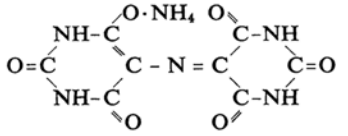
Spectrophotometric Studies on the Kinetics of Decomposition of Tetramethylmurexide in Acid Medium

By R. K. CHATURVEDI

(Received August 12, 1961)

Murexide or ammonium purpurate (I) has found extensive application in analytical procedures for qualitative detection and quantitative estimation[1-8] of various metal ions. For these purposes murexide is used both in acidic[9] as well as alkaline media[10], but in highly acidic or alkaline solutions this metal ion indicator is quite unstable. A research programme was therefore, undertaken on the kinetics of decomposition of murexide in acidic[11] and alkaline[12] media in order to elucidate the reaction mechanism (s) and to formulate proper conditions for the analytical use of murexide[13]. These kinetic studies are now being extended to various compounds related to murexide. The present communication refers to the study on the kinetics of decomposition of tetramethylmurexide (ammonium tetramethylpurpurate) in acid solutions.



(I)

Experimental

Tetramethylmurexide was prepared by the method of Gysling and Schwarzenbach[15] by the action of ammonium carbonate on tetramethylalloxantin. This last named compound was obtained by careful degradation of caffeine following the procedure outlined by Biltz[16]. Repeated recrystallization of ammonium tetramethylpurpurate by salting it out from its aqueous solution[17] by the addition of ammonium chloride gave a sample of satisfactory purity. Hydrochloric acid used was an Analar sample; diluted solutions of this acid were standardized against a standard sodium carbonate solution. Throughout these studies redistilled water was used for preparing the solutions etc. All the spectrophotometric measurements were made with a Beckman DU spectrophotometer using corex cells of 1 cm. width. The temperature of the reaction system was maintained at desired value with an accuracy of ±0.05°C (or better) by employing a Beckman dual thermospacer set in combination with an externally connected thermostat; this last was operated by a micro relay.

Results and Discussion

Absorption Spectra of Tetramethylmurexide.—Figure 1 gives a typical set of results on the absorption of tetramethylmurexide solution in the visible region; the curve in this figure refers to a concentration of 0.05 mM. It may be noted from Fig. 1 that tetramethylmurexide exhibits an absorption maximum at $\lambda = 530\ \mathrm{m}\mu$.

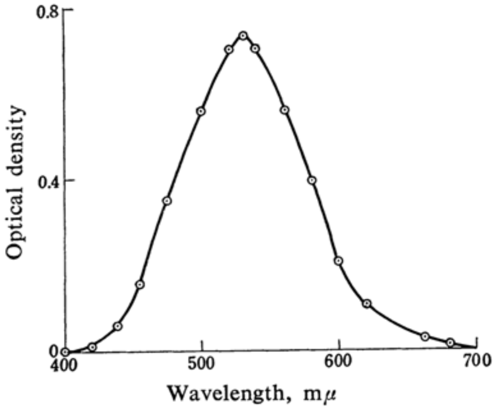


Fig. 1. Absorption spectrum of tetramethylmurexide in the visible region.

---

[1] C. A. Kumins, *Anal. Chim. Acta*, **1**, 69 (1947).

[2] H. Ostertag and D. E. Rinck, *Chim. Anal.*, **34**, 108 (1952).

[3] W. Th. G. M. Smeets and L. Seekles, *Nature*, **169**, 802 (1952).

[4] W. F. Harris and T. Sweet, *Anal. Chem.*, **24**, 1062 (1952).

[5] G. Schwarzenbach, W. Biederman and F. Bangerter, *Helv. Chim. Acta*, **29**, 811 (1946).

[6] M. G. Williams and J. E. Moser, *Anal. Chem.*, **25**, 1414 (1953).

[7] H. Flaschka and F. Huditz, *Z. anal. Chem.*, **197**, 104 (1952).

[8] N. A. Ramaiah, Vishnu and R. K. Chaturvedi, *Naturwiss.*, **45**, 290 (1958).

[9] S. P. Sangal and A. K. Dey, *Proc., Natl. Acad. Sci. (India), Symposium*, 12 (1961).

[10] Cf. Ref. 5.

[11] N. A. Ramaiah, S. L. Gupta and Vishnu, *Z. physik. Chem.*, **106**, 254 (1956); R. K. Chaturvedi, ibid., communicated.

[12] R. K. Chaturvedi, Doctoral Thesis, Agra University, 1960; R. K. Chaturvedi, *Z. physik. Chem.*, communicated.

[13] N. A. Ramaiah and R. K. Chaturvedi, *Curr. Sci.*, **29**, 305 (1960).

[14] R. K. Chaturvedi, *Naturwiss.*, **48**, 643 (1961).

[15] H. Gysling and G. Schwarzenbach, *Helv. Chim. Acta*, **32**, 1484 (1944).

[16] H. Biltz, *Ber.*, **45**, 3674 (1912).

[17] See also D. Davidson, *J. Am. Chem. Soc.*, **58**, 1821 (1936).

Further, this wavelength of maximum absorption was unaffected by the change in the concentration or/and pH of the system. Studies on the variation of optical density with concentration of tetramethylmurexide were carried out in the concentration range 0.01∼0.08 mM. It is evident from Fig. 2 that the absorption of tetramethylmurexide at $\lambda=530\ \mathrm{m}\mu$ is governed by Beer's law; the value of the Beer's constant from the slope corresponds to 1.464×10^4.

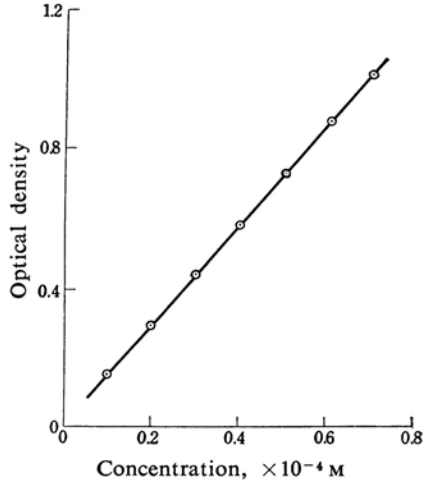


Fig. 2. Application of Beer's law to the absorption of tetramethylmurexide at $\lambda=530\ \mathrm{m}\mu$.

The observations recorded above thus provide a method for following the reaction kinetics by observing the variation with time of the optical density of acidic solution of tetramethyl murexide at $\lambda=530\ \mathrm{m}\mu$. In the experiments reported here the concentration of hydrochloric acid and tetramethyl murexide were 5 and 0.05 mM respectively. Thus the temperature was the only variable parameter in these experiments; the range of temperature investigated in these experiments was from 25∼45°C.

Determination of the Rate Constants.—For a first order reaction, we have

$$\ln C=\ln C_0-kt \qquad \text{(i)}$$

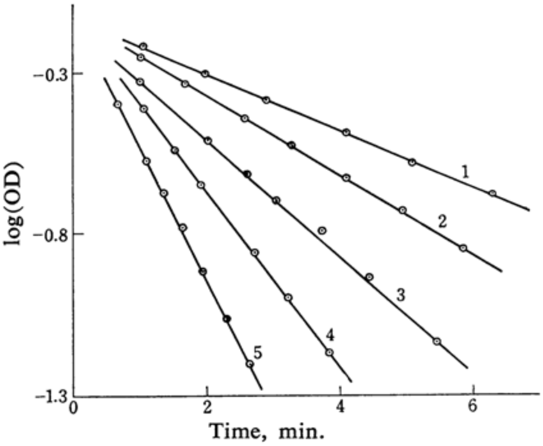


Fig. 3. log(OD) vs. time plots: concentration of HCl, 5 mM; concentration of tetramethylmurexide, 0.05 mM. Temperature: curve 1, 25°C; curve 2, 30°C; curve 3, 35°C; curve 4, 40°C; curve 5, 45°C.

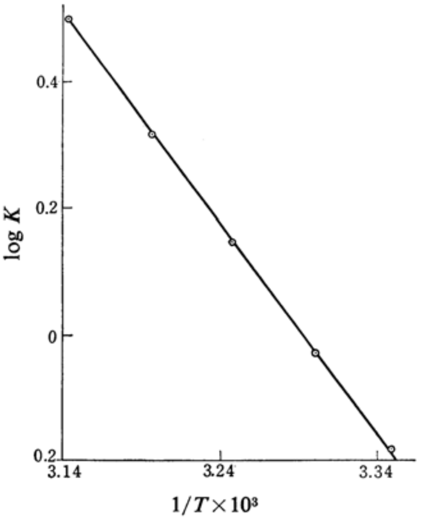


Fig. 4. Arrhenius plot.

Where C_0 is the initial concentration of the substance and C is the concentration after a time interval t. Equation 1 suggests that $\log C$ vs. t plots should be linear with slope being equivalent to $-k/2.303$. Instead of concentration one may also employ a physical property linearly dependent upon C such as optical density (vide supra). Figure 4 gives the plots of log(OD) vs. t for the reaction under investigation; from the slope of these plots, we get the value of the apparent rate constant (in time^{-1}) for the reaction. As pointed out previously[18], these k values are linearly variant with hydrogen ion concentration $[\mathrm{H}^+]$ and refer here to the experimental conditions under which the initial concentration of H^+ is taken in much excess over that of the tetramethylmurexide. The reaction is in fact bimolecular and the rate constant characteristic of the reaction is given by the relationship.

$$k=\frac{2.303\ \varDelta \log(\mathrm{OD})}{[\mathrm{H}^+]\times\varDelta t}$$

which differs from the values obtained from the slope in Fig. 4 by the factor $[\mathrm{H}^+]$ which appears in the denominator. These values of k (expressed in $\mathrm{sec}^{-1}\ \mathrm{l.\ mol}^{-1}$) corresponding to

---

[18] Cf. Ref. 14.

TABLE I. RATE CONSTANTS OF DECOMPOSITION OF TETRAMETHYLMUREXIDE IN ACID SOLUTIONS

Temp., °K	298	303	308	313	318
Rate constant l.mol^{-1}sec^{-1}	0.67	0.93	1.39	2.07	3.17

TABLE II. FREQUENCY FACTOR, ENERGY AND THERMODYNAMIC QUANTITIES OF ACTIVATION (25°C)

E	15.49 kcal.
$\log A$	11.18
$\Delta F^{\neq}$	17.69 kcal. deg^{-1} mol^{-1}
$\Delta S^{\neq}$	-4.75 e. u.
$\Delta H^{\neq}$	16.27 kcal. mol^{-1}

different temperatures are returned in Table I. Thus for example the values of k were 0.937 and 3.17 at 30°C and 45°C respectively.

Determination of Quantities of Activation.—For computation of the energy of activation of the reaction, the well-known Arrhenius equation was applied. Figure 4 gives the linear plot of $\log k$ vs. $1/T$; from the slope of this plot, we get a value of 15.49 kcal. for the energy of activation. This value of E along with the values of k in Table I, gives a value of 11.18 for the Arrhenius frequency factory ($\log A$)[19]. Also presented in this table are the data on the free energy and entropy of activation calculated from the relationship[20]

$$\Delta F^{\neq}=2.303\,\boldsymbol{RT}\left(\log\frac{\boldsymbol{RT}}{\boldsymbol{Nh}}-\log k\right) \qquad (1)$$

$$\Delta S^{\neq}=2.303\,\boldsymbol{R}\left(\log A-\log e\frac{\boldsymbol{RT}}{\boldsymbol{Nh}}\right) \qquad (2)$$

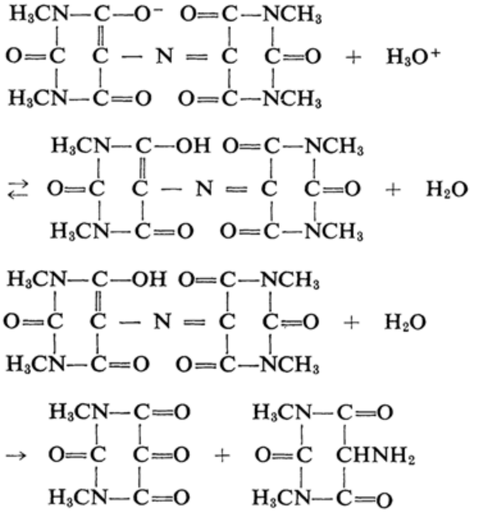
and the value of $\Delta H^{\neq}$ obtained from the values of $\Delta F^{\neq}$ and $\Delta S^{\neq}$.

Comparison with Collision Theory.—From consideration based on the collision theory one can shown that for a biomolecular reaction the kinetic collision frequency is given by[21]

$$Z=\frac{\boldsymbol{N}}{1000}\,\sigma^{2}\left[8\Pi\boldsymbol{RT}\left\{\frac{1}{M_{\mathrm{a}}}+\frac{1}{M_{\mathrm{b}}}\right\}\right]^{1/2} \qquad (3)$$

where σ is the distance of approach between the reactions for effective collision, M_{a} and M_{b} are molecular weights of the reactants and the terms $\boldsymbol{N}$, Π, $\boldsymbol{R}$ and T have their usual significance. From Eq. 3 we get a value of 11.62 for $\log Z$ in good agreement with a value of 11.18 obtained experimentally for the Arrhenius frequency factor. Similar agreements have been obtained for various reactions in solutions[22] which are free from complications arising out of ionic interactions etc.

Possible Reaction Mechanism.—In view of the above observations and with the knowledge of the reaction products, a possible mechanism of the reaction can be formulated. The problem of the products formed as a result of the decomposition of murexide and substituted murexides in acid solutions has been widely discussed[23]. The presence of alloxan and uramil along with some alloxantin has been detected[24]. In fact it was a controversial point as to whether the alloxan and uramil are primary products or the alloxantin. It was, however, convincingly shown by Davidson[25] that the decomposition of murexide in acid medium gives alloxan and uramil; these compounds then undergo secondary reactions to give alloxantin. For tetramethylmurexide the products of the primary reaction would thus be dimethyl substituted alloxan and uramil. It is suggested that this process takes place according to the following mechanism:



It is of interest to point out here that it has not been so far possible to isolate the free purpuric acid or substituted purpuric acids because of their extreme instability. On the other hand in case of certain compounds related to murexide for which the corresponding acids are stable, the addition of acids to say ammonium salts results in the liberation of free acid[26]. Further the direct interaction of the tetramethylpurpurate ion and the hydronium

---

[19] Calculated from the equation: $\log A=\log k+E/2.303\boldsymbol{RT}$

[20] S. Glasstone, K. L. Laidler and A Eyring, "The Theory of Rate Processes", McGraw Hill Book Co., Inc. New York (1941), pp. 195, 199.

[21] E. A. Molewyn-Hughes, "The Kinetics of Reaction in Solutions", Macmillan & Co., London (1933), p. 79.

[22] Cf. Ref. 21.

[23] O. Piloty, *Ann.*, **22**, 333 (1904); B. J. R. Mohlau, *Ber.*, **37**, 2686 (1904); M. D. Slimmer and J. Stieglitz, *Am. Chem. J.*, **31**, 661 (1904).

[24] H. E. Wohler and J. Liebig, *Ann.*, **26**, 319 (1838); F. K. Beilstein, ibid., **107**, 176 (1858).

[25] D. Davidson and E. Epstein, *J. Org. Chem*, **1**, 305 (1936).

[26] Cf. Ref. 15.

ion cannot be the rate determing one, as for such a process the kinetic data should be such as to suggest an ionic interaction. Moreover the tetramethylpurpurate is considered to be in equilibrium with tetramethylpurpuric acid as a result of the ionic combination of the former with the hydronium ion. This process will be governed by an equilibrium constant the reciprocal of which will be, according to the modern concepts(27), the ionization constant of tetramethylpurpuric acid; the ionic combination of tetramethylpurpurate and the hydronium ion cannot be considered to be the rate determing one, as such processes are known to be extremely fast(28). Thus it appears very likely that the instability of tetramethylpurpuric acid, the formation of which is favored in presence of excess of acid, is responsible for the decomposition of tetramethylmurexide in acid medium and for the observed kinetics of the reaction.

Summary

Kinetics of the decomposition of tetramethylmurexide in acid solutions have been investigated spectrophotometrically by following the absorption at $\lambda = 530\,\mathrm{m}\mu$. The optical density of tetramethylmurexide at this wavelength has been shown to obey Beer's law with a value of 1.464×10^4 for the Beer's constant. The change in the optical density with time of tetramethylmurexide solutions in presence of hydrochloric acid has been shown to obey a first order law and from these data the rate constants of the reaction have been computed at different temperatures in the range 25∼45°C. These data have been employed to obtain quantities such as energy and entropy of activation etc. and the Arrhenius frequency factor; the comparison of this last has been made with the collision theory. The possible mechanism of the reaction has been discussed.

Author's grateful thanks are due to Dr. H. Trivedi, Principal, for facilities and encouragement and to Dr. J. B. Lal, head of the Chemical Engineering Department, for his kind interest in the work.

Department of Physical Chemistry
H. B. Technological Institute
Kanpur, India

---

27) R. W. Gurney, "Ionic Processes in Solutlon", McGraw Hill Book Co., Inc., New York (1953); see also M. Mandel and C. Decroly, *Trans. Faraday Soc.*, **56**, 29 (1960).

28) M. Eigen, *Discussions Faraday Soc.*, **17**, 194 (1954); P. Delahay and T. J. Adams, *J. Am. Chem. Soc.*, **74**, 1437 (1954).