

The Kinetics of the Hydrolysis of Atropine*

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A study has been carried out on the chemical kinetics of the hydroxyl ion catalyzed hydrolysis of atropine in solution. The mechanism of this hydrolysis has been shown to consist of two reactions, the effect of each being governed by the hydroxyl ion concentration. The temperature dependency of each reaction has been established, and equations have been proposed which enable the approximation of the half-life of atropine undergoing hydrolysis at constant temperature and constant hydroxyl ion concentration. Half-life values calculated by these equations have been shown to compare favorably with those obtained experimentally.

INSTABILITY of atropine in solution is due mainly to the hydrolytic reaction yielding tropine and tropic acid. Several investigators have reported atropine to be very stable at low pH, even at autoclave temperatures, but at higher pH values hydrolysis occurred and was enhanced by increased temperature and hydroxyl ion concentration (1-4). However, no attempt has been made to study the problem from a chemical kinetic viewpoint.

The object of this study is to establish a basis for predicting the rate of hydrolytic reactions of atropine in solution at various hydroxyl ion concentrations and temperatures. Studies were made at temperatures ranging from 25 to 60° and at hydroxyl ion concentrations covering roughly a million fold range, i. e., 10^{-1} to 10^{-7} N. Periodic quantitative determinations of the residual ester were made at a number of hydroxyl ion concentrations for each of the temperatures used. Specific velocity constants and activation energies were determined and these were used to derive an equation that appears to be valid for widely differing conditions of temperature and hydroxyl ion concentration.

THEORETICAL CONSIDERATION

The proton catalyzed hydrolysis of atropine is slow. We have found the reaction velocity constant to be approximately $3 \times 10^{-6} \text{ min.}^{-1}$ in 0.1 N HCl. For the purpose of this investigation, it may be assumed that the hydroxyl ion catalyzed reaction is the mechanism responsible for hydrolysis of atropine in solution. Two hydrolytic pathways are possible for atropine because in solution it can occur as the free base or as the charged ion. For the purpose of this study, hydrolysis of the free base will be designated Reaction 1; that of the charged ion will be designated Reaction 2.

This study is based considerably on the theoretical concepts employed by Higuchi, *et al.* (5), in their study on procaine. Using the derivations of these

authors, the overall rate of hydrolysis of atropine may be expressed as

$$\frac{d \ln a}{dt} = \frac{(\text{OH}^-)}{k_b + (\text{OH}^-)} [k_1(\text{OH}^-) + k_2k_b] \quad (1)$$

where a = total drug concentration; k_1 = specific velocity constant for Reaction 1; k_2 = specific velocity constant for Reaction 2; and k_b = dissociation constant of the base.

The right side of Eq. 1 is a constant at any given hydroxyl ion concentration. If Reactions 1 and 2 are essentially responsible for the hydrolysis of atropine, the overall rate of hydrolysis would be first order with respect to drug concentration. Therefore, at any constant hydroxyl ion concentration the half-life of the drug would be expressed as follows:

$$\frac{1}{t_{1/2}} = \frac{(\text{OH}^-)}{k_b + (\text{OH}^-)} \left[\frac{k_1(\text{OH}^-) + k_2k_b}{0.693} \right] \quad (2)$$

Equation 2 may be expressed logarithmically:

$$-\log t_{1/2} = \log (\text{OH}^-) - \log [k_b + (\text{OH}^-)] + \log \left[\frac{k_1(\text{OH}^-) + k_2k_b}{0.693} \right] \quad (3)$$

At relatively high hydroxyl ion concentration the equation reduces to:

$$-\log t_{1/2} = \log (\text{OH}^-) + \log k_1/0.693 \quad (4)$$

and at low hydroxyl ion concentrations the equation simplifies to:

$$-\log t_{1/2} = \log (\text{OH}^-) + \log k_2/0.693. \quad (5)$$

Equations 4 and 5 indicate that at both ends of the pH scale the rate of hydrolysis would be directly proportional to the hydroxyl ion concentration. In the intermediate region of the pH scale there would be an area of nonproportionality.

EXPERIMENTAL

Method of Analysis.—The experimental method used in this study consisted of a series of ultraviolet spectrophotometric determinations of atropine solutions undergoing hydrolysis at constant temperatures. Atropine sulfate U. S. P., recrystallized twice from ethanol, was used; the melting point was 189 to 190°.

The ultraviolet absorption spectra of atropine is practically identical with that of its hydrolytic product, tropic acid; therefore, a separation of the two compounds before scanning the solutions was

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necessary. The absorption curve for atropine in acid solution is shown in Fig. 1. Absorption measurements were made with a Beckman Model DU Quartz Spectrophotometer at wavelengths of 256.5 and 262 $m\mu$. The molar extinction coefficients at these wavelengths were found to be 198 and 149, respectively. The ratio of the absorbances at the two wavelengths, being constant over a wide range of drug concentration, served as a check on the analyses.

Buffer system.—The hydroxyl ion concentration of the solutions was maintained by using either buffers or an excess of strong base. The change of hydroxyl ion concentration during the experimental period was limited to 0.1 pOH unit.

Buffer systems used in this study are listed in Table I. The pH of each buffer was determined at the temperature of the run.

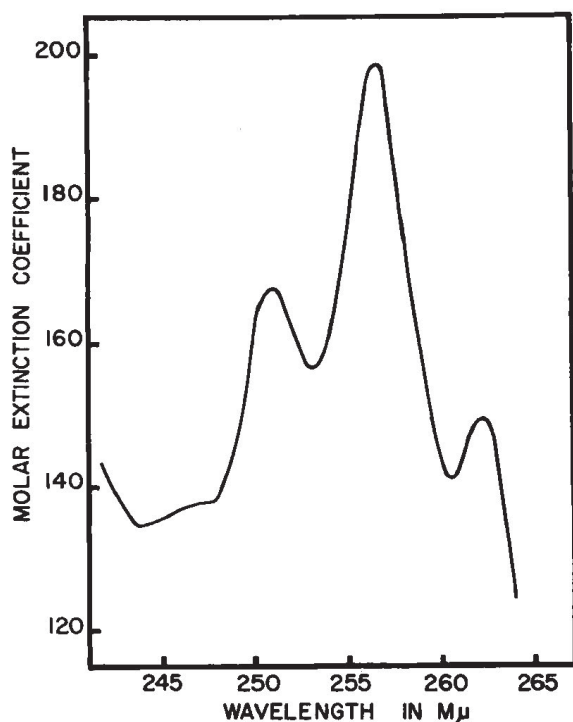


Fig. 1.—Ultraviolet absorption curve of atropine in 1.0 N HCl .

Experimental Procedure.—The procedure outlined below is essentially the one followed in this investigation.

Buffer solution was placed in a thermostatically controlled bath and allowed to reach temperature equilibrium. Sufficient standard solution, containing 10 mg./cc. of atropine sulfate, was then added to give in the reaction mixture a concentration of 1 mg./cc. The progress of the reaction mixture was followed by periodically removing 10-cc. samples for residual ester determination.

Tropic acid was separated from the residual ester by a modification of the U. S. P. method for purification of alkaloids (6). The sample for analysis was made alkaline with ammonium hydroxide; atropine, in base form, was extracted with two 10-cc. portions of chloroform. The chloroform extracts were

TABLE I.—LIST OF BUFFERS

pH Range	Composition
6.99–7.97	$M/15 KH_2PO_4 - Na_2HPO_4$
8.03–9.89	$M/10 H_3BO_3 + M/10 KCl + NaOH$
10.01–10.51	$M/20 Na_2B_4O_7 - Na_2CO_3$

combined, and atropine, in charged form, was then extracted with exactly 10 cc. of 0.1 N hydrochloric acid. The extent of hydrolysis was then calculated from the ultraviolet absorption of the acid extract.

RESULTS AND DISCUSSION

Influence of Ester Concentration.—At constant temperature and hydroxyl ion concentration, the observed rate of hydrolysis of atropine is first order with respect to ester concentration. This is illustrated in Fig. 2, 3, and 4 representing, respectively, neutral, slightly alkaline, and moderately alkaline reaction conditions. These results are in accord with Eq. 1.

Influence of Hydroxyl Ion Concentration.—Figure 5 illustrates the effect of hydroxyl ion concentration on the hydrolytic reaction rate of atropine. The points were determined experimentally at 40°, whereas the smooth curve, which apparently fits the experimental points, represents Eq. 3. Values for k_1 and k_2 in this equation were obtained by substituting in Eq. 4 and 5, respectively, the extreme points of Fig. 5; the k_b for atropine was determined in 1% $NaCl$ at 40°. These constants are: $k_1 = 0.95 \text{ min.}^{-1}$; $k_2 = 33.6 \text{ min.}^{-1}$; $k_b = 7.3 \times 10^{-5}$.

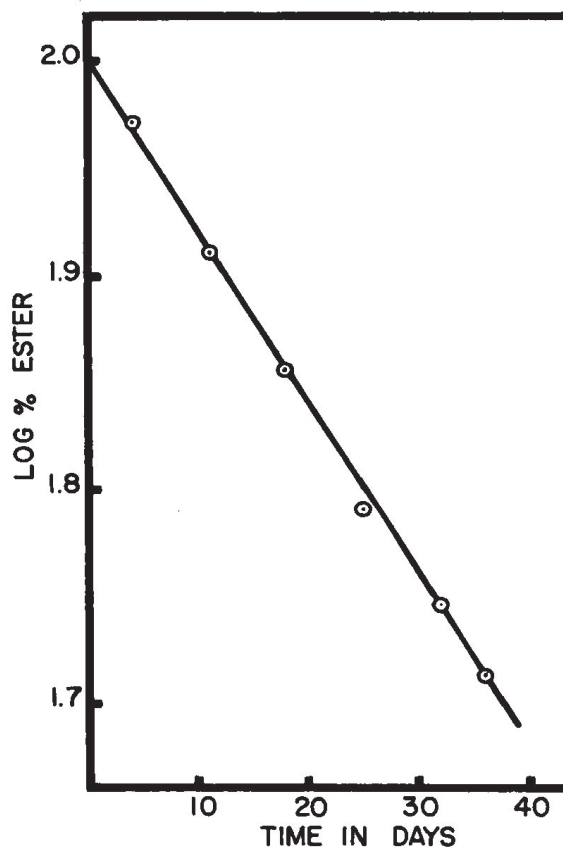


Fig. 2.—Hydrolysis of atropine in phosphate buffer at pH 7.10 and at 40°.

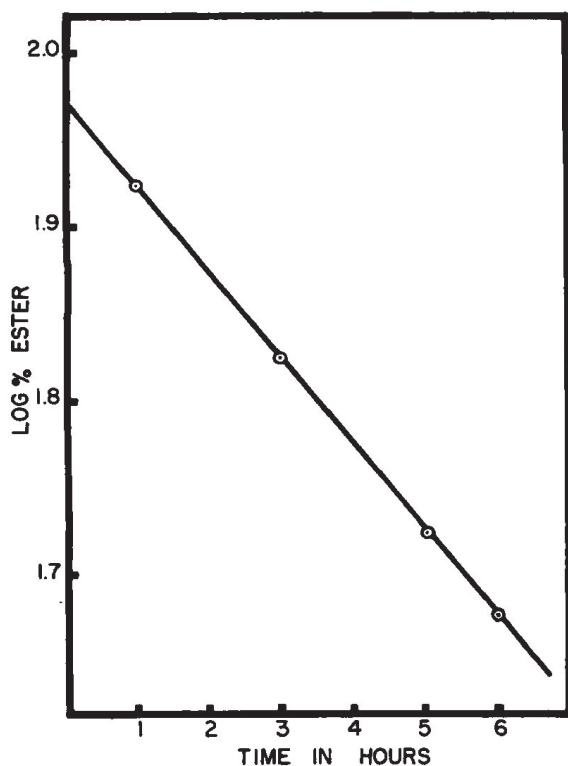


Fig. 3.—Hydrolysis of atropine in borate buffer at pH 9.89 and at 40°.

Figure 5 shows that above pH 11.5 the mechanism of hydrolysis is that of Reaction 1, or its equivalent; below pH 8.5 the mechanism is that of Reaction 2, or its equivalent. An area of nonproportionality lies between these two pH values.

The following study was carried out in order to determine the temperature dependency of Reactions 1 and 2, and to ascertain whether or not a change in the mechanism of hydrolysis of atropine is associated with a change in temperature.

Temperature Dependency of Reaction 1.—Velocity constants were determined at 25, 32.5, and 40° on three alkaline solutions of atropine that were 0.010 *N*, 0.020 *N*, and 0.040 *N* with respect to hydroxyl ion concentration. These measurements, which theoretically should represent a Type 1 reaction, were used for calculating the velocity constant at 1 *N* hydroxyl ion concentration for each of the temperatures used. In Fig. 6 the velocity constants are plotted against the reciprocal of the absolute temperature. The straight line relationship indicates that the mechanism responsible for the ester hydrolysis, essentially that of Reaction 1 or its equivalent, is not altered with changes in temperature. The slope of this line was calculated and by setting it equal to $-E_a/2.303 R$, the energy of activation, E_a , was found to be approximately 7.7 Kcal. The frequency factor in the Arrhenius equation $k = Ze^{-E_a/RT}$ was found by substituting the conditions at 40°, i. e., $k_1 = 0.016 \text{ sec.}^{-1}$ and $E_a = 7,700 \text{ calories}$; Z comes out to be approximately $3.6 \times 10^3 \text{ sec.}^{-1}$,

$$\left. \begin{array}{l} \text{or } k_1 = 3.6 \times 10^3 e^{-7,700/RT} \text{ sec.}^{-1} \\ \text{and } k_1 = 2.1 \times 10^5 e^{-7,700/RT} \text{ min.}^{-1} \end{array} \right\} \quad (6)$$

TABLE II.—EXPERIMENTAL HALF-LIVES OF ATROPINE AT HIGH pH COMPARED WITH PREDICTED VALUES

Temp.	pOH	Half-Life in Minutes	
		By Experiment	By Equation
25.0	2.00	128.0	137.0
	1.70	63.6	68.3
	1.40	34.7	34.1
32.5	2.00	92.0	100.0
	1.70	46.2	49.8
	1.40	26.0	24.9
40.0	2.00	66.3	73.8
	1.70	34.1	36.7
	1.40	18.7	18.4

On substituting the value for Eq. 6 in Eq. 4 a formula is obtained for estimating the half-life of atropine above pH 11.5 at constant temperature and hydroxyl ion concentration. A comparison of the experimental values and those predicted by formula for Reaction 1 is given in Table II.

Temperature Dependency of Reaction 2.—Half-lives were determined at 40, 49.8, and 59.8° on solutions of atropine of pH 7.0, 7.5, and 8.0, since Fig. 5 indicates Reaction 2 to be the mechanism responsible for the hydrolytic reaction at these pH values. In Fig. 7 the logs of these half-lives are plotted against the reciprocal of the absolute tem-

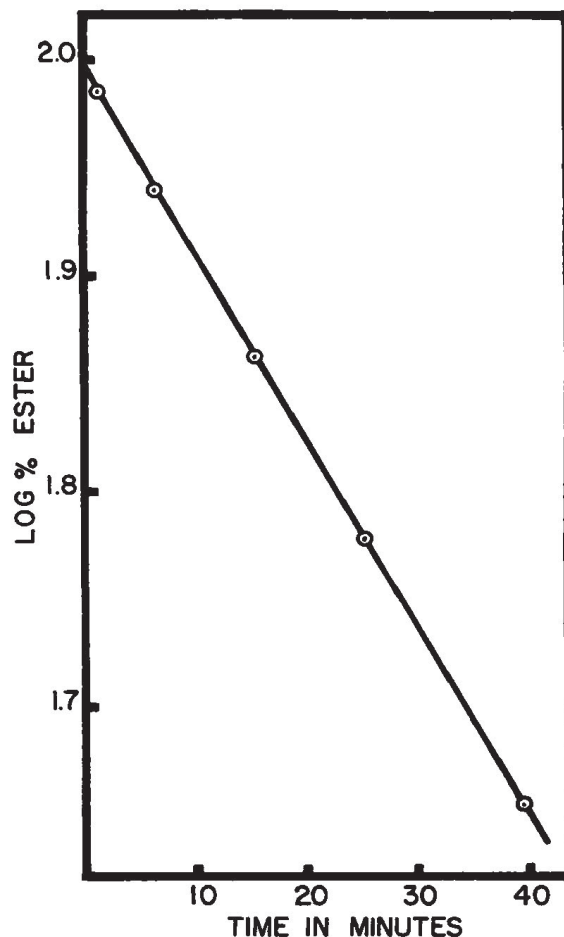


Fig. 4.—Hydrolysis of atropine in 0.02 *N* Ba(OH)₂ and at 40°.

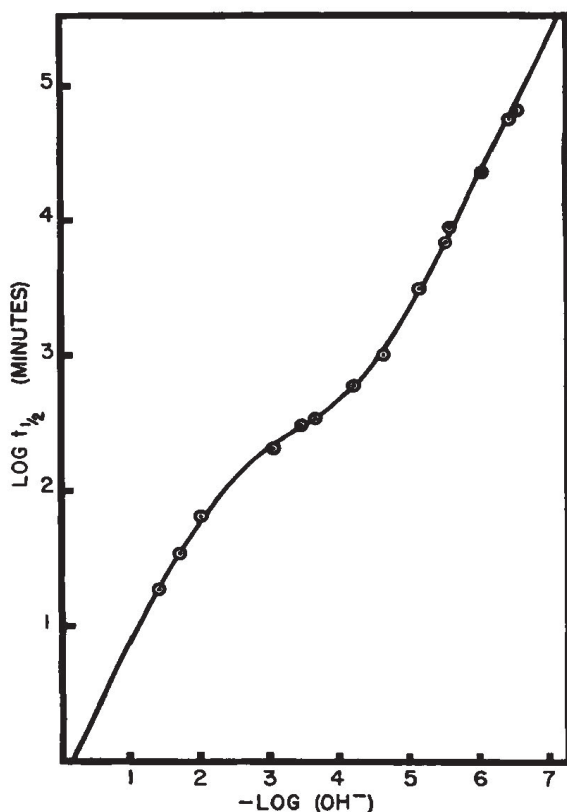


Fig. 5.—A Relation of half-life of atropine and hydroxyl ion concentration at 40°.

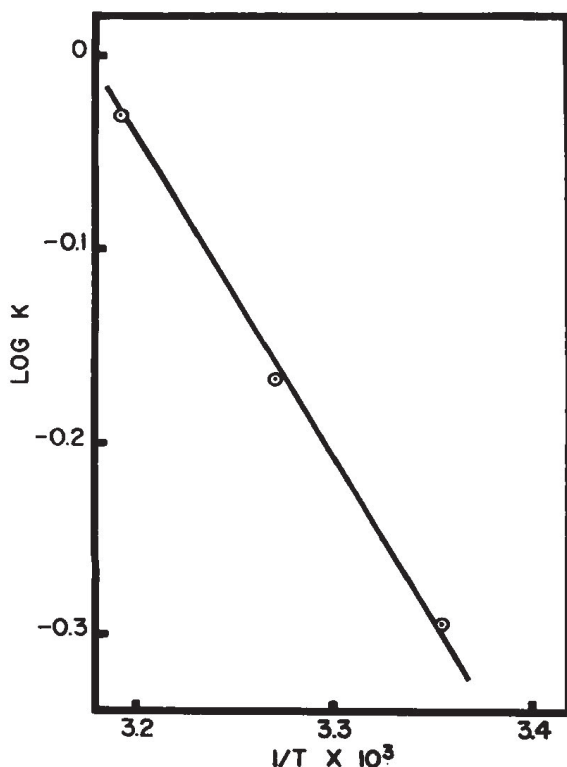


Fig. 6.—Temperature dependency of reaction 1. Log K min.⁻¹ vs. reciprocal of the absolute temperature of the run.

perature, $1/T$; from the slope of the lines the activation energy, E_a , was calculated. The experimental data, when subjected to an analyses of covariance (7), gave an average slope of 5,400, with a standard error of 250. The activation energy, calculated from the slope, comes out to be 24.7 ± 1.1 Kcal. Taking into account the heat of ionization of water, which is approximately 12 Kcal. at 40°, the activation energy for Reaction 2 is 24.7 minus 12, or 12.7 Kcal. per mole.

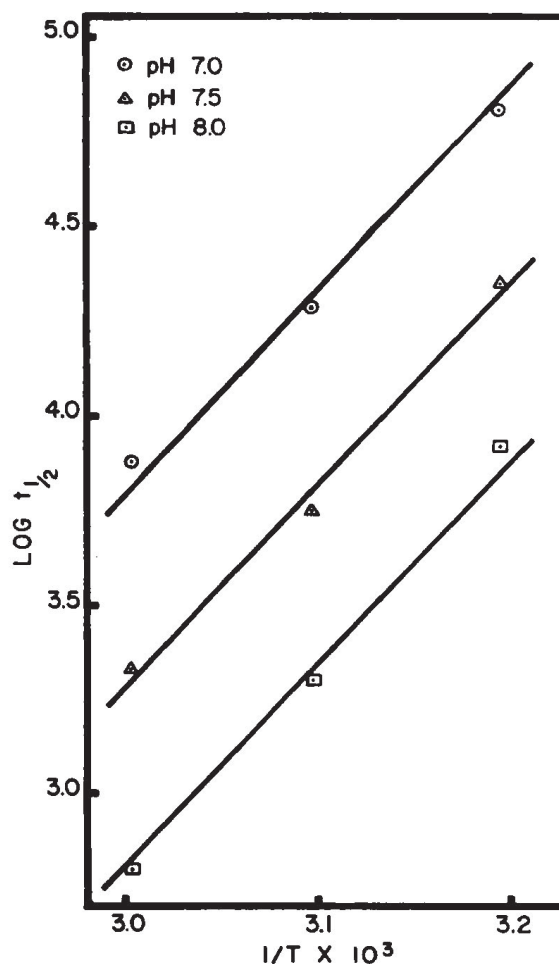


Fig. 7.—Temperature dependency of reaction 2. Log $t_{1/2}$ in minutes vs. reciprocal of the absolute temperature of the run.

The corresponding Arrhenius frequency factor for Reaction 2, when calculated from values for 40°, i. e., $k_2 = 0.56 \text{ sec.}^{-1}$ and $E_a = 12,700$ calories comes out to be $3.5 \times 10^8 \text{ sec.}^{-1}$,

$$\left. \begin{array}{l} \text{or} \quad k_2 = 3.5 \times 10^8 e^{-12,700/RT} \text{sec.}^{-1} \\ \text{and} \quad k_2 = 2.1 \times 10^{10} e^{-12,700/RT} \text{min.}^{-1} \end{array} \right\} (7)$$

When the value for Eq. 7 is substituted in Eq. 5, one obtains a simple relationship that expresses the half-life of atropine solutions buffered below pH 8.5 as a function of pOH and temperature. In Table III half-lives of atropine solutions determined experimentally are compared with those predicted by Eq. 5.

The complete formula for the hydroxyl ion catalyzed hydrolysis of atropine may be obtained by substituting Eq. 6 and 7 into Eq. 3, i. e.

$$-\log t_{1/2} = \log (\text{OH}^-) - \log [7.3 \times 10^{-6} + (\text{OH}^-)] + \log [3.1 \times 10^6 e^{-7,700/RT} (\text{OH}^-) + 2.2 \times 10^6 e^{-12,700/RT}]$$

TABLE III.—EXPERIMENTAL HALF-LIVES OF ATROPINE AT NEUTRAL pH COMPARED WITH PREDICTED VALUES

Temp.	pH	Half-Life in Hours	
		By Experiment	By Equation
40.0	7.01	1044.0	1165.0
	7.10	906.0	947.0
	7.50	377.0	377.0
	7.97	149.0	128.0
49.8	7.01	315.0	346.0
	7.49	95.0	115.0
	7.94	38.4	40.7
59.8	6.99	128.0	110.0
	7.47	37.5	36.8
	7.97	11.3	11.6

This formula can be used to calculate the half-life in minutes of atropine solutions falling within the nonproportional section of the hydrolysis curve. Experimental half-lives of atropine in solution covering this range of pOH at 40° are compared with those calculated by this formula in Table IV.

This work has been concerned only with the hydrolysis of atropine in solution catalyzed by hydroxyl ions. Although the proton catalyzed hydrolysis of atropine is very slow by comparison,

TABLE IV.—EXPERIMENTAL HALF-LIVES OF ATROPINE AT MODERATE pH AND 40° COMPARED WITH PREDICTED VALUES

pH	Half-Life in Hours	
	By Experiment	By Equation
8.03	114.0	116.0
8.37	50.8	55.4
8.91	16.1	19.2
9.34	10.0	9.9
9.89	5.8	5.7
10.01	5.1	5.0
10.51	3.4	3.7

it does exist and indications are that it may become of practical significance at a pH value of about 4 and lower. A kinetic study of the proton-catalyzed reaction is presently being carried out in this laboratory. Data which will be obtained from this study, along with those presently reported, should provide a basis for predicting the pH range over which atropine in solution is least prone to the overall effect of both hydroxyl ion and proton-catalyzed hydrolysis, i. e., most stable during prolonged storage.

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Assay of 2-Aminoheptane and Methyl Hexane Amine in Plastic Inhalers by Titration in Glacial Acetic Acid*

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The 2-aminoheptane and methyl hexane amine content of plastic inhalers can be determined rapidly and accurately by titrimetric assay in glacial acetic acid solution. The amine is extracted directly from the saturated pledget with glacial acetic acid and titrated with perchloric acid. 4-Aminopyridine was found to offer advantages over potassium acid phthalate for the standardization of the perchloric acid-glacial acetic acid solution.

THE ASSAY of 2-aminoheptane¹ and methyl hexane amine² in plastic inhalers by the usual aqueous acid extraction and titration is made

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¹ Inhaler Tuamine® (tuaminoheptane, Lilly).

² Inhaler Forthane® (methyl hexane amine, Lilly).

difficult by two factors. The amine is so strongly adsorbed on the cotton pledget that it requires a prolonged extraction on the steam bath in acid solution to completely extract the amine. The hot acid extraction also has been found to remove basic constituents from the plastic inhaler. This makes it necessary to perform a blank titra-