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KINETICS AND MECHANISM OF HYDROLYSIS OF FUROSEMIDE

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SUMMARY

Furosemide, a potent diuretic, has been found to give a low bioavailability profile when administered orally which potentially could be due to hydrolysis of the drug prior to absorption. An in vitro kinetic study was performed to elucidate the kinetics and hydrolysis mechanism of furosemide as a function of pH and temperature. In the acidic pH region, below the reported pKa of 3.8, the log K–pH profile indicated specific hydrogen ion catalysis on the undissociated species. In the basis pH region, furosemide hydrolysis is extremely slow. Using the Arrhenius parameters obtained from the studies, furosemide would have a half-life of 178 min under physiological conditions in the stomach of pH 1.0 and temperature of 37°C.

INTRODUCTION

Furosemide is a potent orally effective diuretic that is widely prescribed to treat edema associated with congestive heart failure, cirrhosis of the liver, and renal diseases. An anthranilic acid derivative, 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid, furosemide is excreted in the urine both unchanged and as a hydrolysis product (Yakatan et al., 1976; Hajdu et al., 1964; Seno et al., 1969), 4-chloro-5-sulfamoylanthranilic acid (saluamine). However, little information is available in the literature on the stability of furosemide in aqueous solution and its hydrolysis mechanism.

Furosemide is considerably less than 100% bioavailable from oral dosage forms (Beerman et al., 1975; Yakatan et al., 1976; Calesnick et al., 1966). This in vitro kinetic study was initiated to investigate whether the cause of this phenomenon could be attributed to hydrolysis of furosemide prior to absorption.

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MATERIALS AND METHODS

Materials

Furosemide [1] and saluamine [1] were used without further purification. All other chemicals used were of reagent grade quality.

The buffer systems used were pH 1.0–3.0, hydrochloric acid; pH 3.2–5.6, acetate buffer; pH 4.7–7.4, phosphate buffer; pH 6.9–9.5, borate buffer. The ionic strength of each buffer solution was adjusted to 1.0 with potassium chloride. The pH values were measured with a digital pH meter [2] at the temperature of the kinetic run.

Kinetic measurements

Furosemide was dissolved in methanol in a light-resistant flask to procedure a 1×10^{-3} M stock solution of the drug. In a typical kinetic run, an aliquot of the stock solution was added to a reaction-temperature-equilibrated buffer solution to produce approximately a 1×10^{-5} M solution of the drug. The ionic strengths of the buffered solutions were adjusted to 1.0 by adding appropriate amounts of KCl. Light-resistant flasks were used as reaction vessels to minimize photolytic effects on the drug solution (Rowbotham et al., 1976). The reaction flasks were maintained at the reaction temperature (60–80°C ± 0.01°C) in an oil bath [3]. Samples were taken at different times during the course of the reaction for at least 10 half-lives of the drug degradation process. The samples were cooled rapidly in a light-protected ice bath to quench the reaction and the ultraviolet spectrum obtained on a spectrophotometer [4]. Kinetic runs were conducted throughout the pH regions 1–9. Beer's Law plots were constructed to insure that absorbance was directly proportional to concentration.

Product identification

For identification of the hydrolysis product, saluamine, a solution of furosemide at pH = 1.14 was degraded at 70°C. After the reaction was quenched in an ice bath, the solution was extracted with ether. The ether extract was evaporated to dryness. The dried extract was reconstituted with a few drops of ether–methanol mixture and applied on a cellulose TLC plate and developed in the solvent system, 2-propanol : butyl acetate : water : concentrated ammonia (50 : 30 : 15 : 5 v/v) (Yakatan et al., 1976). In this TLC system, furosemide has an R_f value of 0.6 and saluamine an R_f of 0.2. Authentic samples of furosemide and saluamine were also applied on the plate next to the dried extract. After plate development, the chromatogram was dried and visualized under UV light [5].

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[1] Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J. 08876, U.S.A.

[2] Orion Research Model 701 equipped with a high temperature electrode Cambridge, Mass., U.S.A.

[3] Sargent model oil bath equipped with a Sargent thermonitor (model ST), Dallas, Texas, U.S.A.

[4] Coleman, Maywood, Ill., U.S.A.

[5] Ultra-Violet Products, Inc., San Gabriel, Calif., U.S.A.

RESULTS AND DISCUSSION

Spectral changes and rate constant determinations

The kinetics of hydrolysis of furosemide were monitored by following the change in absorbance, A, of the ultraviolet chromophore as a function of time. In the acidic pH region the furosemide ultraviolet spectrum has peaks at 274 nm and 234 nm. As the degradation proceeds the 234 nm peak shifts to 230 nm with a clearly defined isosbestic point at 232 nm. This is indicative of a 1 : 1 transformation of the furosemide chromophore. The ultraviolet spectrum of saluamine has a maximum at 230 nm. Fig. 1 shows typical ultraviolet spectra of furosemide hydrolysis as a function of time in 0.01 N HCl at 70°C.

The logarithms of the difference between the final absorbance, A_∞, and the absorbance at any time, A_t, at the desired wavelength were plotted against time. The apparent first-order rate constants were calculated from the slopes of the linear segment according to Eqn. 1:

$$\ln(A_t - A_\infty) = \ln(A_0 - A_\infty) - kt \quad (1)$$

where A_0 is the absorbance at zero time and k is the apparent first-order rate constant. Fig. 2 shows a typical first-order kinetic plot for the hydrolysis of furosemide in 0.01 N HCl at 70°C. Table 1 presents the rate constants derived from these plots.

In the pH region above 7.2, the hydrolysis reaction is extremely slow. After weeks of incubation at 70°C at pH 8.5, no spectral changes were observed. Furosemide hydrolysis is apparently negligible in the basic pH region.

From the observed spectral changes and kinetic behavior of furosemide, the hydrolysis of furosemide may be expected to proceed as depicted in Scheme 1, where I represents

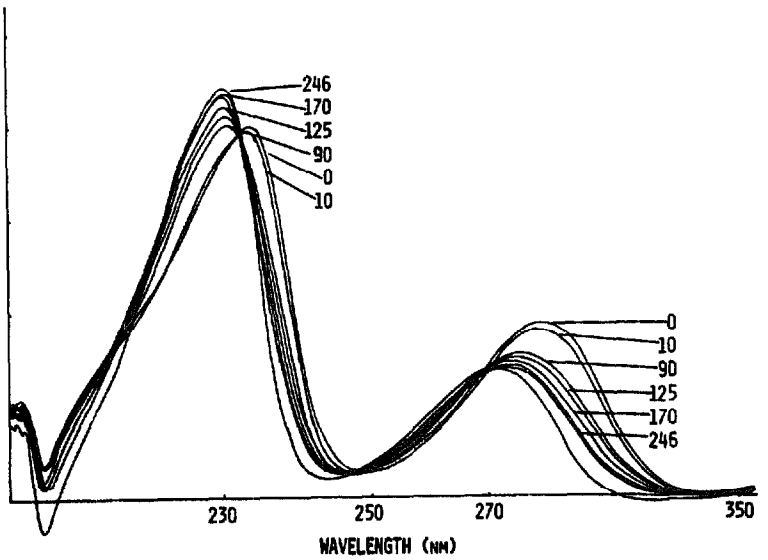


Fig. 1. Typical spectral changes for the hydrolysis of 10^{-5} M furosemide in 0.1 N HCl at 70°C and μ = 1.0. The curves are labeled as to minutes after the start of the reaction.

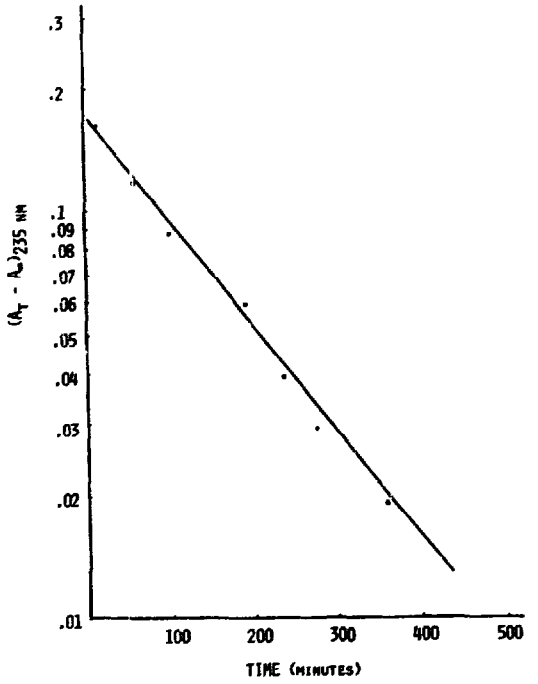
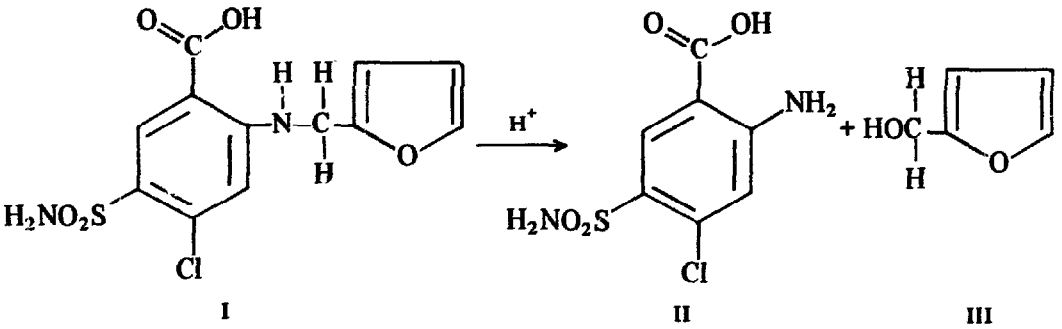


Fig. 2. Typical apparent first-order plot for the hydrolysis of 10^{-5} M furosemide in 0.01 N HCl at 70°C and μ = 1.0. The absorbance values, A_t, were measured at 235 nm.

furosemide, II represents saluamine and III represents the furfuryl function which was not isolated.



Scheme 1.

This scheme suggests that the basic amino nitrogen is protonated followed by nucleophilic attack on the furfuryl carbon to give the hydrolysis product, saluamine. Saluamine was reported to be a major metabolite of furosemide in humans, dogs and the rat (Yakatan et al., 1976; Seno et al., 1969; Hajdu et al., 1964).

Rate–pH profile

The log k–pH profile for the hydrolysis of furosemide (Fig. 3) was constructed from the logarithm of the apparent first-order rate constants and pH values at 70°C with ionic strength of 1.0. In the acidic pH region, the log k–pH profile indicates specific hydrogen

TABLE 1

APPARENT FIRST-ORDER RATE CONSTANTS AT 70°C AND IONIC STRENGTH 1.0

pH	k_{obs} (min^{-1})
1.50	67.02×10^{-3}
2.45	5.22×10^{-3}
3.46	0.67×10^{-3}
4.24	0.22×10^{-3}
7.17	0.13×10^{-3}

ion catalysis on the neutral species. In this region, the log k–pH profile may be described by:

$$k_{OBS} = k_{H^+}[H^+] \tag{2}$$

or

$$\log k_{OBS} = \log k_{H^+} - pH \tag{3}$$

Least squares analysis of the data at the lower pH values gave a slope of −1.09 and an intercept (log k_{H^+}) of 0.307 (k_{H^+} = 2.03 liter mol^{-1} min^{-1}) in accordance with Eqn. 3.

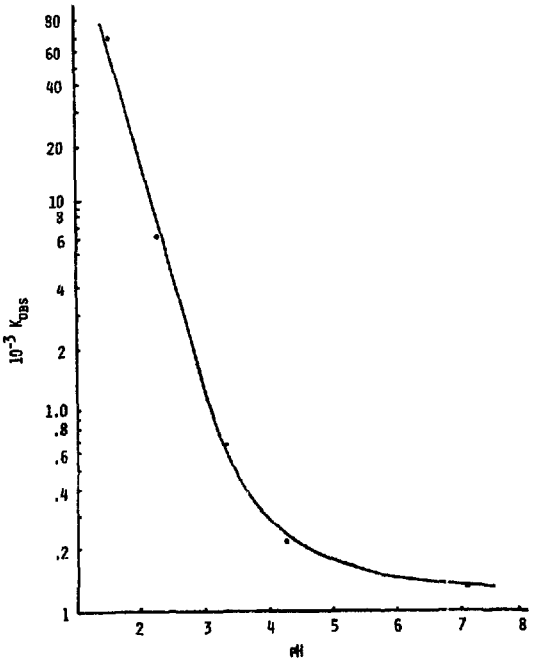


Fig. 3. Log rate–pH profile for the hydrolysis of furosemide at 70°C and μ = 1.0.

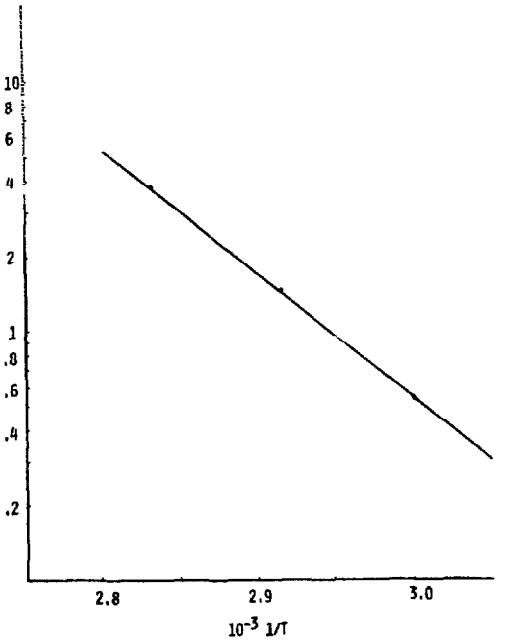


Fig. 4. Arrhenius plot for furosemide hydrolysis in 0.01 N HCl.

A plot of k_{OBS} against $[H^+]$ in accordance with Eqn. 2 gave an intercept of zero and a slope of 2.14 liter mol^{-1} min^{-1} which is the bimolecular rate constant, k_{H^+}. Beyond pH 4.0. the log k–pH profile deviates from the theoretical line described by Eqn. 3. The reported pKa for furosemide due to the carboxyl group is 3.8 (Orita et al., 1976). Due to the slow hydrolysis rate in the neutral and basic pH regions, there are too few data points to construct a complete log k–pH profile. The data obtained at pH values above the pKa indicate that the complete log k–pH profile may be described by:

$$k_{OBS} = (k_{H^+}[H^+] + k_{H_2O})\, f_{HS} \tag{4}$$

where k_{H_2O} represents the rate constant for water attack on the fraction of the drug existing as the undissociated species, f_{HS}.

Product identification

The hydrolysis product, saluamine, was identified using the procedure described in the methods section. The TLC system used easily separates furosemide from saluamine. After development of the chromatogram and subsequent analysis under long wavelength UV light, the extract from the hydrolysis reaction gave a spot with the same R_f value as the saluamine standard. The UV spectrum of the extract from the hydrolysis reaction was the same as the UV spectrum of authentic saluamine.

Stability parameters

Hydrolysis kinetics as a function of buffer concentration and temperature were studied to give a complete profile of furosemide stability. Buffer catalysis was ascertained by varying the original buffer concentrations in a gradient manner while keeping the buffer pH constant. No significant buffer catalysis was observed.

The Arrhenius parameters were obtained from a plot of the logarithm of the biomolecular rate constant, k_{H^+}, against the reciprocal of the absolute temperature, T, in accordance with the expression:

$$\ln k = \ln A - \frac{Ea}{RT} \tag{5}$$

where R is the gas constant (1.987 cal deg^{-1} mol^{-1}) and ln A is related to the entropy of activation and E_a is related to the enthalpy of activation of the hydrolysis. To generate data for this plot, reactions were run at different temperatures in buffered solutions at constant pH and buffer concentration. Least-squares analysis of the Arrhenius plot gave a slope (Ea) of 23.5 kcal/mol and an intercept (ln A) of 34.9. These values will allow stability predictions in the acidic pH region where the degradation is hydrogen-ion catalyzed.

Knowledge of the Arrhenius parameters permitted the estimation of the rate constant for furosemide hydrolysis under physiological conditions in the stomach. Such a calculation, based on a stomach pH of 1.0 and a body temperature of 37°C, produced a half-life of furosemide of 178 min. Under these conditions, furosemide would hydrolyze to an extent of approximately 20% if it remained at pH 1 for 1 h at 37°C. Thus, although

hydrolysis of furosemide to saluamine in the stomach could account for some of the bioavailability loss, it appears that other mechanisms would have to be involved to account for the low bioavailability of the drug.

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