

Acid Dissociation Constants and Related Thermodynamic Functions of Protonated 2,2-Bis(Hydroxymethyl)-2,2',2''-Nitrilotriethanol (BIS-TRIS) from (278.15 to 328.15) K

Rabindra N. Roy, Lakshmi N. Roy, Katherine E. Hundley, John J. Dinga, Mathew R. Medcalf, Lucas S. Tebbe, Ryan R. Parmar, Jaime A. Veliz

Hoffman Department of Chemistry, Drury University, Springfield, USA
Email: roy@drury.edu

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Abstract

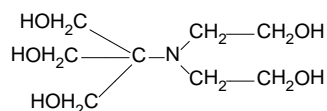
Thermodynamic dissociation constants pK_a of 2,2-bis(hydroxymethyl)-2,2',2''-nitrilotriethanol have been determined at 12 temperatures from (278.15 to 328.15) K including the body temperature 310.15 K by the electromotive-force measurements (emf) of hydrogen-silver chloride cells without liquid junction of the type: Pt(s), H₂(g), 101.325 kPa|BIS-TRIS (m) + BIS-TRIS·HCl (m)|AgCl(s), Ag(s), where m denotes molality. The pK_a values for the dissociation process of BIS-TRIS·H⁺ + H₂O = H₃O⁺ + BIS-TRIS given as a function of T in Kelvin (K) by the equation $pK_a = 921.66 (K/T) + 14.0007 - 1.86197 \ln(T/K)$. At 298.15 and 310.15 K, the values of pK_a for BIS-TRIS were found to be 6.4828 ± 0.0005 and 6.2906 ± 0.0006 respectively. Thus buffer solutions composed of BIS-TRIS and its hydrochloride would be useful as secondary pH buffer standards and for control of acidity in the pH range 6 to 8. At 298.15 K the thermodynamic functions ΔG° , ΔH° , ΔS° and ΔC_p° for the dissociation process of BIS-TRIS·H⁺ are $\Delta G^\circ = 37,005 \text{ J}\cdot\text{mol}^{-1}$, $\Delta H^\circ = 28,273 \text{ J}\cdot\text{mol}^{-1}$, $\Delta S^\circ = 29.3 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ and $\Delta C_p^\circ = 36 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$. These results are compared with the dissociation of protonated bases structurally related to BIS-TRIS·H⁺.

Keywords

Dissociation Constant, pH, Buffer, emf, Thermodynamic Function, Ionic Strength

1. Introduction

Many biochemical reactions that normally occur in living systems are highly sensitive to pH. For example, some enzyme catalysts involved in important biochemical reactions become very significant and effective with a narrow range of pH [1]. The phosphate buffer has been widely used as a biological pH standard, but it is not an ideal pH standard in the pH range 6 to 8 for physiological application [2]-[5]. The buffer substances recommended by Good *et al.* [6] have proved useful for clinical pH measurements in the range 7 to 9. These compounds, mostly protonated amines or N-substituted amino acids, are compatible with many media including isotonic saline solution of physiological importance. BIS-TRIS is a free base. The nitrogen atom is protonated to form BIS-TRIS·H⁺ which dissociated to BIS-TRIS + H⁺. The dissociation constant is abbreviated pK_a. The biological buffers listed in the text have pK₁ and pK₂ values. However, only pK₂ values are mentioned because these values lie in the physiological pH range, whereas the pK₁ values do not. In the present study BIS-TRIS has only one pK₁ designated as pK_a, whose value does lie in the physiological pH range of interest. In previous work from the authors laboratory and by other investigators, the pK_a values and associated thermodynamic functions of N-substituted glycine including N-tris(hydroxymethyl)methylglycine (TRICINE) [7] and N,N-bis(2-hydroxyethyl)glycine [8] have been reported. Other substances that have been studied include tris(hydroxymethyl)aminomethane (TRIS or THAM) [9], and 2-ammonium-2-methyl-1,3-propanediol (AMP) [10]. The pK_a values of N, N-bis(2-hydroxyethyl) known as BIS-TRIS and a derivative of (TRIS) have been determined from (273.15 to 323.15) K by Paabo and Bates [11]. At 298.15 K, its pK_a value is 6.483. Thus it has a great potential to be a useful buffer standard for pH control in the pH region of 6 to 8 close to that of blood serum. In order to provide very accurate, reliable and reproducible pH data in isotonic saline solutions we have now undertaken to study in detail the acid-base and thermodynamic properties of protonated (BIS-TRIS) at 12 temperatures from (278.15 to 328.15) K at 5° intervals. The free base (BIS-TRIS) has the following structure.



The precise electromotive force (emf) measurements were made using hydrogen and silver-silver chloride electrodes without liquid junction of the type:



For all 16 buffer solutions, the molalities of (BIS-TRIS) and (BIS-TRIS·HCl) were of equal molality (m). It is to be noted that the solubility of the silver chloride in the presence of [BIS-TRIS] base to form silver complex ion is negligible [12], hence no molality correction is necessary for the chloride ion.

2. Experimental

The commercial sample of (BIS-TRIS) obtained from Research Organics (Cleveland, Ohio) was used without further purification. It was 99% pure. Preliminary measurements gave emf data within ± 0.04 mV for the purified and unpurified sample. Thus the unpurified material was used as received and was always dried and stored in a desiccator. Hydrochloric acid was prepared from the constant boiling twice distilled 6 M acid. The middle fraction of the distillate was used. The concentration of the hydrochloric acid solution was determined gravimetrically by weighing as AgCl from the precipitation with AgNO₃. All sixteen different buffer concentrations were prepared by adding a standard solution of hydrochloric acid to a solution of (BIS-TRIS) base in such amount as to produce equal molalities of the base and its hydrochloride. The molality range was 0.009 to 0.08 mol·kg⁻¹. The buffer ratio of (BIS-TRIS) and [BIS-TRIS·HCl] was 1 and the pH of the buffer solution was very close to neutral. Thus hydrolysis correction of the basic species to m was unnecessary. Vacuum corrections were applied to all these masses.

3. Methods and Results

In previous communications [7] [13] [14] the preparation of the hydrogen and silver-silver chloride electrode (thermal electrolytic type), cell design, preparation of cell solutions followed usual procedure of the author's la-

boratory. The cell voltage (emf) of the cell (A) for 16 solutions from (278.15 to 328.15) K corrected to 1 atm hydrogen partial pressure, is given in **Table 1**. At 298.15 K, agreement of the emf readings among the initial, middle and sometimes final (at the end of the temperature series) on the average were 0.04 mV. The thermodynamic dissociation constant of pK_a for cell (A) is expressed as:

$$pK_a = (E - E^\circ)F / (RT \cdot \ln 10) + \log m_{\text{BTH}^+} + \log(\gamma_{\text{BTH}^+} \gamma_{\text{Cl}^-}) \quad (1)$$

Table 1. Electromotive force (emf) of Cell A (in volts): Pt(s); H₂(g), 1 atm|BIS-TRIS (m)+ BIS-TRIS·HCl(m)|AgCl(s), Ag(s).

m^a	278.15 K	283.15 K	288.15 K	293.15 K	298.15 K	303.15 K
0.008998	0.72877	0.72982	0.73071	0.73138	0.73193	0.73228
0.009989	0.72656	0.72757	0.72843	0.72906	0.72956	0.72988
0.014998	0.71770	0.71856	0.71926	0.71974	0.72010	0.72026
0.020003	0.71143	0.71219	0.71278	0.71314	0.71341	0.71346
0.024994	0.70668	0.70735	0.70786	0.70814	0.70833	0.70830
0.030001	0.70289	0.70350	0.70395	0.70416	0.70429	0.70420
0.035044	0.69962	0.70017	0.70057	0.70072	0.70080	0.70065
0.039982	0.69678	0.69729	0.69764	0.69774	0.69779	0.69759
0.044982	0.69433	0.69480	0.69511	0.69517	0.69518	0.69493
0.049940	0.69223	0.69266	0.69293	0.69295	0.69293	0.69265
0.055064	0.69013	0.69052	0.69076	0.69074	0.69069	0.69038
0.059975	0.68842	0.68879	0.68901	0.68896	0.68888	0.68853
0.065167	0.68664	0.68698	0.68716	0.68709	0.68699	0.68661
0.069991	0.68517	0.68549	0.68565	0.68555	0.68542	0.68502
0.074995	0.68381	0.68410	0.68425	0.68413	0.68399	0.68356
0.080022	0.68240	0.68267	0.68279	0.68264	0.68248	0.68203
m^a	308.15 K	310.15 K	313.15 K	318.15 K	323.15 K	328.15 K
0.008998	0.73250	0.73254	0.73256	0.73238	0.73207	0.73163
0.009989	0.73006	0.73009	0.73008	0.72986	0.72951	0.72904
0.014998	0.72029	0.72026	0.72016	0.71979	0.71929	0.71867
0.020003	0.71339	0.71332	0.71315	0.71267	0.71207	0.71133
0.024994	0.70815	0.70805	0.70783	0.70727	0.70659	0.70578
0.030001	0.70398	0.70385	0.70358	0.70297	0.70222	0.70135
0.035044	0.70038	0.70022	0.69993	0.69925	0.69845	0.69752
0.039982	0.69726	0.69709	0.69676	0.69604	0.69515	0.69422
0.044982	0.69456	0.69437	0.69402	0.69325	0.69237	0.69135
0.049940	0.69225	0.69204	0.69166	0.69086	0.68994	0.68889
0.055064	0.68994	0.68971	0.68932	0.68848	0.68753	0.68644
0.059975	0.68807	0.68783	0.68742	0.68655	0.68557	0.68446
0.065167	0.68611	0.68586	0.68543	0.68453	0.68352	0.68237
0.069991	0.68449	0.68423	0.68378	0.68286	0.68183	0.68065
0.074995	0.68301	0.68274	0.68227	0.68132	0.68027	0.67907
0.080022	0.68145	0.68117	0.68069	0.97972	0.67864	0.67742

^aUnits of m , mol·kg⁻¹.

The “apparent” thermodynamic constant pK'_a is rearranged from Equation (2).

$$pK'_a = pK_a - \log(\gamma_{\text{BTH}^+} \gamma_{\text{Cl}^-}) = \frac{(E - E^\circ) F}{RT \cdot \ln 10} + \log m_{\text{BTH}^+} \quad (2)$$

where BTH^+ stands for $\text{BIS-TRIS}\cdot\text{H}^+$, m is the molality of $\text{BIS-TRIS}\cdot\text{H}^+$, pK_a is the thermodynamic dissociation constant, γ is the activity coefficient of the corresponding species, E is the corrected emf to 1 atm, E° is the standard electrode potential of silver-silver chloride, R , T , F have their usual physical significances. Since hydrolysis of $\text{BIS-TRIS}\cdot\text{H}^+$ is negligible at the almost neutral pH of these buffer solutions, so only the stoichiometric molality m appears in Equation (1).

The term $\log(\gamma_{\text{BTH}^+} \gamma_{\text{Cl}^-})$ is expected to vary linearly with I (ionic strength) because of the charge type. Hence, the quantity pK'_a is defined by:

$$pK'_a = pK_a - \beta I \quad (3)$$

and the ionic strength is given by

$$I = m \quad (4)$$

The values of pK_a (the intercept at $I = 0$), β (the slope parameter) were found by the least-squares methods and are entered in **Table 2** together with the standard deviation in the temperature range (278.15 to 328.15) K which were fitted to the following equation

$$pK_a = A/T + B + C \ln T \quad (5)$$

by the method proposed by Ives and Moseley [15]. The final Equation (6) takes the form:

$$pK_a = 921.66/T + 14.0007 - 1.86197 \ln T \quad (6)$$

with a residual standard deviation of 0.0004. Quantities for the dissociation of $\text{BIS-TRIS}\cdot\text{H}^+$ were computed by applying the usual thermodynamic equations from the constants of Equation (6). The values of ΔG° , ΔH° , ΔS° and ΔC_p° along with standard deviations were calculated by the method of Please [16], and are summarized in **Table 3**. At 298.15 K, the estimated uncertainties of these values are; ΔG° , 3 J·mol⁻¹; ΔH° , 22 J·mol⁻¹; ΔS° , 0.07 J·K⁻¹·mol⁻¹; and ΔC_p° , 2 J·K⁻¹·mol⁻¹.

Table 2. Dissociation constant pK_a of BIS-TRIS from (278.15 to 328.15) K.

T/K	pK_a	$\sigma(pK_a)^a$	β	$\sigma(\beta)^b$
278.15	6.8352	0.0006	0.145	0.021
283.15	6.7430	0.0006	0.142	0.020
288.15	6.6536	0.0006	0.138	0.021
293.15	6.5671	0.0006	0.134	0.021
298.15	6.4828	0.0005	0.130	0.019
303.15	6.4011	0.0006	0.136	0.020
308.15	6.3214	0.0006	0.143	0.020
310.15	6.2906	0.0006	0.148	0.020
313.15	6.2443	0.0005	0.155	0.020
318.15	6.1683	0.0005	0.162	0.019
323.15	6.0942	0.0006	0.163	0.020
328.15	6.0214	0.0006	0.170	0.020

^aStandard deviation of pK_a . ^bSlope parameter.

4. Discussion

There are some reliable thermodynamic data with which to compare the present work. The pK_a and standard thermodynamic functions of BIS-TRIS·H⁺ are compared in **Table 4** with the corresponding data for other compounds such as (CH₃)₃CN⁺H₃ [17] and CH₃(CH₂OH)₂CN⁺H₃ [10] [18] which are structurally related to BIS-TRIS·H⁺ (this work). For the interpretation of the data given in **Table 4**, Timimi and Everett [18], in their publication indicated that for isoelectric dissociation process (positively charged acid) hydroxymethyl or hydroxyethyl substitution to primary, secondary, or tertiary amine compounds usually lowers the values of pK_a , ΔG° and ΔH° . This trend is consistent in the present work. For example at 298.15 K, the value of pK_a for (CH₃)₃CN⁺H₃ = 10.685, $\Delta H^\circ = 60,070 \text{ J}\cdot\text{mol}^{-1}$ compared to $pK_a = 6.483$, $\Delta H^\circ = 28,273 \text{ J}\cdot\text{mol}^{-1}$ for BIS-TRIS·H⁺. This enhancement of the acidic strength is through the inductive effect of the oxygen atoms. The dissociation of BIS-TIS·H⁺ is an isoelectric process where no new charge is created. It appears that somewhat bulky hydrophilic substituent groups partially shield the basic nitrogen. Hence the interactions of the substituent groups with hydrogen ions become a problem. This is also consistent with N substitution. It is evident from **Table 4** that the values of ΔS° and ΔC_p° for (CH₃)₃CN⁺H₃ [17] are small ($-3.1 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ and $\Delta C_p^\circ = 15 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$), but when substitution of three hydroxymethyl groups in -(CH₃)₃ occur, as in the case of (CH₂OH)₃CN⁺H₃ [19] $\Delta S^\circ = 4.8 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ and $\Delta C_p^\circ = -78 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ which is substantial. This data suggests a decreased ordering in terms of the destabilization of the solvent structure through hydroxyl groups. This contrary variation of negative value of ΔS° and positive value of ΔC_p° is difficult to explain quantitatively. Usually electrostatic interactions with charged species cause an orientation of polar solvent molecules in the proximity of the ion. This leads to lower both ΔS° and ΔC_p° of the system. However, the present thought is that other hydrophobic interactions between polar water structure and -(CH₃)₃ groups play an important role for a decrease in ΔS° and an increase in

Table 3. Thermodynamic functions for the dissociation of protonated BIS-TIS from (278.15 to 328.15) K.

	278.15 K	288.15 K	298.15 K	308.15 K	318.15 K	328.15 K
ΔG°	36,395 ± 3	36,706 ± 3	37,005 ± 3	37,292 ± 3	37,568 ± 3	37,832 ± 3
ΔH°	27,560 ± 63	27,916 ± 41	28,273 ± 22	28,629 ± 23	28,986 ± 45	29,342 ± 70
ΔS°	-31.8 ± 0.2	-30.5 ± 0.1	-29.3 ± 0.07	-28.1 ± 0.07	-27.30 ± 0.1	-25.9 ± 0.2
ΔC_p°	35 ± 2	35 ± 2	35 ± 2	35 ± 2	35 ± 2	35 ± 2

Units: ΔG° , ΔH° , J·mol⁻¹; ΔS° , ΔC_p° , J·K⁻¹·mol⁻¹.

Table 4. Comparison of the thermodynamic functions for the dissociation of protonated BIS-TRIS and related protonated bases at 298.15 K.

Protonated base	pK_a	ΔH°	ΔS°	ΔC_p°	Ref
(CH ₃) ₃ CN ⁺ H ₃	10.685	60,070	-3.1	15	[17]
(CH ₃) ₂ (CH ₂ OH)CN ⁺ H ₃	9.694	53,948	-4.9	-19	[18]
CH ₃ (CH ₂ OH) ₂ CN ⁺ H ₃	8.797	49,794	-1.3	-45	[10] [18]
(CH ₂ OH) ₃ CN ⁺ H ₃	8.072	47,488	4.8	-78	[9] [19]
OHCH ₂ CH ₂ N ⁺ H ₃	9.498	50,543	-12.1	-4	[12]
(CH ₂ OH) ₃ N ⁺ H	8.075	47,614	5.1	-63	[17]
(CH ₂ CH ₂ OH) ₃ N ⁺ H	7.762	33,472	-36.4	52	[21]
(CH ₂ CH ₂ OH) ₂ N ⁺ H ₂	8.816	42,426	-28	49	[10]
(CH ₂ OH) ₃ CN ⁺ H(C ₂ H ₄ OH) ₂	6.483	28,238	-29.4	24	[11]
	6.483	28,273	-29.3	36	This work

Units: ΔH° , J·mol⁻¹; ΔS° , ΔC_p° , J·K⁻¹·mol⁻¹.

ΔC_p° [20]. As can be seen from **Table 4**, comparisons of the thermodynamic properties of $(\text{CH}_2\text{CH}_2\text{OH})_3\text{NH}^+$ [21], $(\text{CH}_2\text{CH}_2\text{OH})_2\text{NH}_2^+$ [10], and BIS-TRIS [this work] reveal the increase in acidic strength due to the inductive effect in the presence of more oxygen atoms.

5. Conclusion

The emf data are stable, reliable, and very accurate with an uncertainty of ± 0.04 mV. The $\text{p}K_a$ value of BIS-TRIS $\cdot\text{H}^+$ is 6.483 at 298.15 K. Hence this is of great importance in the preparation of a buffer solution composed of BIS-TRIS and BIS-TRIS $\cdot\text{HCl}$ for the control of acidity in the physiological pH range 6 - 8. These pH results will be published in a separate communication. The detailed procedure will be followed as reported in recent publications for (TAPSO) [22] and (ACES) [23].

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