Ionization constants of Carboxyl, Amino and Hydroxy groups in Threonine - pH metry

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PH metric titration technique is by far the most convenient for the determination of ionization constant [1]. Perusal of literature reveals a few references [2] on the ionization constants of amino and carboxyl groups in DL-2-amino-3-hydroxy butanoic acid commonly known as DL-threonine. Some workers [3] have suggested that the dissociation of hydroxy group should also be taken into consideration at pH > 10, because at high pH, it can actively participate in complex formation. Keeping this in view and also the variation in the literature values of ionization constants of amino and carboxyl groups under specific conditions of temperature and ionic strength, it has been considered worthwhile to determine the ionization constants of carboxyl and amino groups along with the hydroxy group under different conditions of temperature and ionic strengths in aqeuous and aquo organic media.

EXPERIMENTAL

DL-Threonine was supplied by BDH chemicals Ltd. Poole England and all other chemicals used were of AnalaR-grade. All solutions were made in conductivity water. pH measurements were made on an EC digital pH meter (accuracy ± 0.01 pH) having a combined glass - calomel electrode assembly. The temperature of the titration cell was maintained constant using a thermostat.

RESULTS AND DISCUSSION

(A) Henderson Hasselbalch Method [4]

A known amount of (0.2 mg) DL-threonine in 20 ml water was titrated with standard acid / alkali to reach pH 1 and 12 respectively (Fig.1). The same amount of water blank was also titrated. The values of pK_1, pK_2 and pK_3 have been evaluated. Detailed method has already been described [5].

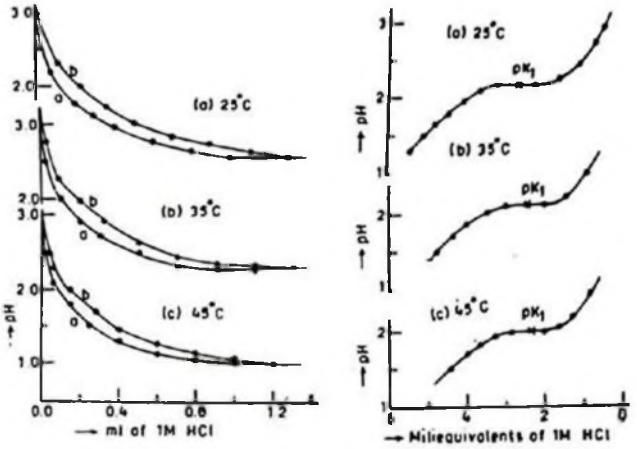


Fig I (B) EVALUATION OF pK_2 & pK_3 OF DL-THREONINE BY HENDERSON HASSELBALCH METHOD AT VARYING TEMPERATURES.

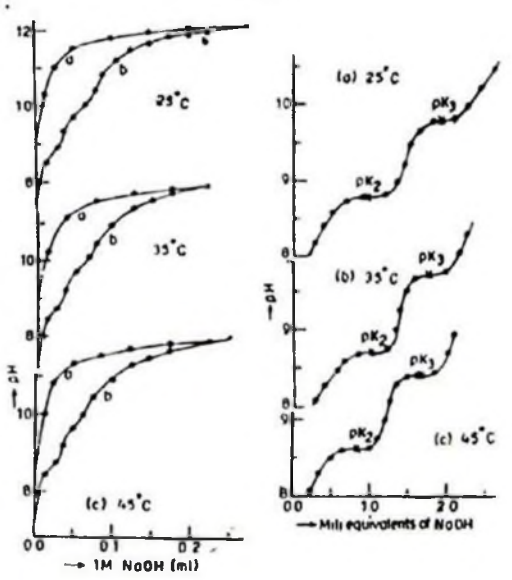


Fig 1 Evaluation of pK_1, pK_2 and pK_3 of DL Threonine
Henderson Hasslbalch Method - at Varying Temperatures

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(B) Albert Serjeants method [6] as refined by Noyes [7]

A known concentration of ligand solution (0.01 M threonine) was titrated with the standard acid in 10 equal portions - After each tenth of an equivalent was added and ionic strength maintained by adding required amount of $NaClO_4$, time was allowed for the system to attain equilibrium pH value after the addition and pH was recorded. The first ionization constant (pK_1) was evaluated by Albert Serjeant's method.

As the difference between the values of pK_2 and pK_3 was found to be less than 2.5, Albert Serjeant's method as refined by Noyes has been adopted for the evaluation of pK_2 and pK_3 values. The solution containing a known concentration of ligand (0.001 M threonine) at three quarter neutralization was titrated with stanadard alkali (0.01 N NaOH). The ionic strength was maintained by adding required amount of $NaClO_4$. The method has been employed for studying the effect of temperature, ionic strength and solvents on the ionization constants of DL-threonine.

(C) Irving - Rossotti Method [8]

The following solutions were titrated pH-metrically with 0.1 M NaOH solution.

(a) 0.05 M $HClO_4$
(b) 0.05 M $HClO_4$ + 0.002 M DL-threonine

The ionic strength was maintained at 0.1 M with $NaClO_4$. The pH was recorded after 10-15 minutes (when the equilibrium was attained). From the pH titration curves $\bar{n}$ values were obtained. The pK values were obtained from pH values at $\bar{n}$ corresponding to 0.5, 1.5 and 2.5 respectively (Fig. 2). This was repeated at different temperatures - 25, 35 and 45° C.

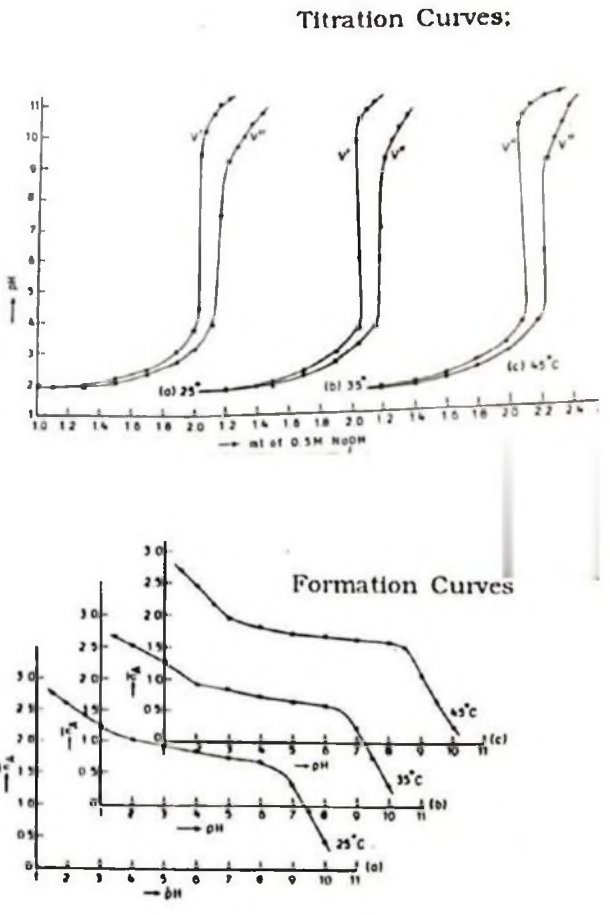


Fig 2. Evaluation of pK of DL Treonine Irving Rossotti Method - at Varying Temperatures

The values of pK_1, pK_2 and pK_3 determined by all three methods described above have been recorded in Table 1.

Table 1

pK Values of DL-Threonine in Aqueous Media
Varying Temperatures/ Ionic Strength

Temp/°C	Method	pK_1	pK_2	pK_3
a. Varying Temperature;		**I = 0.1 M**		
25	A	2.2	8.8	9.8
25	B	2.2	8.8	9.85
25	C	2.21	8.84	9.85
35	A	2.1	8.7	9.7
35	B	2.1	8.7	9.75
35	C	2.15	8.73	9.74
45	A	2.0	8.6	9.6
45	B	2.0	8.6	9.65
45	C	2.0	8.62	9.67
b. Varying Ionic Strength;		**T = 35°C**		
0.1 M	C	2.15	8.73	9.74
0.2 M	C	2.12	8.61	9.65
0.5 M	C	2.04	8.53	9.58
1.0 M	C	1.94	8.41	9.53

Effect of Temperature and Ionic Strength

The values of pK_1, pK_2 and pK_3 at different temperatures and varying ionic strength are recorded in Table 1. From the table, it is noted that the values decrease with increase in temperature. Further plots of pK_1, pK_2 and pK_3 against 1/T were found to be linear which is in agreement with the relationship due to Pitzer [9].

The pK values decrease with increase in ionic strength which is in accordance with Debye Huckel Theory [10].

Effect of Solvents

The three pK values have been determined in different aquo-organic solvents at 20 % V/V - DMSO-water, DMF-water, Acetonitrile-water, Methanol-water, Ethanol-water and Isopropanol-water. As the values are based on numerical value of pH obtained, appropriate corrections were applied to pH meter readings [11] to get the true hydrogen ion concentration in mixed solvents. Results in Table 2 show that pK values in different aquo-organic mixtures follow the sequence:

DMSO-water > DMF - water > Acetonitrile-water > Methanol-water > Ethanol-water > Isopropanol-water. This may be explained on the basis of dielectric constants of the solvent mixture and proton solvation properties of media [12,13].

Table 2

pK values of DL-Threonine in 20% V/V Aquo Organic Media as a Function of Reciprocal Dielectric Constant (D)

(by Albert Serjeant method as refined by Noyes)
Temp = 35°C; I = 0.2 M

Solvent	1/D	pK_1	pK_2	pK_3
i-PrOH	0.05464	2.12	8.63	10.04
EtOH	0.04073	2.13	8.63	10.16
MeOH	0.03058	2.15	8.64	10.18
DMF	0.2724	2.192	8.72	10.25
AN	0.0266	2.15	8.67	10.21
DMSO	0.02146	2.212	8.76	10.33

The three pK values were determined in 20%, 0% and 60% V/V aqueous mixtures with methanol, ethanol, isopropanol, acetonitrile, dimethyl formamide, dimethyl sulphoxide. It was found that the values

increase with increase in organic solvent content in the mixture but the increase has not been found appreciable due to difference in solubilities and ionization of DL-threonine in different aquo organic mixtures.

Thermodynamic Functions

The values of log of the step formation constants at various ionic strengths were extrapolated to zero ionic strength to give step thermodynamic formation constants. These values are calculated and recorded in Table 3 as log K_1, log K_2, log K_3 at I = 0. The sum of these has been shown as log K calculated.

Table 3

Values of Thermodynamic functions in aqueous media

I = 0.1 M; Temp = 35° C

log K at I =0	- ΔG°	-ΔH°	ΔS°
K_1= 2.22	2.96	4.38	-4.59
K_2= 8.82	12.40	4.38	+26.07
K_3= 9.93	13.74	4.58	+29.76

ΔG° and ΔH° in K cals/mole; S° in cal/deg/mole

log K_c Calculated = 20.97
log K_c Experimental = 20.98

From the variation of thermodynamic formation constants with temperature, the values of related thermodynamic functions of free energy (ΔG°), enthalpy (ΔH°) and entropy (ΔS°) have been determined at 35°C in aqueous media with the help of standard equations [14] and are shown in Table 3. The negative values of ΔG° indicate that the dissociation is spontaneous under standard state conditions. The decreasing pK values with temperature results in negative value of ΔH° [15] indicating the reaction as exothermic. The positive ΔS° values account for no charge separation during dissociation.

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