147. *The Acid Dissociation Constant of the Protonated Form of Tri(hydroxymethyl)methylamine.*

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Dissociations constants for this base at 0—60° have been measured. Discrepancies from published results are discussed.

Tri(hydroxymethyl)methylamine (" tris ") is widely used in biochemical research for the preparation of buffers because of its low toxicity and the useful range of pH's which can be covered. Therefore, it appeared desirable to make accurate measurements of the dissociation constant over a wide temperature range, and thus define more precisely the pH values of buffers prepared from it. It was also thought that a detailed study of the acid dissociation equilibria of this substance might throw light on the interaction of the hydroxyalkyl substituents with water. Since the completion of this work Bates and Hetzer [1] have described the same equilibrium with substantially similar results.

Chloride-containing buffer solutions were used in cells without liquid junction of the type:

$$\mathrm{Pt,H_2(1\ atm.)|R{\cdot}NH_3^+,\ R{\cdot}NH_2,\ Cl^-|AgCl,\ Ag}$$

The concentrations of the solutions were expressed on the molal scale. From the e.m.f.'s of these cells, values of pwH were derived:

$$\mathrm{pwH} = F(E - E_0)/\boldsymbol{R}T \ln 10 + \log m_{\mathrm{Cl}^-} = -\log a_{\mathrm{H}^+}\gamma_{\mathrm{Cl}^-}.$$

The values of pK_a are related to pwH as follows:

$$\mathrm{p}K_a = \mathrm{pwH} + \log [\mathrm{R{\cdot}NH_3^+}]/[\mathrm{R{\cdot}NH_2}] + \log \gamma_{\mathrm{R\cdot NH_3^+}}\gamma_{\mathrm{Cl^-}}/\gamma_{\mathrm{R\cdot NH_2}}, \qquad (1)$$

where the activity coefficient term is represented [2] by:

$$-\log \gamma_{\mathrm{R\cdot NH_3^+}}\gamma_{\mathrm{Cl^-}}/\gamma_{\mathrm{R\cdot NH_2}} = 2AI^{\frac{1}{2}} + BI + CI^{\frac{3}{2}}, \qquad (2)$$

in which A is the Debye–Hückel constant and B and C are empirical parameters.

Corrections were applied for the hydrolysis of the basic species and for complex formation by the silver ion.[2] The hydroxide-ion concentration was calculated from:

$$m_{\mathrm{OH}^-} = \mathrm{antilog}\ (\mathrm{pwH} - \mathrm{p}K_w),$$

where pK_w is the negative logarithm of the ionic product of water calculated from Robinson and Stokes's equation.[3] The first and second stability constants for the tris-silver complexes were determined by titration in cells with liquid junction from 0° to 60° at 10° intervals by the method described previously.[4]

The thermodynamic values of pK_a were found by extrapolating to zero ionic strengths the function:

$$y = \mathrm{pwH} + \log [\mathrm{R{\cdot}NH_3^+}]/[\mathrm{R{\cdot}NH_2}] - 2AI^{\frac{1}{2}} = \mathrm{p}K_a - BI - CI^{\frac{3}{2}}, \qquad (3)$$

which is obtained by combining equations 1 and 2. The extrapolation was carried out by fitting experimental values of y to the above equation by the method of least squares.

The values of the E_0 of the Ag,AgCl electrodes used were determined by measurements of the e.m.f. of cells of the type:

$$\mathrm{Pt,H_2\ (1\ atm.)|HCl|AgCl,Ag}$$

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[1] Bates and Hetzer, *J. Phys. Chem.*, 1961, **65**, 667.

[2] Datta and Grzybowski, *Trans. Faraday Soc.*, 1958, **54**, 1179.

[3] Robinson and Stokes, " Electrolyte Solutions," Butterworths Scientific Publns., London, 1955, p. 496.

[4] Datta and Grzybowski, *J.*, 1959, 1091.

in some cases at 25° (Series 1) only, but in most cases at 5°, 25°, and 45° (Series 2). In the former instance the temperature coefficient given by Bates and Bower [5] was used but, in the latter, the E_0's at the other temperature were interpolated by comparison with Bates and Bower's results.[5] The concentrations of the hydrochloric acid solutions used were in the range 0·01—0·03m and their activity coefficients were calculated from the data of Bates and Bower [5] by a method described previously.[2]

The values of $(E - E_0)$, the molalities, ionic strengths, and extrapolation functions y from equation 3 are given in Table 1. The observed values of pK_a, its standard error, and the constants B and C of equation 3 are given in Table 2. When the values of pK_a were related to temperature by Harned and Robinson's [6] equation,

$$\mathrm{p}K_a = A/T - D + CT, \tag{4}$$

the fit was unsatisfactory, as is evident from the differences between the observed and the calculated values of pK_a shown in Table 2. It was found, however, that the experimental pK_a's can be represented adequately by an equation of the form

$$\mathrm{p}K_a = A/T - D + CT + ET^2. \tag{5}$$

As can be seen from Table 2 the differences between the observed and the calculated values of pK_a are then much smaller and more random. $V(\log K)$ is 752×10^{-8} for equation 4 and 214×10^{-8} for equation 5, where $V(\log K)$ is the variance of the observed values of pK_a about the respective equations in temperature. $V(\log K) = \sum\Delta^2/(m - 3)$ for equation 4, and $\sum\Delta^2/(m - 4)$ for equation 5, where m is the number of temperatures at which observations were made.

With the type of experimental procedure used, $V(\log K)$ is usually smaller than $V(y_o)$, the variance of the intercept in the extrapolation curves against ionic strength, owing to correlation of errors. This is because, when measurements are made at different temperatures on the same solution, errors in the stoicheiometry and failure in the reproducibility of the electrodes are repeated at each temperature. When the extrapolation against ionic strength is linear, $V(y_o)$ is given [7] by:

$$V(y_o) = \left\{\frac{1}{n} + \frac{\bar{I}^2}{\sum\limits_1^n (\mathrm{I} - \bar{I})^2}\right\}\frac{1}{n-2}\sum_1^n \Delta^2. \tag{6}$$

However, when a second arbitrary parameter, in $I^{3/2}$, is introduced, a more complex formula must be used:

$$V(y_o) = \left\{\frac{1}{n} + \frac{\bar{I}^2 \sum\limits_1^n (I^{3/2} - \bar{I}^{3/2})^2 + \bar{I}^{3/2}\sum\limits_1^n (I - \bar{I})^2 - 2\bar{I}^{5/2}\sum\limits_1^n (I - \bar{I})(I^{3/2} - \bar{I}^{3/2})}{\sum\limits_1^n (I - \bar{I})^2 \sum\limits_1^n (I^{3/2} - \bar{I}^{3/2})^2 - \left[\sum\limits_1^n (I - \bar{I})(I^{3/2} - \bar{I}^{3/2})\right]^2}\right\}\frac{1}{n-3}\sum_1^n \Delta^2 \tag{7}$$

When the full Debye–Hückel equation is used to represent the activity coefficient term in the extrapolation function, the adjustable ion-size parameter a^* is itself arbitrary. Hence, although a function linear in ionic strength is ultimately obtained by adjusting a^*, it is not permissible to use equation 6 for $V(y_o)$, since the variance in a^* is not then included. To test these relations, the full Debye–Hückel equation was applied to our results at 5° and 25°, a^* being found by minimising $\sum\Delta^2$ about the extrapolation lines. The intercepts, values of a^*, $V(y_o)$ from equation 6, and the values of $V(y_o)$ from equation 7 for the corresponding extrapolations based on equation 3 are in Table 3. It is clear that the values

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[5] Bates and Bower, *J. Res. Nat. Bur. Stand.*, 1954, **53**, 283.

[6] Harned and Robinson, *Trans. Faraday Soc.*, 1940, **36**, 973.

[7] Please, *Biochem. J.*, 1954, **56**, 196.

TABLE 1.

Molalities and ionic strengths of solutions, e.m.f.'s of cell (1) (abs. v), and extrapolation functions y [eqn. (3)].

At each temperature the first line represents $10^5(E - E_0)$, and the second represents y.

Series 1.

m_1 = molality of tri(hydroxymethyl)methylamine; m_2 = molality of HCl; m_3 = molality of KCl; $y = \text{pwH} + \log [m_2/(m_1 - m_2)]$; $I = m_2 + m_3$.

$10^2 m_1$	1·2163	1·6369	1·9026	2·5378	3·5568	4·1044	5·4334	6·1349$_5$	6·4177
$10^2 m_2$	0·9267	1·2471	1·4496	1·9334$_8$	2·7098	3·1270	4·1395	4·6740	4·8894
$10^2 m_3$	0·9183	1·2358	1·4364	1·9160	2·6853	3·0987	4·0806	4·6317	4·8452
25°	55,729	55,057	54,725	54,089	53,352	53,036	52,464$_6$	52,235	52,100
	8·1919	8·2071	8·2164	8·2339	8·2561	8·2648	8·2889	8·3040	8·3007
35°	55,958	55,286	54,934$_9$	54,289	53,520	53,202$_5$	52,612	52,374	52,237
	7·9236	7·9426	7·9506	7·9700	7·9908$_5$	8·0011	8·0541	8·0401	8·0373
45°	56,201	55,537	55,162	54,503	53,711	53,388	52,783	52,539	52,388
	7·6744$_7$	7·6982	7·7042	7·7249	7·7459	7·7569	7·7817$_5$	7·7970	7·7927
55°	56,489	55,807	55,407	54,742	53,916	53,586	52,966	52,715	52,560
	7·4473$_5$	7·4716	7·4754	7·4983	7·5181	7·5297	7·5550	7·5704$_6$	7·5662

Series 2.

m_1 = molality of tri(hydroxymethyl)methylamine hydrochloride; m_2 = molality of KOH; $y = \text{pwH} + \log [(m_1 - m_2 + m_{\text{OH}^-})/(m_2 - m_{\text{OH}^-} - \Delta_R)]$, where Δ_R is the decrease in the molality of the free base due to complex-formation with Ag$^+$ ions; I_{av} (the average ionic strength) = $(m_1 + \Delta_{\text{Cl}^-})$, where Δ_{Cl^-} is the increase in the molality of Cl$^-$ ions due to increased solubility of AgCl.

$10^2 m_1$	1·6725	2·4330	3·1394	3·1733	4·0022$_5$	4·0022$_5$	4·5919	5·1697	6·3676$_5$	6·6008	7·2743	8·2762$_5$
$10^2 m_2$	0·8536	1·2364$_7$	1·6040	1·6182$_5$	2·0412	2·0412	2·3291	2·6216	3·2436	3·3614$_6$	3·7019	4·1664
$10^2 I_{\text{av}}$	1·6728	2·4334	3·1399	3·1738	4·0029	4·0029	4·5926	5·1705	6·3687	6·6017	7·2755	8·2776
0°	58,302$_5$	57,512		56,992		56,528	56,231	55,979	55,590	55,514	55,339	55,057
	8·9636	8·9842		9·0005$_5$		9·0155	9·0254	9·0305$_5$	9·0454	9·0470$_5$	9·0576	9·0712
5°	58,421	57,615		57,092	56,611	56,619	56,321	56,061	55,675	55,599$_5$	55,414	55,118
	8·7917$_5$	8·8121		8·8297	8·8431	8·8446	8·8552	8·8598	8·8764	8·8784$_8$	8·8877$_5$	8·8996
10°	58,560	57,739		57,208	56,723	56,727	56,424	56,157	55,772	55,694	55,504$_8$	55,201
	8·6296	8·6499		8·6676	8·6820	8·6827	8·6932	8·6975	8·7155	8·7174	8·7266	8·7380$_5$
15°	58,710$_5$	57,875	57,357	57,334	56,843	56,846	56,538	56,268	55,877$_5$	55,799	55,606	55,295
	8·4751	8·4954$_5$	8·5107	8·5131	8·5278	8·5284	8·5390	8·5434$_7$	8·5617	8·5638	8·5728	8·5841
20°	58,872	58,023	57,496	57,470	56,973	56,975	56,661	56,389	55,989	55,912	55,714	55,399
	8·3278	8·3482	8·3636	8·3654	8·3807	8·3811	8·3915	8·3965	8·4143	8·4168	8·4257	8·4370
25°	59,036	58,177	57,638	57,603	57,108	57,105	56,791	56,518	56,107	56,030	55,830	55,509
	8·1858	8·2069	8·2218	8·2222	8·2392$_5$	8·2387	8·2501	8·2556	8·2728	8·2755	8·2846	8·2958
30°	59,208	58,340	57,788	57·747	57,253	57,238	45,924$_8$	56,652	56,234	56,154	55,954	55,627
	8·0497	8·0718	8·0860	8·0854$_6$	8·1041	8·1016	8·1140	8·1204	8·1374	8·1400	8·1494$_9$	8·1607
35°	59,379	58,502	57,934	57,909	57,402		57,050	56,787	56,361		56,080	55,747$_5$
	7·9181	7·9410	7·9540	7·9563	7·9740		7·9810	7·9897	8·0064$_6$		8·0192	8·0304
40°	59,567	58,679	58,094$_6$	58,071$_5$	57,563		57,201	56,937	56,502		56,218	55,879
	7·7932	7·8167	7·8286	7·8312	7·8500$_5$		7·8563	7·8654$_8$	7·8820		7·8949$_9$	7·9059
45°	59,758$_5$	58,862$_5$	58,264	58,245	57,726		57,345$_5$	57,085	56,653		56,362	56,019
	7·6729	7·6974	7·7086	7·7118$_5$	7·7304		7·7345$_5$	7·7450	7·7630		7·7755	7·7868
50°	59,949	59,042	58,434	58,407	57,888		57,508	57,240	56,808$_5$		56,511	56,161$_5$
	7·5563	7·5812	7·5928	7·5944	7·6142		7·6194	7·6293	7·6485		7·6608	7·6718
55°	60,142$_5$	59,227	58,610	58,567	58,056		57,681	57,401	56,960		56,661	56,298
	7·4435	7·4693$_5$	7·4810	7·4837	7·5024		7·5093	7·5180	7·5368		7·5495	7·5594
60°	60,337	59,407$_5$		58,765	58,225		57,854	57,561	57,110		56,813	56,438
	7·3344$_7$	7·3602		7·3760$_5$	7·3943		7·4027	7·4100	7·4283		7·4419	7·4508

of $V(y_0)$ from equation 6 are one-tenth (standard error about one-third) of those calculated from equation 7. Hence in the calculation of standard errors it is important to bear in mind the nature of the extrapolation function employed. Although the error in the thermodynamic pK_a's is relatively large, it is probable that the temperature coefficient

TABLE 2.

The values of pK_a and the constants B and C of eqn. (3).

pK_a (obs.) was obtained from eqn. (3); pK_a (obs.) (Bates) are the observed values of Bates and Hetzer;[1] $[V(y_o)]^{\frac{1}{2}}$ was obtained from eqn. (7); Δ_1 = pK_a (4) − pK_a (obs.); Δ_2 = pK_a (5) − pK_a (obs.); Δ_3 = pK_a (5) (Bates) − pK_a (obs.) (Bates); Δ_4 = pK_a (5) − pK_a (5) (Bates); where pK_a (4) = $3037{\cdot}61/T - 3{\cdot}9321 + 0{\cdot}00608510T$; p$K_a$ (5) = $6574{\cdot}61/T - 39{\cdot}0879 + 0{\cdot}1223040T - 0{\cdot}0001277861T^2$; p$K_a$ (5) (Bates) = $5362{\cdot}10/T - 27{\cdot}6550 + 0{\cdot}0865295T - 0{\cdot}0000906287T^2$ (calc. from the observed values of Bates and Hetzer [1]); $\sigma^2 = V$ (log K) is the variance about the curves of eqns. (4) or (5).

Temp. (c)	pK_a (obs.)	No. of solns.	$10^4[V(y_o)]^{\frac{1}{2}}$ [eqn. (7)]	B (β)	C (γ)	$10^4\Delta_1$	$10^4\Delta_2$	pK_a (obs.) (Bates)	$10^4\Delta_3$	$10^4\Delta_4$
0°	8·8553	10	47	+1·2104	−1·2900	−50	−8	8·8500	−11	+56
5	8·6792	11	32	+0·9630	−0·5802	+16	+19	8·6774	+14	+23
10	8·5158	11	30	+0·9481	−0·5324	+26	+8	8·5164	+3	−1
15	8·3602	12	27	+0·9604	−0·5647	+25	0	8·3616	+3	−17
20	8·2124	12	24	+1·0049	−0·6534	+10	−12	8·2138	0	−26
25	8·0686	21	34	+0·9199	−0·2268	+14	+2	8·0746	−28	−30
30	7·9336	12	25	+1·1348	−1·0210	−12	−12	7·9344	+9	−28
35	7·8006	19	31	+1·0145	−0·4900	−3	+9	7·8031	+9	−25
40	7·6756	10	38	+1·1667	−1·0532	−23	−2	7·6772	0	−18
45	7·5515	19	41	+0·9734	−0·3375	−2	+21	7·5543	+4	−11
50	7·4380	10	46	+1·3123	−1·4878	−40	−24	7·4365	−5	−4
55	7·3204	19	41	+0·9525	−0·2028	+8	+6		+3 *	+3
60	7·2098	9	39	+0·8976	−0·0878	+29	−5		−14 *	+9
			Av. 36			$\sigma = 2{\cdot}74 \times 10^{-3}$	$\sigma = 1{\cdot}46 \times 10^{-3}$		$\sigma = 1{\cdot}29 \times 10^{-3}$	

* pK_a (5) (Bates) − pK_a (obs).

TABLE 3.

The values of pK_a obtained by using the full Debye–Hückel equation to represent the activity coefficients.

pK_a (5) was obtained using eqn. (5); pK_a (D. H.) was obtained using the full Debye–Hückel equation. a^* = Ion-size parameter. b = Slope in the full Debye–Hückel equation. $V(y_0)$ = Variance of the intercept.

Temp. (c)		pK_a (5)	pK_a (D. H.)	a^* (Å)	b	$10^8V(y_o)$ [eqn. (6)]	$10^8V(y_o)$ [eqn. (7)]
5°	(This work)	8·6792	8·6792	2·8	−0·0809	116	1024
25	(This work)	8·0686	8·0713	3·2	−0·0602	136	1170
25	(Ref. 1)	8·0768	8·0746	0	—	40	1003

of the pK_a is known with much greater accuracy, as suggested by the value of V(log K). This means that the errors in $\Delta S°$ and $\Delta C_p°$, which do not depend on the absolute values of the thermodynamic pK_a's, should be calculated from V(log K) rather than from $V(y_o)$.

It is interesting that Bates and Hetzer found a^* to be 0 since in this work 2·8 and 3·2 Å were obtained at 5° and 25°, respectively. A possible reason for this extraordinary discrepancy is the fact that potassium chloride was used in all the buffer solutions in this work, whereas the only ions present in Bates and Hetzer's solutions were the ions from the amine and chloride ions. The thermodynamic quantities associated with the acid dissociation of the former ion at a few representative temperatures are collected in Table 4. On

TABLE 4.

Thermodynamic quantities for the acid dissociation of the protonated form of tri(hydroxymethyl)methylamine.

Temp. (c)	$\Delta G°$ (kJ mole^{-1})	$\Delta H°$ (kJ mole^{-1})	$\Delta S°$ (J mole^{-1} deg.$^{-1}$)	$\Delta C_p°$ (J mole^{-1} deg.$^{-1}$)
5°	46·229	50·01	13·58	166·9
15	46·121	48·52	8·31	130·6
25	46·058	47·40	4·52	91·4
35	46·025	46·70	2·19	49·2
45	46·009	46·43	1·32	4·1
55	45·994	46·63	1·93	−44·0

the face of it they appear to be rather different from Bates and Hetzer's values, but this apparent discrepancy largely disappears when the latters' results are recalculated from a cubic equation similar to equation (5). Further evidence in support of equation 5 comes from the following observation. When Bates and Hetzer's data are extrapolated to 55° and 60° by means of their three-parameter equation, the pK_a's differ from ours by $+0{\cdot}0041$ and $+0{\cdot}0061$, respectively, whereas the same data extrapolated by means of a four-parameter equation similar to equation (5) yield values differing by only $+0{\cdot}0003$ and $-0{\cdot}0014$, respectively from our pK_a's.

Bates and Hetzer have discussed the possible interpretations of these quantities. Their main points are: (*a*) the low pK_a, compared with those of other acids of this charge type, is due to the electron-attracting effect of the hydroxyl groups; (*b*) the positive entropy change may be due to the restricted rotation about the carbon–carbon bonds caused by the solvation shell in the cation (" the chain stiffening effect "), part of the increase being also due to the hydroxyl groups (by analogy with triethanolammonium). They do not put forward any convincing explanation of the contrary variation in $\Delta C_p°$ and $\Delta S°$. Other facts to be explained are the decrease in $\Delta S°$ to a minimum at about 46°.

EXPERIMENTAL

Series 1.—Nine solutions were prepared by diluting a stock solution (tris : HCl : KCl $\approx$ 3 : 1 : 1), the ionic strengths ranging from 0·018 to 0·097. The e.m.f. measurements were made at 5°, 15°, 25°, 35°, and 45°, but only those between 25° and 45° inclusive were considered sufficiently reliable and were used in the final calculations.

Series 2.—Ten solutions were prepared individually by weighing out appropriate amounts of the amine hydrochloride, ~0·86D-KOH, and water, the ionic strengths ranging from 0·017 to 0·083. The e.m.f. measurements were made from 0° to 60° at 5° intervals.

Tri(hydromethyl)methylamine (*Tris*).—(*a*) *Free base for series* (1). Commercial material (L. Light & Co., Colnbrook), recrystallised from aqueous methanol, was kindly supplied by Dr. B. R. Rabin. Analysis by titration with hydrochloric acid (addition by weight of ~1·0D-HCl to within 96—98% of the end-point followed by titration with ~0·02N-HCl) gave a purity of 99·76% (99·870%, 99·656%). Titration of the stock showed the purity to be 99·71% (99·594%, 99·830%), and this value was applied as a correction factor in calculating the molalities.

(*b*) *Hydrochloride.* Commercial base (L. Light & Co., Colnbrook) was gradually added to bromine-free ~6N-hydrochloric acid. The product was recrystallised from 50% ethanol and then from 70% ethanol (152 g. in 250 ml.; recovery ~82%), and its purity was checked by gravimetric analysis for chloride (as AgCl) which showed it to be 99·24% (99·189%, 99·339%, and 99·188%). A correction was applied to the weights used. The density of the material (determined to correct the weights for the buoyancy of air) was 0·88.

Hydrochloric Acid (*for Series* 1).—Constant-boiling acid (20·21%) was used.

Miscellaneous.—The preparation of potassium chloride[8] and carbonate-free potassium hydroxide, the measurement of the e.m.f.'s of the cells without liquid junction,[9] and the measurement of temperature[10] have been described previously.

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[8] Datta and Grzybowski, *J.*, 1962, 3068.

[9] Ashby, Crook, and Datta, *Biochem. J.*, 1954, **56**, 190.

[10] Clarke, Cusworth, and Datta, *Biochem. J.*, 1954, **58**, 146.