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Stability of Pipethanate Hydrochloride in Aqueous Solution

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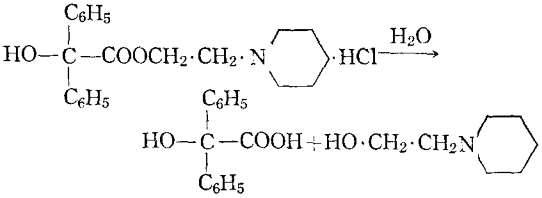
Abstract □ In the present investigation, an attempt has been made to apply the methods of classical chemical kinetics to the hydrolytic reaction of pipethanate hydrochloride. By successively keeping all but one variable essentially constant, it has been possible to resolve the overall effect of the individual contributing factors. Since nearly all commercial pipethanate preparations are formulated with antacid, studies were made at several constant hydrogen ion concentration ranging pH 0.4 to 7.5. Rate measurement was also carried out in temperature ranging from 25°C to 60°C. The hydrolysis of pipethanate is found to be of first order with respect to pipethanate concentration over an experimental range of hydrogen ion concentration (pH 0.4—7.5). The apparent activation energy(Ea) at pH 7.5 is 18.30 Kcal/mole and the frequency factor is 1.1408×10^9 sec^{-1}. The rate of the hydrolysis has a minimum at pH 2.5–3.5. In this region the half-life of pipethanate was about 15.3 days at 60 °C. The catalytic effect of water was found to be $K_{H_2O}=3.16\times10^{-5}$ min^{-1} at 60 °C. The catalytic constants of the hydroxyl ions and hydrogen ions at 60 °C were also found to be $K_{OH^-}=4.5519\times10^5$ min^{-1} and $K_{H^+}=1.1568\times10^{-2}$ min^{-1}, respectively. This reaction appears to be primarily base catalyzed hydrolysis and pipethanate is relatively reluctant toward acid catalyzed hydrolysis. A positive primary salt effect was noted in the solution of pipethanate at pH 7.5 and at 60 °C.

Key phrases □ Pipethanate hydrochloride-assay in aqueous solution, stability, degradation kinetics. Stability pipethanate hydrochloride, effect of pH, temperature, ionic strength, acid-base catalysis.

The pipethanate hydrochloride has been used as parasympathetic and antispasmodic agent. Pipethanate was first synthesized in 1947[1)] and the synthetic process was modified by Lespagnol et al[2)] and Lemoine[3)]. Kawashina et al[4)] reported the effect of pipethanate on central nervous system and studied drug interaction with drugs acting on the central nervous system like barbiturate and morphine. Tanaka et al[5)] reported that the treatment of rabbits with pipethanate caused hypotension which was not blocked by dichloroisoproterenol or atropine, and pipethanate decreased respiration and heart rates.

The scope of the present work was to study the hydrolysis of pipethanate hydrochloride both in acidic and in alkaline solution, and to determine to what extent the hydrolysis is catalyzed by hydrogen and/or hydroxyl ions.

It is known from the literature that the hydrolysis of esters is subject to both hydrogen ion and hydroxyl ion catalysis[6-8)]. It is assumed that the breakdown of pipethanate can be attributed solely to the hydrolytic cleavage of the ester linkage according to the following equation



yielding N-2-piperidinoethanol and benzilic acid.

Although the rate of hydrolysis of pipethanate has been studied[9], no attempt seems to have been made to study the whole problem from a chemical kinetics point of view. In the present investigation, an attempt has been made to apply the methods of classical chemical kinetics to the hydrolytic reaction of pipethanate hydrochloride.

EXPERIMENTAL

Materials

The pipethanate hydrochloride used was purchased from Il-Yang Pharm. Co. & recrystallized from benzene.

All chemicals used were of analytical reagent grade.

Analytical Procedure

The study of the hydrolysis of pipethanate required an assay method which permitted an accurate determination of pipethanate in the presence of degradation procucts. 100 mg of pipethanate hydrochloride was dissolved in 100 ml of phosphate buffer (1/15M, pH 7.5). This solution was diluted with same phosphate buffer to make a series of different concentrations. 10 ml of each solutions was taken and after the addition of 3 g of sodium chloride, the pipethanate presented in 10 ml of solution was extracted with 50 ml of chloroform.

Chloroform layer of solution was taken and filtrated to remove the solid residue. 10 ml of chlorofrom layer of sample was mixed with 1.5 ml of 0.1% B.C.G. solution and then readjusted to pH 5.6 by adding 5 ml of Mcllvain buffer.

This solution was agitated by mechanical shaker for 5 min and allowed to equilibrate at least 15 min after agitation. Chloroform layer was then taken, dehydrated by the addition of 0.2 g of disodium sulfate anhydrous and the chlorofrom layer was filtrated. The ultraviolet absorbance of the resulting solution was measured at wavelength of 418 nm on a model SP 1750 Pye Unicam Spectrophotometer. The calibration curve was constructed with UV absorbance data of five different concentration. Fig. 1 indicates the calibration curve of pipethanate HCl which is linear within the concentration

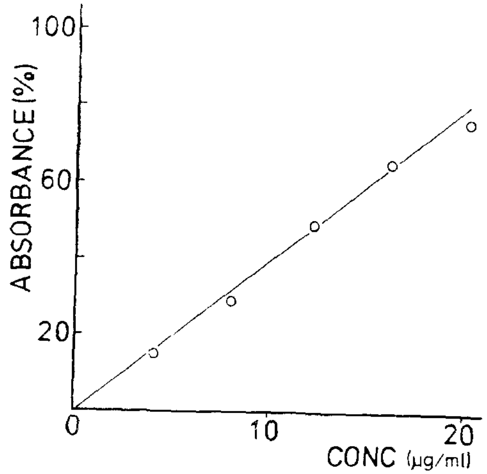


Fig. 1: Calibration curve of pipethanate·HCl.

range exhibited.

Control of pH

The buffer solutions were prepared by precalculation of the buffer concentration utilizing the pKa values of the buffer components. In all cases, the ionic strength was adjusted by the addition of sodium chloride. Buffers used in this study are listed in Table I.

Table I: List of buffers employed and composition of each.

pH	Composition	Dilution
0.4		
1	Adjust pH with conc-HCl	
1.8	0.2 M HCl 100 ml+0.2 M KCl 33.3 ml	As is
2.65	0.2 M HCl 1 ml+0.2 M KCl 132.3 ml	As is
3.6	0.1 M HAc 160ml+0.1 M NaAc 400 ml	As is
4.5	0.1 M HAc 60 ml+0.1 M NaAc 140 ml	As is
5.0		
6.0	Make buffer solution with 1/15 M	
6.5	NaH_2PO_4 and 1/15 M Na_2HPO_4	
7.0		
7.5		

Hydrolysis Experiments

The experimental method employed in the present study consisted essentially of making a series of UV absorption measurements on a pipethanate solution undergoing hydrolysis in a constant temperature bath. The following procedure was employed: A 100 ml volumetric flask containing slightly less than 80 ml of buffer solution was placed in a thermostatically controlled bath which was maintained at the desired temperature, ±0.5 °C. When the flask content had reached the temperature of bath, 20 ml of 0.1 % pipethanate hydrochloride was added. The flask was then filled to the mark with buffer solution and shaken. After allowing 5 min for temperature equilibrium, the zero-hour samples were removed and further samples were withdrawn at definite time intervals depending on the rate of hydrolysis. 10 ml of sample solution in each time was pipetted.

The reaction was quenched by immersion of the samples in ice water. The concentration of pipethanate remained was determined from the calibration curve.

RESULT AND DISSCUSSION

Order of Reaction with Respect to Pipethanate

The rate of disappearance of pipethanate from the solution at pH 7.5 was found to be strictly first order with respect to the concentration of pipethanate at temperature from 25 °C to 60 °C. There was a linear relationship between time and logarithm of

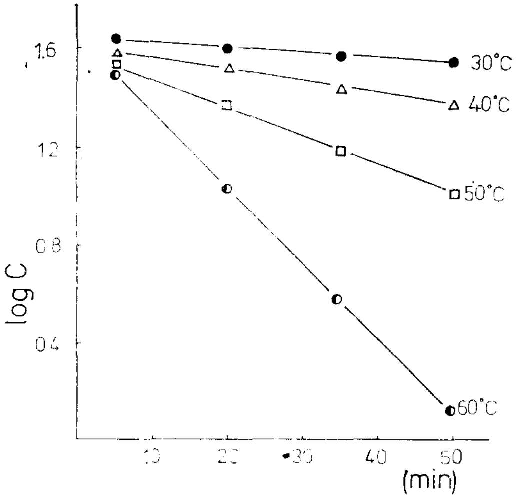


Fig. 2: The effect of temperature on the hydrolysis of pipethanate·HCl with $\mu=0.266$ at pH 7.5.

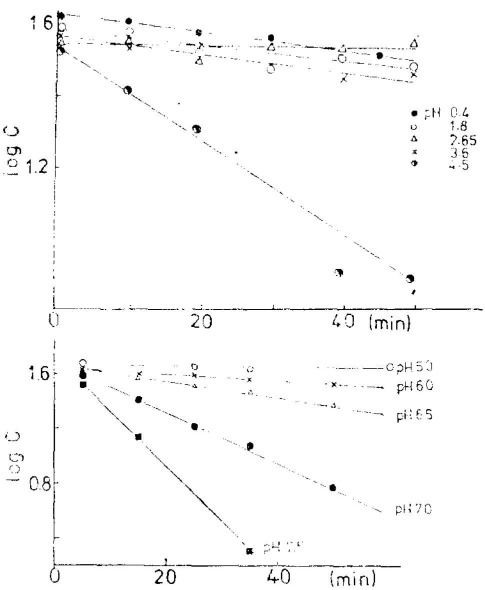


Fig. 3: The effect of pH on the hydrolysis of pipethanate·HCl with $\mu=0.266$ at 60°C.

residual pipethanate concentration as shown in Fig. 2. It was also found that the rate of disappearance was first order with respect to the concentration of pipethanate at pH values from 0.4 up to 7.5 as shown in Fig. 3.

Determination of the Apparent Activation Energy of the Hydrolytic Reactions

The apparent activation energy of the reaction was determined by measuring the observed rate constants at varying temperatures under constant pH condition. The result shown in Fig. 4 was obtained from studies at pH 7.5, based on Fig. 2. Linear relationship was observed when log K^{obs} was plotted against the reciprocal of the absolute temperature.

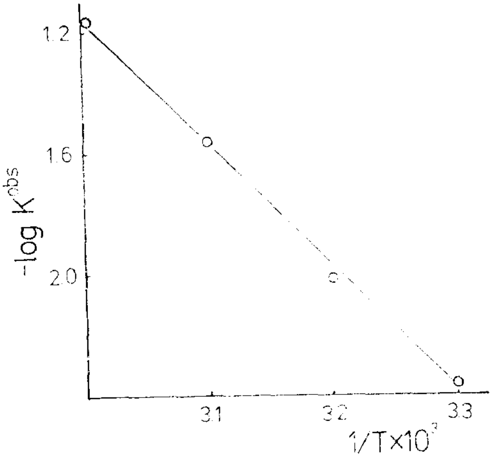


Fig. 4: Arrhenius plot for the hydrolysis of pipethanate HCl at pH 7.5.

The linearity of the points over the temperature range indicated the abherence of the observed reactions to the Arrhenius equation and allowed calculation of the apparent activation energy from the slope of the line. The apparent activation energy (Ea) at pH 7.5 was found to be 18.30 Kcal/mole and the frequency factor to be 1.1408×10^9 sec^{-1}.

Effect of Ionic Strength on the Reaction Rate

As expected from the various possibilities of pipethanate to exist in a charged state, it was found that the rate of hydrolytic reaction was influenced by ionic strength. It is of pharmaceutical interest, that where the salt effect was operative, an increase of ionic strength resulted in a linear rate of degradation, as shown in Fig. 5. To conduct these studies, solutions were prepared at constant pipethanate concentration (1% w/v), constant buffer concentration (1/30 M phosphate buffer) and constant pH(7.5), but at varying ionic strength. The ionic strength was controlled by the addition of sodium chloride.

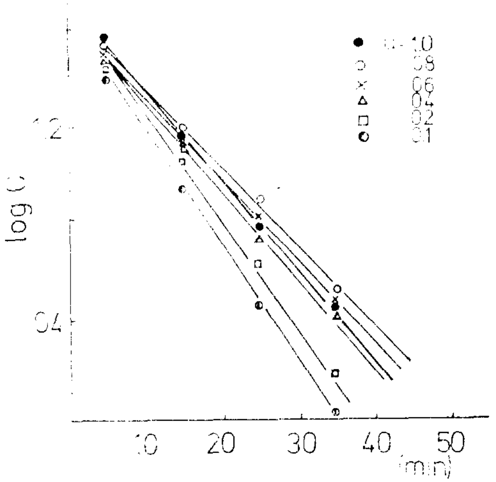


Fig. 5: The effect of ionic strength on the hydrolysis of pipethanate·HCl at 60°C and pH 7.5.

The data were analyzed by utilization of the Brönsted–Bjerrum equation,[10] which relates the primary salt effect on the observed reaction rate to the ionic strength as follows.

$$\log K^{obs} = \log K_0 + 2AZ_a \cdot Z_b \sqrt{\mu}$$

where μ is the ionic strength; K_0 is the intrinsic reaction rate constant without ionic strength; A is a constant; Z_a and Z_b are the valence charge of the reactants.

This relationship was derived on the assumption of low ionic strength ($\mu \leq 0.2$).

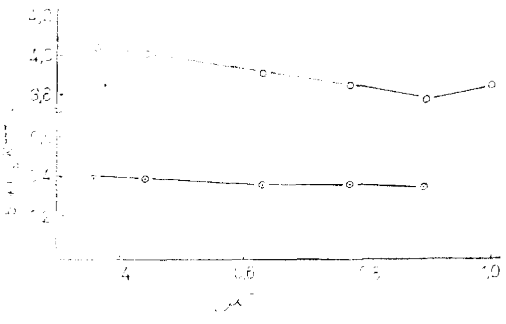


Fig. 6: Brönsted–Bjerrum type plot for the hydrolysis of pipethanate·HCl at 60°C and pH 7.5.

The data were plotted in Fig. 6, based on Brönsted–Bjerrum equation. The value for $(Z_a \cdot Z_b)$ at 60 °C was -0.41 and the intrinsic reaction rate constant at 60° C was 0.1150 min $^{-1}$. As might be expected, the experimental values were deviated from linearity as ionic strength was increased, but the values obtained from the initial slope were of interest. The finding of nonintegral values for $(Z_a \cdot Z_b)$ might be due to high ionic strength, or to a significant contribution by the secondary salt effect. Because of the primary salt effect in pipethanate solution, all experiments were carried out at the same ionic strength, namely, 0.266.

pH–Rate Profile of Pipethanate Hydrolysis

The pH–rate profile shown in Fig. 7 indicates the relation between pH and log K^{obs}. pH of the acid solution at 60 °C and $\mu = 0266$ was calculated from the following equation.

$$\begin{aligned} pH &= -\log[H^+] - \log \gamma \\ &= -\log[H^+] + 0.103 \end{aligned}$$

The above relationship between pH and $[H^+]$ was obtained as the result of pH measurements in acids of known hydrogen ion concentration at 60 °C and $\mu = 0.266$. As with all kinetic relationship, a number of different chemical reactions can lead to the same observed experimental dependencies. At zero buffer concentration, the following reactions will contribute to the overall velocity of the hydrolysis.

Reaction I: protonated pipethanate

$$[P^+] + [H^+] \xrightarrow{K_{H^+}}$$

Reaction II: protonated pipethanate

$$[P^+] + [H^+] \xrightarrow{K_{H_2O}}$$

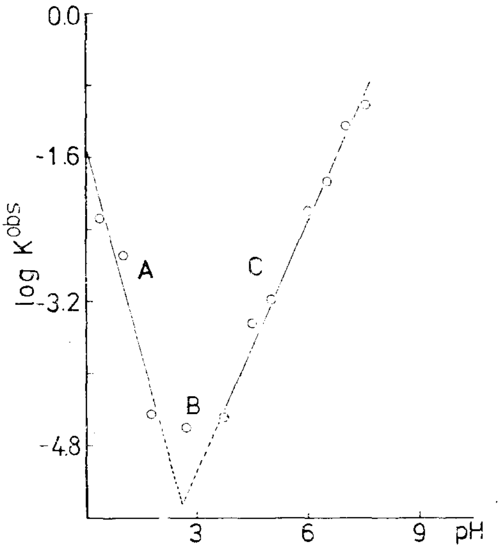


Fig. 7: pH–rate profile for the hydrolysis of pipethanate·HCl with $\mu=0.266$ at 60°C.

Reaction III: protonated pipethanate

$$[P^+]+[OH^-]\xrightarrow{K_{OH^-}}$$

The overall velocity is equal to the sum of the rates of all these reactions.

$$-\frac{dp_t}{dt}=K_{H^+}[P^+][H^+]+K_{H_2O}[P^+]+K_{OH^-}[P^+][OH^-] \quad \cdots\cdots\cdots(1)$$

where K_{H^+}, K_{H_2O} and K_{OH^-} are the specific catalytic constants, and P_t represents the total concentration of pipethanate hydrochloride. In fairly strong acidic solution the last two terms of EQ(1) will be negligible compared to the first term and may be omitted, thus giving

$$-\frac{dp_t}{dt}=K_{H^+}[P^+][H^+] \quad \cdots\cdots\cdots(2)$$

Because of the overall first-order character of the hydrolysis, the following equation is valid.

$$-\frac{dp_t}{dt}=K^{obs}[P_t] \quad \cdots\cdots\cdots(3)$$

In the strongly acid region, designated by the letter A in Fig. 7, the reaction is first order in respect to hydrogen ion, because the most important step is reaction (I). Thus

$$K^{obs}=K_{H^+}[H^+] \quad \cdots\cdots\cdots(4)$$

$$\log K^{obs}=\log K_{H^+}-pH \quad \cdots\cdots\cdots(5)$$

The pH–rate profile is a nearly straight line from pH 0.4 to 2.5. The catalytic constant of the hydrogen ions was found to be

$$K_{H^+}=1.1568\times10^{-2}\text{min}^{-1} \text{ at } 60°C.$$

In fairly basic region, designated by the letter C in Fig. 7, main reaction in this region is reaction(III).

The first two terms of EQ(I) will be negligible compared to the last term and may be omitted, Thus

$$K^{obs}=K_{OH^-}[OH^-] \quad \cdots\cdots\cdots(6)$$

$$\log K^{obs}=\log K_{OH^-}+14-pH$$

In accordance with this last equation the pH–rate profile is a straight line between pH 3.5 and 7.5. The catalytic constant of the hydroxyl ions was found to be $K_{OH^-}=4.5519\times10^5$ min^{-1} at 60°C. The failure to find any salt effect at pH 2.5 tends to support the suggestion that the most important reaction in the B region in Fig. 7 is reaction(II), cleavage of protonated pipethanate by water. The catalytic constant of the water was found to be

$$K_{H_2O}=3.16\times10^{-5}\text{ min}^{-1} \text{ at } 60°C.$$

SUMMARY

1) The hydrolysis of pipethanate was found to be first order with respect to pipethanate concentration over an experimental range of hydrogen ion concentration (pH 0.4–7.5). The apparent activation energy(Ea) at pH 7.5 was found to bef18.30 Kcal/mole and the frequency factor to be 1.1408 10^9 sec^{-1}.

2) The rate of the hydrolysis has a minimum at pH 2.5–3.5. In this region, the half life of pipethanate·HCl was about 15.3 days at 60 C. The catalytic effect of water was found to be K_{H_2O} 3.16 10^{-5} min^{-1} at 60 °C.

3) The hydrolysis is catalyzed by hydroxyl ions, hydrogen ions and water. The catalytic constant of the hydroxyl ions was found to be $K_{OH^-} = 4.5519 \times 10^5 min^{-1}$ at 60° C. The catalytic effect of the hydrogen ions was also found to be $K_{H^+} = 1.1568 \times 10^{-2}$ min^{-1} at 60°C. This reaction appears to be primary base catalyzed hydrolysis and pipethanate is relatively reluctant toward acid-catalyzed hydrolysis.

4) A positive primary salt effect was noted in the solution of pipethanate at pH 7.5 and at 60°C The value for $Z_a.Z_b$ under these conditions was –0.41 and the intrinsic reaction rate constant was 0.1150 min^{-1}.

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