

## CHEMISTRY

### Physical Chemistry

# DISSOCIATION EQUILIBRIA IN ADENOSINE TRIPHOSPHATE (DISODIUM SALT) AND THE KINETICS OF THEIR ALKALI CATALYSED DEGRADATION PART II : KINETIC STUDIES

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A comprehensive kinetic study has been made of the degradation of Adenosine Triphosphate (ATP) at  $pH$ 's 8.0, 8.5, 9.0, 9.5, 10.0, 10.7, 11.0 and 11.8, at temperatures 50°, 60°, 65° and 70 °C maintained within  $\pm 0.05$  °C at constant ionic strength ( $\mu = 0.1$ ). The reaction was found to be of 1st order in  $[\text{triphosphate}]_{\text{total}}$ ,  $k/[\text{H}^+]$  values are not constant and are found to increase with increasing  $pH$  values. The overall rate constant ( $k$ ) has been resolved into the individual rate constants using the values of the dissociation constants obtained under the conditions of the kinetic measurements. The activation parameters for the overall reactions, as also for the stepwise reactions, have been calculated.

**Keywords:** Kinetics; Alkali Catalysed Degradation; Adenosine Phosphate.

## INTRODUCTION

In our earlier paper (Malhotra & Sharma—*Under publication*), a comprehensive study of the kinetics and energetics of the degradation of Adenosine Triphosphate (ATP) has been reported at  $pH$ 's ranging from 1.50 to 6.0 at temperatures 50°, 60°, 65°, and 70 °C maintained within  $\pm 0.05$  °C at constant ionic strength ( $\mu=0.1$ ). The study is now being extended in the alkaline medium at  $pH$ 's varying from 8 to 11.8 and temperatures 50°, 60°, 65°, and 70 °C at constant ionic strength ( $\mu=0.1$ ). The overall rate constant has been resolved into the individual rate constants of the various species present in the  $pH$  range 8.0 to 11.8 and the activation parameters reported. A survey of the literature reveals that earlier investigations (Alberty, 1968; Couture & Ouellet, 1957; Taqui Khan & Srinivas Mohan, 1974, 1976; and Tatas & Lowensteine, 1963) on the kinetics of alkali catalysed degradation of ATP have been restricted upto  $pH$  9.0.

## MATERIALS AND METHODS

Disodium salt of adenosine triphosphate obtained from Biochemicals unit of Vallabh Bhai Patel Chest Institute, Delhi 110 007, India was used. Its purity was found to be 99 per cent (on phosphorus basis). All other chemicals used were of A. R. or B. D. H. grade. The studies were carried out at a constant temperature by using an Ultra Thermostat type NBE (German). The temperature of the thermostat could be controlled within  $\pm 0.05$  °C.

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## KINETIC MEASUREMENTS

The reaction mixture containing ATP and adjusted to the desired pH value by adding NaOH was placed in a 250 ml Pyrex measuring flask and suspended in the thermostat. It attained the temperature of the bath within fifteen minutes. Aliquots (5.0 to 10.0 ml) of the reaction mixture were taken out at different time intervals and analysed for orthophosphate formed by the degradation of ATP, spectrophotometrically by the method due to Bernhart and Wreath (1955). A Coleman spectrophotometer was used for this purpose. The amount of orthophosphate was read from a standard linear plot of optical density versus [orthophosphate]. Light of wavelength 430 m $\mu$  was used for measuring the optical densities.

In order to know the initial concentration of ATP, the reaction mixture (5 ml) was refluxed with 5 ml of conc. HNO<sub>3</sub> for about 3-4 hrs. to convert all the triphosphate completely into orthophosphate and the total amount of orthophosphate estimated. From this, the amount of orthophosphate present initially was subtracted and the difference corresponded to the amount of triphosphate present initially.

## RESULTS AND DISCUSSIONS

The results of the kinetic studies are given in Table I. The degradation rate ( $k$ ) of ATP showed a first order dependence on [triphosphate]. A linear dependence of  $\log(a)/(a-x)$  on time further supports this point.

TABLE I

*Acid dissociation constants of ATP at ionic strength ( $\mu$ ) = 0.1*

Temp.	$pK_3$	$pK_4$
50 $\pm$ 0.05 °C	4.06 $\pm$ 0.01	7.02 $\pm$ 0.01
60 $\pm$ 0.05 °C	4.00 $\pm$ 0.01	7.08 $\pm$ 0.01
65 $\pm$ 0.05 °C	4.00 $\pm$ 0.02	7.14 $\pm$ 0.01
70 $\pm$ 0.05 °C	3.97 $\pm$ 0.01	7.17 $\pm$ 0.01

The variation of  $k$  with pH indicates that the relation between (H<sup>+</sup>) and  $k$  is not a simple one. The rate decreases upto pH 10.00 and then slightly increases. Increase in rate constant above pH 10.00 indicates that a reaction is being catalysed by hydroxyl ions while a decrease in rate constant with increase in pH upto pH 10.00 indicates an acid catalysed and an uncatalysed water reaction.

In the pH range 8.00-11.80, the reactant form of ATP would consist largely of HATP<sup>3-</sup> and ATP<sup>4-</sup>. The amount of these species as fractions ( $F_{\text{HATP}^{3-}}$ ,  $F_{\text{ATP}^{4-}}$ ) of the [triphosphate]<sub>total</sub> present in the reaction mixture at any given pH were calculated using eqns. (1) & (2)

$$F_{\text{HATP}^{3-}} = \frac{\text{HATP}^{3-}}{\sum_{n=0}^4 \text{H}_n\text{ATP}} = \frac{1}{\frac{[\text{H}^+]^3}{K_1 K_2 K_3} + \frac{[\text{H}^+]^2}{K_2 K_3} + \frac{[\text{H}^+]}{K_3} + 1 + \frac{K_4}{[\text{H}^+]}} \quad \dots (1)$$

$$F_{\text{ATP}^{4-}} = \frac{\text{ATP}^{4-}}{\sum_{n=0}^4 \text{H}_n\text{ATP}} = \frac{1}{\frac{[\text{H}^+]^4}{K_1 K_2 K_3 K_4} + \frac{[\text{H}^+]^3}{K_2 K_3 K_4} + \frac{[\text{H}^+]^2}{K_3 K_4} + \frac{[\text{H}^+]}{K_4} + 1} \dots (2)$$

where  $K_i$ 's are the dissociation constants.

TABLE II  
Fractions of the various species and average 1st order rate constants ( $k$ ) at different pH's and temperatures

Initial ATP concentration (moles/litre)	pH	$F_{\text{HATP}^{3-}}$	$F_{\text{ATP}^{4-}}$	$k$ (min <sup>-1</sup> )
Temp. 50 ± 0.05 °C				
9.218 × 10 <sup>-3</sup>	8.00	0.0948	0.9052	(1.70 ± 0.02) × 10 <sup>-5</sup>
10.032 × 10 <sup>-3</sup>	8.50	0.0356	0.9644	(1.60 ± 0.12) × 10 <sup>-5</sup>
9.218 × 10 <sup>-3</sup>	9.00	0.0104	0.9896	(1.52 ± 0.03) × 10 <sup>-5</sup>
9.885 × 10 <sup>-3</sup>	9.50	0.0035	0.9965	(1.25 ± 0.10) × 10 <sup>-5</sup>
9.040 × 10 <sup>-3</sup>	10.00	0.0010	0.9989	(4.98 ± 0.04) × 10 <sup>-6</sup>
9.536 × 10 <sup>-3</sup>	11.70	—	0.9992	(5.46 ± 0.12) × 10 <sup>-6</sup>
9.221 × 10 <sup>-3</sup>	11.00	—	0.9998	(5.87 ± 0.12) × 10 <sup>-6</sup>
9.852 × 10 <sup>-3</sup>	11.80	—	0.9999	(7.07 ± 0.22) × 10 <sup>-6</sup>
Temp. 60 ± 0.05 °C				
9.756 × 10 <sup>-3</sup>	8.0	0.1073	0.8926	(4.64 ± 0.10) × 10 <sup>-5</sup>
9.658 × 10 <sup>-3</sup>	8.50	0.0398	0.9602	(4.50 ± 0.24) × 10 <sup>-5</sup>
10.871 × 10 <sup>-3</sup>	9.00	0.0118	0.9881	(4.26 ± 0.07) × 10 <sup>-5</sup>
9.853 × 10 <sup>-3</sup>	9.50	0.0039	0.9961	(3.70 ± 0.15) × 10 <sup>-5</sup>
10.882 × 10 <sup>-3</sup>	10.00	0.0012	0.9988	(1.30 ± 0.01) × 10 <sup>-5</sup>
9.686 × 10 <sup>-3</sup>	10.70	—	0.9992	(1.35 ± 0.22) × 10 <sup>-5</sup>
10.875 × 10 <sup>-3</sup>	11.00	—	0.9998	(1.41 ± 0.06) × 10 <sup>-5</sup>
10.882 × 10 <sup>-3</sup>	11.80	—	0.9999	(1.46 ± 0.07) × 10 <sup>-5</sup>
Temp. 65 ± 0.05 °C				
10.509 × 10 <sup>-3</sup>	8.00	0.1213	0.8787	(6.76 ± 0.17) × 10 <sup>-5</sup>
10.032 × 10 <sup>-3</sup>	8.50	0.0408	0.9592	(6.55 ± 0.20) × 10 <sup>-5</sup>
10.518 × 10 <sup>-3</sup>	9.00	0.0136	0.9864	(6.25 ± 0.18) × 10 <sup>-5</sup>
10.193 × 10 <sup>-3</sup>	9.50	0.0050	0.9950	(5.30 ± 0.04) × 10 <sup>-5</sup>
10.880 × 10 <sup>-3</sup>	10.00	0.0013	0.9986	(3.31 ± 0.02) × 10 <sup>-5</sup>
9.567 × 10 <sup>-3</sup>	10.70	—	0.9992	(3.42 ± 0.21) × 10 <sup>-5</sup>
10.880 × 10 <sup>-3</sup>	11.00	—	0.9998	(3.51 ± 0.01) × 10 <sup>-5</sup>
10.881 × 10 <sup>-3</sup>	11.80	—	0.9999	(3.83 ± 0.04) × 10 <sup>-5</sup>
Temp. 70 ± 0.05 °C				
9.579 × 10 <sup>-3</sup>	8.00	0.1288	0.8711	(1.12 ± 0.12) × 10 <sup>-4</sup>
9.872 × 10 <sup>-3</sup>	8.50	0.0418	0.9582	(1.08 ± 0.15) × 10 <sup>-4</sup>
9.759 × 10 <sup>-3</sup>	9.00	0.0145	0.9854	(1.02 ± 0.21) × 10 <sup>-4</sup>
10.146 × 10 <sup>-3</sup>	9.50	0.0055	0.9945	(9.15 ± 0.20) × 10 <sup>-5</sup>
10.656 × 10 <sup>-3</sup>	10.00	0.0014	0.9985	(4.30 ± 0.10) × 10 <sup>-5</sup>
10.541 × 10 <sup>-3</sup>	10.70	—	0.9992	(4.50 ± 0.12) × 10 <sup>-5</sup>
9.941 × 10 <sup>-3</sup>	11.00	—	0.9998	(4.62 ± 0.04) × 10 <sup>-5</sup>
9.97 × 10 <sup>-3</sup>	11.80	—	0.9999	(4.80 ± 0.24) × 10 <sup>-5</sup>

For this purpose, the values of the dissociation constants of adenosine triphosphoric acid determined by us (Malhotra & Sharma, 1979) under the conditions of the kinetic experiments were utilised. These values are listed in Table I.

The values of fractions of the various species along with the average 1st order rate constants ( $k$ ) at different temperatures are given in Table II.

The activation energy ( $E_a$ ) and the frequency factor ( $A$ ) calculated from the linear plots of  $\log k$  vs.  $1/T$  for the overall reaction are given in Table III.

TABLE III  
Activation energies  $E_a$  and the frequency factors ( $A$ ) for the overall reaction

pH	$E_a$ (K cal/mole)	Log A
8.00	21.9 ± 0.6	10.0 ± 0.5
8.50	21.4 ± 0.4	9.7 ± 0.5
9.00	21.3 ± 0.4	9.6 ± 0.4
9.50	22.8 ± 0.7	10.5 ± 0.5
10.00	23.7 ± 0.8	10.7 ± 0.5
10.70	23.3 ± 1.0	10.4 ± 0.5
11.00	22.9 ± 0.9	10.1 ± 0.4
11.80	21.1 ± 0.4	9.0 ± 0.6

#### Calculation of the Individual Rate Constants

For the pH range under consideration, the significant species are  $\text{HATP}^{3-}$  and  $\text{ATP}^{4-}$  (cf. Table II). The rate constants characteristic of these species were calculated using eqn. (3)

$$\text{Rate} = k_4 [\text{HATP}^{3-}] + k_5 [\text{ATP}^{4-}] + k'_4 [\text{HATP}^{3-}] \ln [\text{OH}^-] + k'_5 [\text{ATP}^{4-}] [\ln \text{OH}^-] \quad \dots(3)$$

where  $k_i$ 's are the rate constants for the uncatalysed process and  $k'_i$ 's refer to the same process catalysed by  $\text{OH}^-$  ions. Further, the third term in eqn. (3) can be neglected because  $\text{OH}^-$  ions in any significant amount would not be present along with  $\text{HATP}^{3-}$ .  $k_4$  has been reported in our earlier communication (Malhotra & Sharma, *unpublished*) on acid catalysed hydrolysis of ATP.

Also above pH 10.00, the fraction of the triphosphate species existing as  $\text{HATP}^{3-}$  is negligible at all temperatures (cf. Table II). Therefore, for the pH range 10–11.8, eqn. (3) reduces to

$$\text{Rate} = k_5 [\text{ATP}^{4-}] + k'_5 [\text{ATP}^{4-}] \ln [\text{OH}^-] \quad \dots(4)$$

The overall rate constant values at pH's 10.00 and 11.00 at different temperatures from Table I were used in eqn. (4) to obtain values of  $k_5$  and  $k'_5$ . These values along with  $k_4$  have been given in Table IV.

These individual rate constants were used to evaluate the overall rate constants at pH's 8.0, 8.5, 9.0, 9.5, 10.7 and 11.8 at different temperatures. For this purpose,

TABLE IV

*Values of individual rate constants of the species HATP<sup>3-</sup> and ATP<sup>4-</sup> at different temperatures*

Reaction	Rate constant min <sup>-1</sup> at			
	50 °C	60 °C	65 °C	70 °C
(a) *HATP <sup>3-</sup> + H <sub>2</sub> O $\xrightarrow{k_4}$ Product	5.35 × 10 <sup>-5</sup>	1.51 × 10 <sup>-4</sup>	2.07 × 10 <sup>-4</sup>	3.49 × 10 <sup>-4</sup>
(b) ATP <sup>4-</sup> + H <sub>2</sub> O $\xrightarrow{k_5}$ Product	8.51 × 10 <sup>-6</sup>	2.24 × 10 <sup>-5</sup>	4.31 × 10 <sup>-5</sup>	5.61 × 10 <sup>-5</sup>
(c) ATP <sup>4-</sup> + OH <sup>-</sup> $\xrightarrow{k'_5}$ product	3.86 × 10 <sup>-7</sup>	7.00 × 10 <sup>-7</sup>	8.68 × 10 <sup>-7</sup>	1.39 × 10 <sup>-6</sup>

\*H. C. Malhotra and L. K. Sharma *J. inorg. nucl. Chem. (under publication)*.

TABLE V

*Comparison of overall rate constants with those calculated from individual rate constants*

Temp. °C	pH	Overall 1st order rate constants min <sup>-1</sup>	
		Calculated	Measured
50	8.00	1.76 × 10 <sup>-5</sup>	1.70 × 10 <sup>-5</sup>
	8.50	1.50 × 10 <sup>-5</sup>	1.60 × 10 <sup>-5</sup>
	9.00	1.34 × 10 <sup>-5</sup>	1.52 × 10 <sup>-5</sup>
	9.50	1.25 × 10 <sup>-5</sup>	1.25 × 10 <sup>-5</sup>
	10.70	5.58 × 10 <sup>-6</sup>	5.46 × 10 <sup>-6</sup>
	11.80	6.55 × 10 <sup>-6</sup>	7.07 × 10 <sup>-6</sup>
60	8.00	4.37 × 10 <sup>-5</sup>	4.64 × 10 <sup>-5</sup>
	8.50	3.64 × 10 <sup>-5</sup>	4.50 × 10 <sup>-5</sup>
	9.00	3.18 × 10 <sup>-5</sup>	4.26 × 10 <sup>-5</sup>
	9.50	3.00 × 10 <sup>-5</sup>	3.70 × 10 <sup>-5</sup>
	10.7	1.70 × 10 <sup>-5</sup>	1.35 × 10 <sup>-5</sup>
	11.80	1.89 × 10 <sup>-5</sup>	1.46 × 10 <sup>-5</sup>
65	8.00	7.54 × 10 <sup>-5</sup>	6.76 × 10 <sup>-5</sup>
	8.50	6.04 × 10 <sup>-5</sup>	6.55 × 10 <sup>-5</sup>
	9.00	5.60 × 10 <sup>-5</sup>	6.25 × 10 <sup>-5</sup>
	9.50	5.31 × 10 <sup>-5</sup>	5.30 × 10 <sup>-5</sup>
	10.70	3.65 × 10 <sup>-5</sup>	3.42 × 10 <sup>-5</sup>
	11.80	3.87 × 10 <sup>-5</sup>	3.83 × 10 <sup>-5</sup>
70	8.00	11.10 × 10 <sup>-5</sup>	11.20 × 10 <sup>-5</sup>
	8.50	8.50 × 10 <sup>-5</sup>	10.80 × 10 <sup>-5</sup>
	9.00	7.70 × 10 <sup>-5</sup>	10.20 × 10 <sup>-5</sup>
	9.50	7.10 × 10 <sup>-5</sup>	9.15 × 10 <sup>-5</sup>
	10.70	4.55 × 10 <sup>-5</sup>	4.50 × 10 <sup>-5</sup>
	11.80	4.90 × 10 <sup>-5</sup>	4.80 × 10 <sup>-5</sup>

to calculate  $k$  at pH's 10.7 and 11.8, eqn. (4) was used. However, at all other pH's i.e., in the range (8.0–10.0), since the rate is found to decrease with increase in

pH, unlike in the pH range 10.0–11.8 where the rate is found to increase with increase in pH, eqn. (3) in the form

$$\text{Rate} = k_4 [\text{HATP}^{3-}] + k_5 [\text{ATP}^{4-}] - k'_5 [\text{ATP}^{4-}] \ln [\text{OH}^-] \quad \dots(5)$$

was employed to calculate the overall rate constants.

These values alongwith the experimentally measured values have been tabulated in Table V.

It can be seen from Table V, the calculated values of the overall rate constants are in good agreement with the experimental values.

#### Energies and Entropies of Activation

The activation energy and the frequency factor (A) for reactions (a), (b) and (c) in Table IV were calculated from the linear plots of  $\log k_4$ ,  $\log k_5$  and  $\log k'_5$  vs.  $1/T$ . These parameters alongwith entropies of activation are given in Table VI.

TABLE VI  
Activation energies, frequency factors and entropies of activation (at 60 °C)

Reaction	$E_a$ (Kcal mole <sup>-1</sup> )	$\log A$	$\Delta S^*$ (e.u.)
(a)	20.7 ± 0.5	9.7 ± 0.6	-16.4 ± 1.0
(b)	20.5 ± 0.5	8.7 ± 0.6	-20.8 ± 0.6
(c)	16.3 ± 0.7	4.5 ± 0.8	-40.4 ± 1.0

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