength of the buffer solution, “ $A$ ” and “ $B$ ” are slope parameters known as the Debye-Hückel constants, and “ $C$ ” is an adjustable parameter whose explanation will follow shortly.  $Ba^\circ$  was assumed to be  $1.38 \text{ kg}^{1/2}\cdot\text{mol}^{-1/2}$  for all the experimental temperatures. This corresponded to an ion size parameter,  $a^\circ$ , of  $4.2 \text{ \AA}$  [7,9]. The empirical equation that follows is used for the calculation of the adjustable parameter “ $C$ ” and was obtained from a curve-fitting method [7,9].

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

where  $C_{298.15} = 0.032 \text{ kg}\cdot\text{mol}^{-1}$  at (298.15) K [7] and  $T$  is the absolute temperature in Kelvin.

The  $\text{p}a_{\text{H}}$  values listed in **Table 5** for the five TAPSO buffer solutions without the presence of  $\text{Cl}^-$  were calculated using the following equations with their respective solution denotations:

$$\text{p}a_{\text{H}} = 7.280 + (-2.23 \times 10^{-2})(T - 298.15) + (8.32 \times 10^{-5})(T - 298.15)^2 \quad (8)$$

$$\text{p}a_{\text{H}} = 7.125 + (-2.24 \times 10^{-2})(T - 298.15) + (1.02 \times 10^{-4})(T - 298.15)^2 \quad (9)$$

$$\text{p}a_{\text{H}} = 7.471 + (-2.19 \times 10^{-2})(T - 298.15) + (7.92 \times 10^{-5})(T - 298.15)^2 \quad (10)$$

$$\text{p}a_{\text{H}} = 7.493 + (-2.20 \times 10^{-2})(T - 298.15) + (1.15 \times 10^{-4})(T - 298.15)^2 \quad (11)$$

$$\text{p}a_{\text{H}} = 7.460 + (-2.21 \times 10^{-2})(T - 298.15) + (6.51 \times 10^{-5})(T - 298.15)^2 \quad (12)$$

for the temperature range of (278.15 to 328.15) K. The standard deviations of regression for the  $\text{p}a_{\text{H}}$  of the chloride-free buffer solutions are obtained from lines of best

**Table 3.**  $p(a_{\text{H}^+}/\text{Cl})^\circ$  of (TAPSO + NaTAPSO) buffer solutions from 278.15 K to 328.15 K obtained by extrapolation for chloride-free solutions.

<i>T</i> /K	0.04 <i>m</i> TAPSO + 0.02 <i>m</i> NaTAPSO <i>I</i> = 0.02 <i>m</i>	0.06 <i>m</i> TAPSO + 0.03 <i>m</i> NaTAPSO <i>I</i> = 0.03 <i>m</i>	0.05 <i>m</i> TAPSO + 0.05 <i>m</i> NaTAPSO <i>I</i> = 0.05 <i>m</i>	0.06 <i>m</i> TAPSO + 0.06 <i>m</i> NaTAPSO <i>I</i> = 0.06 <i>m</i>	0.08 <i>m</i> TAPSO + 0.08 <i>m</i> NaTAPSO <i>I</i> = 0.08 <i>m</i>
278.15	7.818	7.684	8.024	8.069	8.027
283.15	7.692	7.554	7.901	7.942	7.906
288.15	7.570	7.426	7.781	7.814	7.790
293.15	7.451	7.309	7.669	7.696	7.673
298.15	7.338	7.196	7.556	7.584	7.561
303.15	7.231	7.085	7.449	7.477	7.452
308.15	7.126	6.985	7.347	7.379	7.350
310.15	7.085	6.943	7.307	7.338	7.306
313.15	7.027	6.883	7.246	7.283	7.248
318.15	6.930	6.789	7.156	7.193	7.149
323.15	6.836	6.702	7.064	7.110	7.055
328.15	6.748	6.617	6.974	7.032	6.962

<sup>a</sup>*m* = 1 mol·kg<sup>-1</sup>.**Table 4.**  $p(a_{\text{H}^+}/\text{Cl})$  of (TAPSO + NaTAPSO) buffer solutions from 278.15 K to 328.15 K, computed using Eq.4<sup>a</sup>.

<i>T</i> /K	0.02 <i>m</i> TAPSO + 0.04 <i>m</i> NaTAPSO + 0.12 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.03 <i>m</i> TAPSO + 0.06 <i>m</i> NaTAPSO + 0.10 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.04 <i>m</i> TAPSO + 0.02 <i>m</i> NaTAPSO + 0.14 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.06 <i>m</i> TAPSO + 0.03 <i>m</i> NaTAPSO + 0.13 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>	0.04 <i>m</i> TAPSO + 0.04 <i>m</i> NaTAPSO + 0.12 <i>m</i> NaCl <i>I</i> = 0.16 <i>m</i>
278.15	8.473	8.397	7.887	7.873	8.192
283.15	8.344	8.272	7.759	7.745	8.066
288.15	8.220	8.151	7.640	7.626	7.945
293.15	8.102	8.031	7.524	7.507	7.827
298.15	7.989	7.921	7.406	7.393	7.713
303.15	7.879	7.811	7.295	7.284	7.605
308.15	7.772	7.710	7.188	7.177	7.500
310.15	7.732	7.670	7.146	7.137	7.460
313.15	7.671	7.606	7.084	7.076	7.400
318.15	7.574	7.513	6.987	6.975	7.302
323.15	7.479	7.418	6.892	6.882	7.208
328.15	7.387	7.326	6.802	6.793	7.117

<sup>a</sup>*m* = 1 mol·kg<sup>-1</sup>.**Table 5.**  $p a_{\text{H}^+}$  of (TAPSO + NaTAPSO) buffer solutions from 278.15 K to 328.15 K, computed using Eqs.4-7<sup>a</sup>.

<i>T</i> /K	0.04 <i>m</i> TAPSO + 0.02 <i>m</i> NaTAPSO <i>I</i> = 0.02 <i>m</i>	0.06 <i>m</i> TAPSO + 0.03 <i>m</i> NaTAPSO <i>I</i> = 0.03 <i>m</i>	0.05 <i>m</i> TAPSO + 0.05 <i>m</i> NaTAPSO <i>I</i> = 0.05 <i>m</i>	0.06 <i>m</i> TAPSO + 0.06 <i>m</i> NaTAPSO <i>I</i> = 0.06 <i>m</i>	0.08 <i>m</i> TAPSO + 0.08 <i>m</i> NaTAPSO <i>I</i> = 0.08 <i>m</i>
278.15	7.759	7.615	7.940	7.979	7.927
283.15	7.633	7.485	7.817	7.851	7.807
288.15	7.511	7.357	7.697	7.724	7.689
293.15	7.392	7.239	7.584	7.606	7.573
298.15	7.279	7.125	7.470	7.492	7.460
303.15	7.171	7.015	7.363	7.385	7.350
308.15	7.065	6.914	7.260	7.286	7.247
310.15	7.024	6.872	7.220	7.245	7.203
313.15	6.966	6.811	7.158	7.190	7.145
318.15	6.868	6.717	7.068	7.098	7.045
323.15	6.774	6.629	6.975	7.015	6.950
328.15	6.685	6.543	6.885	6.936	6.856

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

fit using Eqs.3-7. These standard deviations are 0.0009, 0.0014, 0.0016, 0.0012 and 0.0012, respectively.

For the five buffer solutions containing Cl<sup>-</sup>, with an isotonic saline media ionic strength of  $I = 0.16 \text{ mol}\cdot\text{kg}^{-1}$ , the  $p\text{a}_\text{H}$  values were also calculated using Eqs.3-7. The acidity function data from Table 4 was also used to generate the  $p\text{a}_\text{H}$  data residing in Table 6. These values of  $p\text{a}_\text{H}$  are expressed by use of the following equations:

$$(a) \quad p\text{a}_\text{H} = 7.863 + (-2.26 \times 10^{-2})(T - 298.15) + (7.94 \times 10^{-5})(T - 298.15)^2 \quad (13)$$

$$(b) \quad p\text{a}_\text{H} = 7.795 + (-2.22 \times 10^{-2})(T - 298.15) + (7.61 \times 10^{-5})(T - 298.15)^2 \quad (14)$$

$$(c) \quad p\text{a}_\text{H} = 7.279 + (-2.26 \times 10^{-2})(T - 298.15) + (7.64 \times 10^{-5})(T - 298.15)^2 \quad (15)$$

$$(d) \quad p\text{a}_\text{H} = 7.267 + (-2.25 \times 10^{-2})(T - 298.15) + (7.59 \times 10^{-5})(T - 298.15)^2 \quad (16)$$

$$(e) \quad p\text{a}_\text{H} = 7.588 + (-2.24 \times 10^{-2})(T - 298.15) + (7.76 \times 10^{-5})(T - 298.15)^2 \quad (17)$$

The observed standard deviations of regression from Eqs.13-17 are 0.0012, 0.0019, 0.0019, 0.0011 and 0.0010, respectively.

## 4. DISCUSSION

The operational pH values at (298.15 and 310.15) K were evaluated from cells with a liquid junction (cells B

and C) using the flowing junction cell [7,9]. The cell voltage values of cells B and C at (298.15 and 310.15) K are given in Table 7. The values of  $E_j$  listed in Table 8 were obtained using Eq.1. The widely used equation for the calculation of  $\log \gamma_{\text{Cl}^-}^\circ$  is based on the Bates-Guggenheim convention [3,6,7,10] and is valid for concentrations of  $I \leq 0.1 \text{ mol}\cdot\text{kg}^{-1}$ . The total standard uncertainty for the  $p\text{a}_\text{H}$  values was accounted for by combining various known sources of error: 1) extrapolation of the  $p(\text{a}_\text{H}/\gamma_{\text{Cl}^-})^\circ$  plot for Cl<sup>-</sup> free solutions (error is  $\leq \pm 0.002$  pH unit); 2) assumption for the calculation of  $\log \gamma_{\text{Cl}^-}^\circ$  using Eq.6 (error of  $\pm 0.004$  pH unit); and 3) the error in the experimental measurement from the multimeter (an error of  $\pm 0.02$  mV). This yields an overall error of  $\pm 0.012$  pH and  $\pm 0.006$  pH unit for buffer solutions with and without the presence of Cl<sup>-</sup>, respectively. Errors in values of  $E_j$  are irrelevant to the values of  $p\text{a}_\text{H}$  determined from cell A without liquid junction; however,  $E_j$  of Eq.2 does affect the operational  $p\text{a}_\text{H}$  values listed in Table 9 at (298.15 and 310.15) K.

## 5. CONCLUSIONS

The four buffer solutions listed in Tables 7-9 are recommended as useful secondary pH standards for calibrating electrodes for pH measuring assembly in the physiological range. The consistency of the three sets of experiments listed in Table 9 leads credibility to the pH values of these TAPSO buffer solutions as primary standards for physiological use.

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**Table 6.**  $p\text{a}_\text{H}$  of (TAPSO + NaTAPSO) buffer solutions from 278.15 to 328.15 K, computed using Eqs.4-7<sup>a</sup>.

$T/\text{K}$	0.02 m TAPSO + 0.04 m NaTAPSO + 0.12 m NaCl $I = 0.16 \text{ m}$	0.03 m TAPSO + 0.06 m NaTAPSO + 0.10 m NaCl $I = 0.16 \text{ m}$	0.04 m TAPSO + 0.02 m NaTAPSO + 0.14 m NaCl $I = 0.16 \text{ m}$	0.06 m TAPSO + 0.03 m NaTAPSO + 0.13 m NaCl $I = 0.16 \text{ m}$	0.04 m TAPSO + 0.04 m NaTAPSO + 0.12 m NaCl $I = 0.16 \text{ m}$
278.15	8.347	8.272	7.762	7.748	8.067
283.15	8.218	8.147	7.634	7.620	7.941
288.15	8.095	8.026	7.514	7.500	7.820
293.15	7.977	7.906	7.399	7.382	7.702
298.15	7.862	7.794	7.279	7.267	7.587
303.15	7.752	7.684	7.168	7.157	7.478
308.15	7.644	7.582	7.061	7.049	7.373
310.15	7.604	7.542	7.018	7.009	7.332
313.15	7.543	7.478	6.955	6.948	7.272
318.15	7.445	7.383	6.858	6.846	7.173
323.15	7.349	7.287	6.762	6.752	7.078
328.15	7.256	7.194	6.671	6.661	6.986

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

**Table 7.** Cell voltage of cell B for TAPSO buffer.

$m_1$	$m_2$	$m_3$	$E/V$	
mol·kg <sup>-1</sup>			298.15 K	310.15 K
0.04	0.02	0.00	0.67440	0.66834
0.03	0.06	0.10	0.70292	0.69838
0.04	0.04	0.12	0.69073	0.68526

Cell Voltage of Cell C<sup>a</sup>

Cell C		$E/V$	
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>		298.15 K	310.15 K
		0.68275	0.69147

<sup>a</sup>Corrected to a hydrogen pressure of 101.325 kPa for physiological phosphate buffer solutions (primary reference standard buffer) at 298.15 K and 310.15 K.

**Table 8.** Values of the liquid junction potentials for TAPSO buffer at (298.15 and 310.15) K.

System	$E_j^a / mV$	
	298.15 K	310.15 K
Physiological phosphate (0.008695 <i>m</i> KH <sub>2</sub> PO <sub>4</sub> + 0.03043 <i>m</i> NaCl)	2.6	2.9
0.04 <i>m</i> TAPSO + 0.02 <i>m</i> NaTAPSO + 0.00 <i>m</i> NaCl	2.3	2.6
0.03 <i>m</i> TAPSO + 0.06 <i>m</i> NaTAPSO + 0.10 <i>m</i> NaCl	0.4	0.8
0.04 <i>m</i> TAPSO + 0.04 <i>m</i> NaTAPSO + 0.12 <i>m</i> NaCl	0.4	0.6

<sup>a</sup> $E_j = E + E_{sce}^+ - k^{\circ} \text{pH}$  from Eq.1 is the Emf from Table 7,  $k$  = Nernst slope with values 0.059156 at 298.15 K, and 0.061538 at 310.15 K; the pH of the primary reference standard phosphate buffer is 7.415 and 7.395 at 298.15 K and 310.15 K, respectively;  $E_{sce}^+$  = electrode potential of the saturated calomel electrode = -0.2415 and -0.2335 at 298.15 K and 310.15 K, respectively; units of  $m$ , mol·kg<sup>-1</sup>.

**Table 9.** Values of pH at (298.15 and 310.15) K for TAPSO buffer solutions.

Cell B				298.15 K			310.15 K		
$m_1$	$m_2$	$m_3$	$I$	Without <sup>a</sup> $E_j$ corr	With <sup>b</sup> $E_j$ corr	Calc <sup>c</sup>	Without <sup>a</sup> $E_j$ corr	With <sup>b</sup> $E_j$ corr	Calc <sup>c</sup>
mol·kg <sup>-1</sup>									
0.04	0.02	0.00	0.04	7.274	7.279	7.279	7.019	7.024	7.024
0.03	0.06	0.10	0.16	7.756	7.794	7.794	7.507	7.541	7.542
0.04	0.04	0.12	0.16	7.550	7.587	7.587	7.294	7.331	7.332

<sup>a</sup>Values obtained from Eq.2 and data in Table 7; <sup>b</sup>Obtained from Eq.2 and  $E_j$  values in Table 8; <sup>c</sup>Obtained from Tables 5 and 6.

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