Pharm. Bull., **23**, 651 (1975).
(19) A. Sioufi, F. Caudal, and F. Marfil, *J. Pharm. Sci.*, **67**, 243 (1978).
(20) N. J. Pound, I. J. McGilveray, and R. Sears, *J. Chromatogr.*, **89**, 23 (1974).
(21) N. J. Pound and R. W. Sears, *J. Pharm. Sci.*, **64**, 284 (1975).

Kinetics of Hydrolysis of Methenamine

J. G. STROM, Jr., and H. WON JUN ˣ

Received March 19, 1979, from the *Department of Pharmaceutics, School of Pharmacy, University of Georgia, Athens, GA 30602.* Accepted for publication April 25, 1980.

Abstract □ The kinetics of degradation of methenamine were studied in citrate–phosphate buffers between pH 2.0 and 7.4 at 37.5°. GLC was used to monitor the rate of hydrolysis. The conversion of methenamine to formaldehyde was found to be pH dependent in the buffers of constant ionic strength, with the reaction half-life decreasing from 13.8 hr at pH 5.8 to 1.6 hr at pH 2.0. The kinetics of degradation also were measured at 47, 57, and 67°, and the reaction obeyed the Arrhenius relationship. At pH 2.0, the activation energy was calculated to be 23.5 kcal/mole; at pH 5.1, it was 12.0 kcal/mole.

Keyphrases □ Methenamine—hydrolysis kinetics in citrate–phosphate buffers □ Hydrolysis kinetics—methenamine in citrate–phosphate buffers □ Kinetics—hydrolysis of methenamine in citrate–phosphate buffers

Methenamine (hexamethylenetetramine) is a urinary antibacterial useful in the long-term therapy of chronic urinary tract infections. The antibacterial activity of methenamine is derived from formaldehyde produced upon hydrolysis of methenamine in the bladder. The amount of hydrolysis is a function of the acidity of the urine (1).

BACKGROUND

Methenamine was introduced for the treatment of urinary infections in 1894 (2). Although this drug has been used for over 80 years and its pharmacological activity depends on the extent and rate of hydrolysis of the parent compounds in acidic media (3), there are few reported studies on the *in vitro* hydrolysis of methenamine. In 1935, Philippi and Löbering (4) studied the hydrolysis of methenamine in buffer solutions. Their assay method was based on the formation of a silver nitrate–methenamine complex. According to Slowick and Kelley (5), this procedure produces inaccurate and undependable results, so the findings of Philippi and Löbering (4) probably are in error.

A paper published by Tada (6) summarized work done from 1951 to 1958 (7–10) in Japan and listed the rates of decomposition of methenamine in hydrochloric acid at pH ≤1.6. Decomposition also was measured in acetic acid–sodium acetate buffer solutions in the pH range of ~4–6. Tada's (6) assay involved the titration of excess acid not consumed in the degradation reaction. Titration procedures normally are tedious and time consuming.

Investigations into the pharmacology of methenamine have been difficult because all of the methods are based on the measurement of formaldehyde liberated from methenamine. Early studies used methods that were relatively insensitive. Many of the analytical procedures are not applicable in the presence of intact methenamine (11). Most studies on urinary formaldehyde concentrations used the colorimetric method of Tanenbaum and Bricker (12). The acidity of the reagents used in this method recently was found to convert 20–30% of the residual methenamine to formaldehyde (13).

Useful scientific data for methenamine compounds generally are not available. The pharmacokinetics and mechanisms of action of this drug have not been well elucidated. Because of the use of other urinary tract antibacterials like nitrofurantoin and the sulfonamides, few recent studies on methenamine have been reported.

This report presents a study of the hydrolysis of methenamine between pH 2.0 and 7.4 in aqueous buffers. The rate of hydrolysis of methenamine was followed by measuring the change in the concentration of intact methenamine determined by the specific and accurate GLC procedure of Strom and Jun (14).

EXPERIMENTAL

Apparatus—The gas chromatograph[1] was equipped with a dual flame-ionization detector. A 1.8-m × 6-mm i.d. glass column was packed with 3% OV-17 on 80–100-mesh Chromosorb W-HP. The operating temperatures were 245° for the injection port, 190° (isothermal) for the column oven, and 250° for the detector. Nitrogen at a flow rate of 45 ml/min was the carrier gas. The flow rates of hydrogen and compressed air were adjusted to optimum sensitivity. The electrometer range was 10^{-10} amp/mv.

Calibration Curve—Methenamine[2] was recrystallized from absolute ethanol. Methylparaben[3] (methyl *p*-hydroxybenzoate), the internal standard, was used as received. Aliquots of 0.5, 1.5, 2.5, 5.0, 7.5, and 10 ml of methenamine solution (2 mg/ml in water containing 2 mg of methylparaben/ml) were added to individual 10-ml volumetric flasks and diluted to volume with water (containing 2 mg of methylparaben/ml). Each solution (1 μl) was injected into the chromatograph. The calibration curve was obtained by plotting the known concentration of methenamine *versus* the corresponding peak height ratio (methenamine/methylparaben).

Buffer Solutions—All buffer solutions were prepared in deionized, distilled water and were adjusted to an ionic strength of 0.5 with potassium chloride. Buffer solutions in the pH range of 2.0–7.4 were prepared by combining various amounts of 0.1 *M* citric acid and 0.2 *M* dibasic sodium phosphate stock solutions (15).

Stability Studies—*Effect of pH*—Amounts of methenamine equal to 0.75 mg/ml were weighed and placed in volumetric flasks. At time zero, the methenamine was dissolved in buffer solutions of pH 2.0, 2.4, 3.4, 4.6, 5.1, 5.5, 5.8, or 7.4 previously equilibrated at 37.5°. After dilution with the respective buffers, 10.0-ml samples were removed and placed in 16 × 125-mm culture tubes with polytef-lined screw caps. These tubes were placed into a constant-temperature water bath.

At suitable time intervals, one culture tube was removed and cooled to room temperature by quenching in an ice bath. One milliliter of the internal standard solution (22 mg of methylparaben/ml in methanol) was added, and the sample was subjected immediately to the GLC analysis. The pH was measured at the beginning and end of each run, and no significant change was found.

Effect of Temperature—Methenamine at 0.75 mg/ml also was studied kinetically at 47, 57, and 67° in pH 2.0, 5.1, and 7.4 buffers.

RESULTS AND DISCUSSION

Figure 1 shows a typical gas chromatogram obtained for an aqueous solution of methenamine (t_R = 0.8 min) and methylparaben, the internal standard (t_R = 1.7 min). Both methenamine and the internal standard gave sharp, well-resolved peaks (resolution, R_s = 5.4) with no tailing. A

[1] Varian Aerograph model 1830, Varian Instruments Division, Palo Alto, CA 94304.
[2] Riker Laboratories, Northridge, CA 91324.
[3] Fisher Scientific Co., Fair Lawn, NJ 07410.

0022-3549/80/1100-1261$01.00/0
© 1980, American Pharmaceutical Association

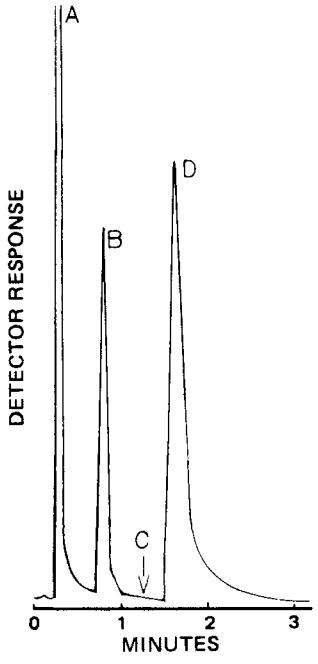

Figure 1—*Gas chromatogram of methenamine and the internal standard in water on a 3% OV-17 column. Key: A, solvent; B, methenamine; C, recurrent unidentified peak; and D, methylparaben.*

peak that appeared repeatedly in all kinetic runs had a retention time of 1.25 min (point C in Fig. 1). This peak, possibly the hydrolysis product formaldehyde or, more probably, a polymer of formaldehyde, would be well resolved from both methenamine (R_s = 4.4) and from the internal standard (R_s = 2.2). A plot of the ratio of the peak height of methenamine to the height of the internal standard *versus* the concentration of methenamine produced a linear relationship between 0.10 and 2.00 mg/ml. The calibration curve had a slope of 0.89 ml/mg, an intercept of 3.24×10^{-2}, and a correlation coefficient of 0.9997.

The kinetics of hydrolysis of methenamine were followed at various pH values by monitoring the decrease of the ratio of the peak height for the peak at the methenamine retention time to the peak height of the internal standard as a function of time. A plot of the hydrolysis data obtained at various pH values is shown in Fig. 2.

The logarithms of the difference of the final peak height ratio, H_∞ (the peak height ratio at the end of 10 half-lives), and the peak height ratio at any time, H_t, *versus* time produced linear plots. The apparent first-order rate constants were calculated from the slopes of the linear regression lines fitted to:

$$\ln(H_t - H_\infty) = \ln(H_0 - H_\infty) - kt \qquad \text{(Eq. 1)}$$

where H_0 is the peak height ratio at time zero and k is the apparent first-order rate constant. At all of the pH values studied, H_∞ was essentially zero after storage for several days at the selected temperature, indicating that the reaction went to completion. The rate constants derived from the data are presented in Table I.

A concentration of 0.75 mg/ml was chosen as the initial concentration because this value is the expected physiological concentration following a normal dose in humans (16). Six hours would be the maximum time a solution of methenamine would remain in the bladder. No decomposition (*i.e.*, no change in the peak height ratio) was observed in the pH 7.4 buffer over a 6-hr period.

Table I—Apparent First-Order Rate Constants for Hydrolysis of Methenamine in Buffer at 37.5°

pH	k, $hr^{-1} \times 10^2$	$t_{1/2}$, hr	r^a
2.0	43.3	1.60	0.9978
2.4	32.7	2.12	0.9848
3.4	22.4	3.09	0.9920
4.6	18.6	3.73	0.9993
5.1	8.36	8.29	0.9882
5.5	6.35	10.9	0.9856
5.8	5.01	13.8	0.9935
7.4	—[b]	—	

[a] Correlation coefficient for the linear regression line. [b] No observable degradation over the time interval studied.

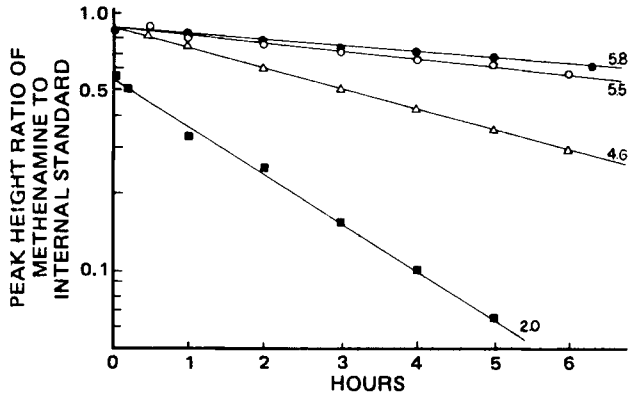

Figure 2—*Semilogarithmic plots of the ratio of the height of the GLC peak for methenamine to the peak for the internal standard* versus *time at specified pH values. Each point is an average of two kinetic runs. Key: ●, pH 5.8; ○, pH 5.5; △, pH 4.6; and ■, pH 2.0.*

The effect of temperature on the hydrolysis rate was investigated at pH 2.0, 5.1, and 7.4 at several temperatures. Table II lists the apparent first-order rate constants calculated at each temperature for each pH value. There was no observable change in the peak height ratio in pH 7.4 buffer at any temperature over a 6-hr period. Arrhenius plots of the logarithms of the observed rate constants *versus* $1/T$ produced straight lines (Fig. 3). The activation energy for the reaction then was calculated from the slopes of the lines fitted to the data (Table II).

The studies of Tada (6) showed that:

$$k_{obs} = (k_w + k_h[H^+])f^+ \qquad \text{(Eq. 2)}$$

where k_{obs} is the observed rate constant; k_w and k_h are the rate constants for the water solvolysis reaction and the hydrogen-ion-catalyzed reaction, respectively; and f^+ is the fraction of methenamine present in the protonated form. Tada (6) calculated k_w and k_h values at 30° of 6.05×10^{-6} sec^{-1} and 6.33×10^{-4} liter/mole/sec, respectively. Tada (6) also determined the dissociation constant of protonated methenamine to be 7.0×10^{-6}, from which values of f^+ as a function of pH can be calculated. This relationship is shown graphically in Fig. 4. The value from Table I for hydrolysis at pH 2.0 and 37.5° was set equal to the value from Tada (6) at pH 2.0 and 30°, and the remaining values from Table I were scaled

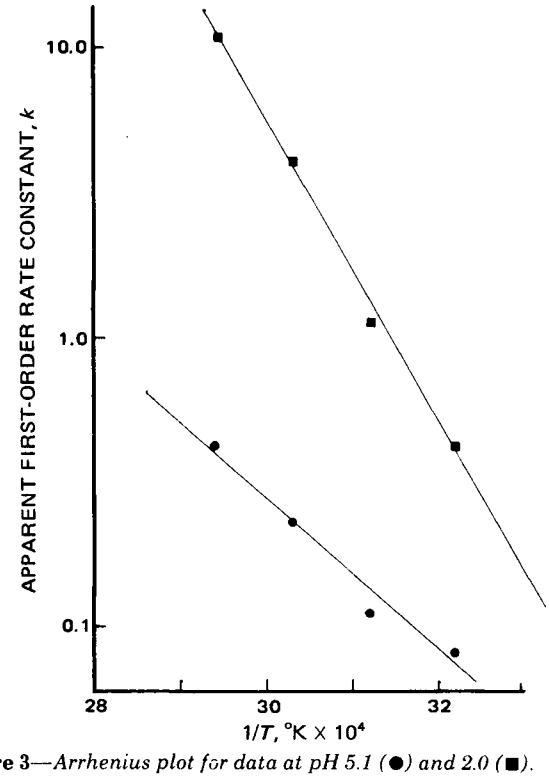

Figure 3—*Arrhenius plot for data at pH 5.1 (●) and 2.0 (■).*

Table II—Apparent First-Order Rate Constants and Thermodynamic Parameters for the Hydrolysis of Methenamine at Various Temperatures

pH	k, $hr^{-1} \times 10$				E_a, kcal/mole	ln A^a	r^b
	37.5°	47°	57°	67°			
2.0	4.33	11.5	41.9	111.0	23.5	37.2	0.9985
5.1	0.836	1.11	2.33	4.27	12.0	16.8	09853

[a] A is the frequency factor. [b] The correlation coefficient for the linear regression line obtained by fitting the data to the Arrhenius relationship.

accordingly and plotted in Fig. 4. The scaled values from this study agreed very well with the values predicted by Tada (6).

The Arrhenius activation energy (E_a) of 23.5 kcal/mole at pH 2.0 and an ionic strength of 0.5 compared favorably with Tada's (6) value of 23.3 kcal/mole in highly acidic media at an ionic strength of 3.08. This E_a value can be largely associated with the hydrogen-ion-catalyzed reaction. The E_a value of 12.0 kcal/mole calculated at pH 5.1 would be almost entirely a function of the rate constant of the water reaction, k_w.

The conversion of methenamine to formaldehyde in biological fluids, particularly urine, is of clinical importance since formaldehyde is the pharmacologically active product and is effective against urinary bacteria only above a concentration of ~20 μg/ml (17). Methenamine solution remains in the bladder for only a certain time (~6 hr), and it is necessary to know the amount of conversion that occurs over that period. It also would be useful for successful therapy to know the effect of the urinary pH on the rate of that conversion. Several recent reports discussed hydrolysis of methenamine in urine (18–20). Possible errors from the assay method used in these studies were minimized in part by use of the Jackson and Stamey method (13), a modification of the Tanenbaum and Bricker (12) procedure, or by use of the Chen and Chafetz method (21). A possibly better method would be use of the Jackson and Stamey procedure to quantitate the free formaldehyde concentration simultaneously with the GLC procedure for intact methenamine.

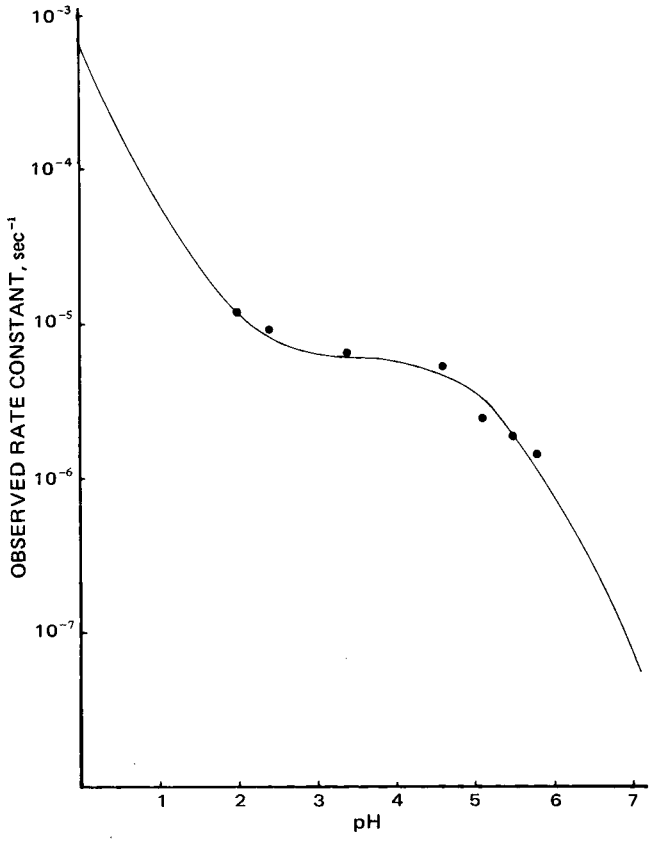

Figure 4—*The pH–rate profile of methenamine degradation. The solid line is the predicted relationship from the work of Tada (6). The points are the data from Table I scaled to 30°.*

According to the data of the present study, methenamine should circulate in the blood as the intact drug and be rapidly converted to formaldehyde when it reaches the acidic urine. If the drug were converted at a similar rate in the urine as in the buffers of this study, sufficient formaldehyde would be produced within 2 hr at pH ≤5.8. However, it may not be possible to make quantitative predictions of the *in vivo* behavior of methenamine from the data of this study because of possible kinetic salt effects. Tada's (6) work demonstrated a linear increase in the observed rate constant with an increase in the ionic strength up to a value of 3 or 4. This effect was shown for about 15 mono- and divalent salts, such as sodium chloride, sodium iodide, potassium chloride, potassium iodide, lithium chloride, calcium chloride, and ammonium chloride.

The reported study demonstrated that the GLC procedure can be used to observe and quantitate the degradation kinetics of methenamine *in vitro* in a buffer system.

REFERENCES

(1) P. J. Hanzlik and A. B. Collins, *Arch. Intern. Med.*, **12**, 578 (1913).

(2) A. Nicolaier, *Dtsch. Med. Wochenschr.*, **21**, 541 (1895).

(3) R. S. A. Heathcote, *Br. J. Urol.*, **7**, 9 (1935).

(4) E. Philippi and J. Löbering, *Biochem. Z.*, **277**, 365 (1935).

(5) E. F. Slowick and R. S. Kelley, *J. Am. Pharm. Assoc., Sci. Ed.*, **31**, 15 (1942).

(6) H. Tada, *J. Am. Chem. Soc.*, **82**, 255 (1960).

(7) S. Kambara and H. Tada, *J. Chem. Soc. Jpn.*, **55**, 360 (1952).

(8) H. Tada, *ibid.*, **56**, 445 (1953).

(9) *Ibid.*, **57**, 37 (1954).

(10) *Ibid.*, **58**, 10 (1955).

(11) J. A. Stamey, "Urinary Infections," Williams & Wilkins, Baltimore, Md., 1972, p. 266.

(12) M. Tanenbaum and C. E. Bricker, *Anal. Chem.*, **23**, 354 (1951).

(13) J. Jackson and T. A. Stamey, *Invest. Urol.*, **9**, 124 (1971).

(14) J. G. Strom, Jr., and H. W. Jun, *J. Pharm. Sci.*, **66**, 589 (1977).

(15) "Scientific Tables," 7th ed., K. Diem and C. Lentner, Eds., Ciba-Geigy, Basel, Switzerland, 1970, pp. 280–282.

(16) V. Knight, J. W. Draper, E. A. Brady, and C. A. Attmore, *Antibiot. Chemother.*, **2**, 615 (1962).

(17) H. Miller and E. Phillips, *Invest. Urol.*, **8**, 21 (1970).

(18) D. M. Musher and D. P. Griffith, *Antimicrob. Agents Chemother.*, **6**, 708 (1974).

(19) D. M. Musher, D. P. Griffith, and Y. Richie, *Invest. Urol.*, **13**, 380 (1976).

(20) J. M. T. Hamilton-Miller and W. Brumfitt, *ibid.*, **14**, 287 (1977).

(21) T. Chen and L. Chafetz, *ibid.*, **10**, 212 (1972).

ACKNOWLEDGMENTS

The authors thank the reviewer for constructive comments and suggestions.