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Photodegradation and hydrolysis of furosemide and furosemide esters in aqueous solutions

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Summary

The stability of furosemide (frusemide) and various furosemide esters has been examined in aqueous solution of various pH values and under different lighting conditions. The furosemide esters were found to be highly unstable in solutions of pH 2–9.5 when exposed to artificial laboratory light or diffuse daylight, the half-lives of degradation being 0.5–1.5 h. Whereas furosemide is quite stable to photodegradation in alkaline solution it was found to be quickly degraded in acidic solution. From the pH–rate profile obtained, the principal photolabile species of furosemide was shown to be the unionized acid form. This form possesses almost the same reactivity as the esters. The acid-catalyzed hydrolysis of furosemide in solution protected against light was found to occur somewhat slower than previously reported in the literature. It was estimated that hydrolysis of furosemide in the stomach prior to absorption cannot account for the incomplete bioavailability of furosemide following peroral intake.

Introduction

Furosemide (frusemide) (I) is a widely used diuretic which is administered both orally and parenterally. Several workers have studied its stability. The furylmethyl group in the molecule is acid-labile (Sturm et al., 1966) and furosemide has been shown to undergo acid-catalyzed hydrolysis in aqueous solution to yield 4-chloro-5-sulphamoyl-anthranilic acid (saluamine) (Sturm et al., 1966; Kovar et al., 1974; Cruz et al., 1979; Toyooka et al., 1981; Andreasen et al., 1982). In alkaline solutions, in contrast, furosemide shows a high stability (Ghanekar et al., 1978; Neil et al., 1984). The compound is also susceptible to photodegradation. Rowbotham et al. (1976) reported that ultraviolet irradiation of alkaline solutions of furosemide produced 4-chloro-5-sulphoanthranilic acid by oxidation of the sulphamoyl group and hydrolysis of the furfuryl group whereas Moore and Tamat (1980) reported complete dechlorination after UV irradiation in deoxygenated neutral aqueous solutions. When irradiated with 365 nm UV light, furosemide in methanol solutions has been shown to undergo both photoreduction to *N*-furfuryl-5-sulphamoyl-anthranilic acid and photohydrolysis to saluamine (Moore and Sithipitaks, 1983). More recently, Yahya et al. (1986) have shown that furosemide infuson solutions, when stored in burette administration sets, are stable over a 48-h period when exposed to diffuse daylight/fluorescent strip room lighting but de-

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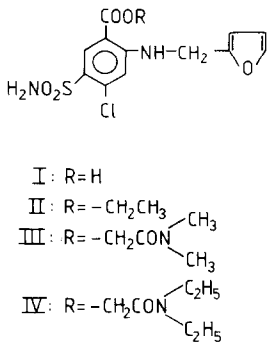


Formulae I–IV.

composed quickly with a half-life of about 4 h when exposed to sunlight. Neil et al. (1984) have also found that furosemide solutions are stable when stored in normal daylight.

In connection with a study of developing ester prodrugs of furosemide, we noted an extremely high susceptibility of furosemide esters to undergo photodegradation in aqueous solutions exposed to normal conditions of artificial light or daylight. This led us to suggest that the photostability of furosemide may be a function of the degree of ionization of its carboxylic acid group (i.e. solution pH) such that the unionized species, resembling the esters in terms of inductive effects, might be much more unstable towards photolysis than the ionized form. The present work, confirming this expectation, reports on the stability of various furosemide esters (II–IV) and furosemide in aqueous solution kept unprotected as well as protected from different lighting conditions. Since some controversy exists concerning the stability of furosemide in acidic solutions, i.e. at gastric pH values (Cruz et al., 1979; Andreasen et al., 1982), we also studied the hydrolysis of furosemide at pH 1–2, using a specific HPLC method.

Materials and Methods

Apparatus

High-performance liquid chromatography (HPLC) was done with a Spectra-Physics Model 3500 B instrument equipped with a variable wavelength detector and a 10-μl loop injection valve. A column, 250 × 4 mm, packed with LiChrosorb RP-8 (7-μm particles (E. Merck, Darmstadt) was used. ^{1}H-NMR spectra were run on a Varian 360 L instrument. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. Melting points were taken on a capillary melting-point apparatus and are uncorrected. Microanalyses were performed at the Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Chemicals

Furosemide was purchased from Sigma Chemical Co. (St. Louis) and was used as received. Buffer substances and solvents used were of reagent grade.

Synthesis of furosemide esters (II–IV)

Ethyl ester (II). A mixture of furosemide (1.65 g, 5 mmol), triethylamine (0.70 ml, 5 mmol) and ethyl iodide (0.41 ml, 5 mmol) in 10 ml of *N,N*-dimethylformamide was stirred at room temperature for 24 h. Water (75 ml) was added and the precipitate formed filtered off, washed with water and recrystallized twice from ethanol to give 1.4 g of the title compound [mp 163–165 °C, rep. mp 165–167 °C (Sturm et al., 1966)].

Anal.: Calculated for $C_{14}H_{15}ClN_2O_5S$: C, 46.86; H, 4.21; N, 7.80, Found: C, 46.74; H, 4.29, N, 7.78.

N,N-Dimethylglycolamide ester (III). A mixture of furosemide (3.30 g, 10 mmol), triethylamine (1.4 ml, 10 mmol), sodium iodide (0.15 g, 1 mmol) and 2-chloro-*N,N*-dimethylacetamide (1.6 g, 13 mmol) in 15 ml of *N,N*-dimethylformamide was stirred at room temperature for 28 h. Water (75 ml) was added and a solid precipitate formed upon standing at 4 °C for 4 h. It was filtered off, washed with water and recrystallized twice from ethanol to give 2.5 g of the title compound, [mp 193–194 °C].

Anal.: Calculated for $C_{16}H_{20}ClN_3O_6S$: C, 45.99; H, 4.82; N, 10.05. Found: C, 45.80; H, 4.92; N, 9.99.

N,N-Diethylglycolamide ester (IV). This was prepared by the same procedure as described for ester III, using 2-chloro-diethylacetamide instead of the dimethyl analogue [mp 135-136 °C (from ethanol–water)].

Anal.: Calculated for $C_{18}H_{24}ClN_3O_6S$: C, 48.48; H, 5.43; N, 9.42. Found: C, 48.55; H, 5.40; N, 9.49.

The ^{1}H-NMR spectra of the esters (in DMSO-d_6) were in agreement with their structures. The identity of the esters was furthermore confirmed by their quantitative conversion to furosemide by alkaline hydrolysis as revealed by HPLC measurements.

Stability experiments

The degradation of furosemide and the esters II–IV was studied in aqueous buffer solutions. Hydrochloric acid, acetate, phosphate and borate were used as buffers; the total buffer concentration was generally 0.05 M and a constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. In one case an ionic strength of 1.0 was used.

The rates of decomposition were followed by using a reversed-phase HPLC procedure. Mobile phase systems of methanol–water–85% phosphoric acid (60 : 40 : 1% v/v) and methanol–0.02 M KH_2PO_4 (60 : 40% v/v) were used in the experiments with furosemide and the esters, respectively. The flow rate was 1.2 or 1.6 ml · min^{-1} and the column effluent was monitored at 235 nm. Quantitation of the compounds was done by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

The reactions were initiated by adding 250 μl of a stock solution of the compounds in acetonitrile (0.5–1 mg · ml^{-1}) to 10 ml of buffer solution in screw-capped test tubes (Pyrex). The stock solutions were protected from light.

In the experiments performed under exclusion of light the reaction solutions were kept in a water-bath at constant temperature (37–70 °C), the test tubes being covered with aluminium foil. At appropriate intervals samples were taken and chromatographed immediately.

In other experiments the test tubes containing the reaction solutions were exposed to sunlight or diffuse daylight. In the former case the test tubes were placed beside a laboratory window, the temperature of the solutions being 25–27 °C. In the last case the test tubes were placed on a laboratory table exposed to normal laboratory light and with diffuse daylight entering the room from the windows. The temperature of the solutions was 20–21 °C.

Results and Discussion

Hydrolytic degradation of furosemide esters

The degradation of the furosemide esters II–IV was studied in acidic and alkaline solutions at 60 °C in the absence of exposure to light. At constant pH and temperature the degradation of the compounds displayed strict first-order kinetics for several half-lives. The pseudo-first-order rate constants (k) were determined from the slopes of linear plots of the logarithm of residual furosemide ester against time.

At pH 1–3 the rate of hydrolysis increased with decreasing pH according to the following equation:

$$k = k_H a_H \quad (1)$$

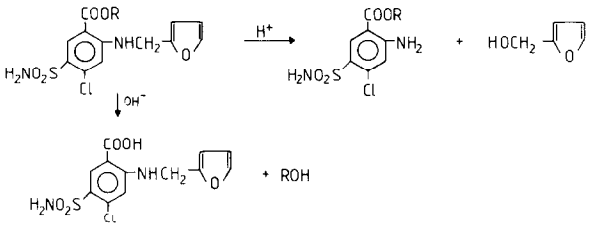
where a_H is the hydrogen ion activity and k_H is the specific acid catalytic rate constant. A plot of k against a_H gave a straight line with an intercept of zero. From the slopes of the lines the k_H values shown in Table 1 were obtained. As revealed by HPLC of the reactions solutions the esters were found to decompose to a single product which was different for each ester. In accordance with the known behaviour of furosemide (Kovar et al., 1974; Cruz et al., 1979), this product is most likely the corresponding 4-chloro-5-sulphamoyl-anthranilic acid ester formed upon acid catalyzed hydrolysis as depicted in Scheme 1.

TABLE 1

Second-order rate constants for the specific acid and base-catalyzed hydrolysis of various furosemide esters at 60 °C and $\mu = 0.5$ in the absence of exposure to light

Compound	k_H ($M^{-1} \cdot min^{-1}$)	k_{OH} ($M^{-1} \cdot min^{-1}$)
II	0.095	31
III	0.092	144
IV	0.085	125

The acid-stability of the esters is very similar to that of furosemide itself. As described below furosemide shows a k_H value of 0.12 $M^{-1} \cdot min^{-1}$ at 60 °C. This similarity supports the suggested mechanism of degradation of the esters and furthermore, it excludes the possibility of any intramolecular catalysis by the carboxyl group in the acid-catalyzed hydrolysis of furosemide.



Scheme 1.

In aqueous solutions of pH 8.8–9.7 the esters were hydrolyzed quantitatively to furosemide (which is stable at these pH values) as determined by HPLC analysis of completed reaction solutions (Scheme 1). In this pH range plots of log k against pH showed straight lines of unity slopes, indicating the occurrence of specific base-catalyzed hydrolysis of the ester bond according to the following rate expression:

$$k = k_{OH} a_{OH} \quad (2)$$

where k_{OH} is a second-order specific base catalytic rate constant and a_{OH} refers to the hydroxide ion activity. The latter was calculated from the measured pH at 60 °C according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = pH - 13.02 \quad (3)$$

The values of k_{OH} for the various esters are listed in Table 1.

Photolytic degradation of furosemide esters and furosemide

When exposed to artificial laboratory light or diffuse daylight the furosemide esters in aqueous solution were found to degrade very quickly. The degradation followed good first-order kinetics for several half-lives as shown by the examples in Fig. 1. At room temperature the half-lives of degradation ranged from about 0.5 to 1.5 h for the esters, the stability being almost independent of pH within the range pH 2–9.5 as seen in Table 2. Ester IV showed almost the same reactivity as ester III. When the solutions were exposed to sunlight the rate of degradation was further accelerated as observed for ester II (Table 2). Part of this rate acceleration can be ascribed to the slightly higher temperature of the solutions exposed to sunlight. When kept in the dark all the solutions remained completely stable for several hours, cf. the stability data obtained at 60 °C.

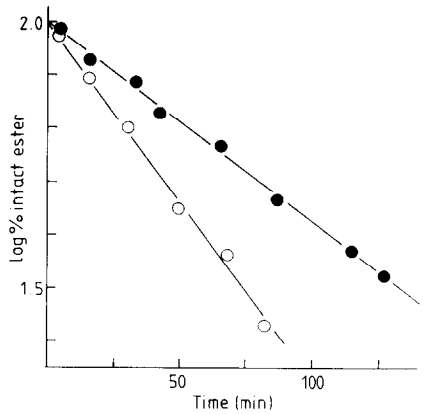


Fig. 1. First-order plots for the photodegradation of ester II (●) and ester III (○) in aqueous solution of pH 6.9 exposed to artificial laboratory light/diffuse daylight at 20 °C.

When aqueous solutions of furosemide of pH 8–10 were exposed to normal laboratory light/diffuse daylight no degradation was observed to occur over 24 h in agreement with previous findings (Neil et al., 1984; Yahya et al., 1986). However, when furosemide was stored in acidic solutions a rapid photolytic degradation took place. Also in this case the disappearance of furosemide followed good first-order kinetics (Fig. 2). The observed half-lives of degradation at various pH values are listed in Table 2. The data show that the rate of degradation is maximal at pH 1–2 and then progressively decreases with increasing pH. In Fig. 3 the logarithm of the observed pseudo-first-order rate constants has been plotted against pH. The shape of this pH–rate profile indicates that the unionized form of furosemide is

TABLE 2

Rate data for the degradation of furosemide esters and furosemide in aqueous solutions exposed to normal laboratory light/diffuse daylight and sunlight

pH	$t_{1/2}$ (min)	
	Lab. light	Sunlight
Ester II		
2.0	51	11
5.0	96	14
6.9	80	17
9.5	57	12
Ester III		
2.0	25	
5.0	39	
6.9	45	
9.5	29	
Furosemide		
1.0	150	
2.0	155	
3.0	201	
3.5	310	
4.0	440	
4.5	1150	
4.9	2010	

the reactive species and that the overall kinetics can be described by:

$$k = k' \frac{a_H}{a_H + K_a} \quad (4)$$

where k' is a first-order rate constant for the photolytic degradation of the unionized form of furosemide, $a_H/(a_H + K_a)$ is the fraction of total furosemide in this form and K_a is the apparent ionization constant of the carboxylic acid group in furosemide. The following values of k' and K_a were obtained:

$$k' = 4.5 \times 10^{-3} \text{ min}^{-1}$$

$$K_a = 10^{-3.8}$$

The line drawn for the pH–rate profile in Fig. 3 was constructed from Eqn. 4 and these values. The good agreement observed between the experimental and calculated data shows that Eqn. 4 adequately describes the kinetics of degradation. Furthermore, the kinetically derived pK_a value (3.8) compares well with the values (3.6, 3.8 and 3.9) reported in the literature (Hajdu and Häussler, 1964; Orita et al., 1976).

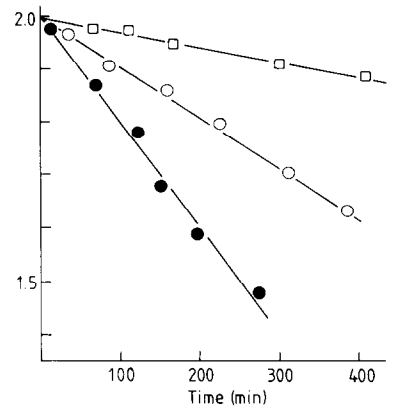


Fig. 2. First-order plots (log % intact furosemide against time) for the degradation of furosemide in aqueous buffer solutions exposed to artificial laboratory light/diffuse daylight at 20 °C. Key: □, pH 4.5; ○, pH 3.5; ●, pH 1.0.

According to Eqn. 4 the stability of neutral and alkaline furosemide solutions can be predicted. Thus, it can be calculated that solutions of pH 7.0 and 8.0 possess $t_{10\%}$ values (i.e. times for 10% degradation) of 26 and 260 days, respectively, when exposed to artificial laboratory light or diffuse daylight. These estimates concur with the stability data given by Yahya et al. (1986) which did not detect any decomposition over a 2-day period for an infusion preparation.

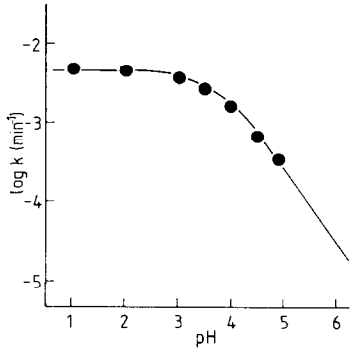


Fig. 3. The pH–rate profile for the photodegradation of furosemide in aqueous solution at 20 °C. The points are experimental and the solid curve calculated from Eqn. 4.

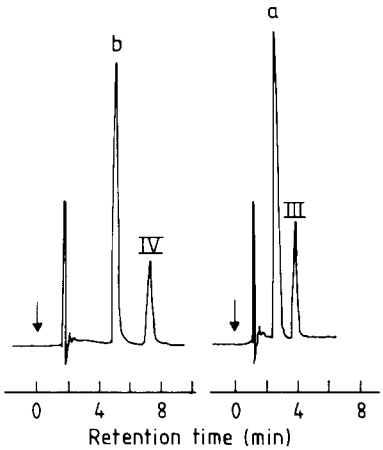


Fig. 4. HPLC tracings of aqueous solutions (pH 7.4) of the furosemide esters III and IV kept at 20 °C with exposure to normal laboratory light/diffuse daylight for 10–20 min. Key: a = photodegradation product from ester III; b = photodegradation product from ester IV.

That the neutral form of furosemide is the principal photolabile species is in accordance with the behaviour of the furosemide esters. The photoinstability of these compounds is almost independent of pH and as seen from the data in Table 2 the neutral form of furosemide has almost the same reactivity as the esters. The findings is also in agreement with previous work by Moore and Burt (1981). These workers have demonstrated that furosemide has a high photosensitizing capacity, being able to initiate both excited-state energy transfer and free radical reactions, and that the principal photosensitizing species is apparently the unionized molecule.

Monitoring the reaction solutions by means of HPLC revealed the formation of a major single product from each ester and from furosemide (Fig. 4). The product was different from that formed by acid-catalyzed hydrolysis and each ester and furosemide gave rise to a different product. Thus, it can be assumed that the ester grouping and the acid group remain intact in the major photolytic product. According to previous findings by Moore and Sithipitaks (1983) the most likely process taking place is dechlorination to give *N*-furfuryl-5-sulphamoyl-anthranilic acid and the corresponding esters. However, substitution of

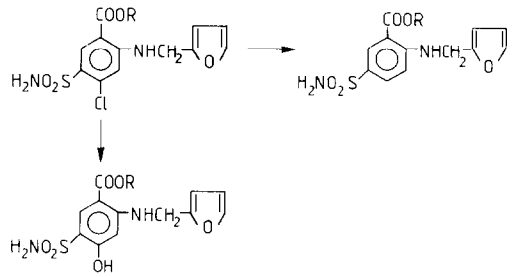


Fig. 5. Proposed pathways of photodegradation of furosemide and furosemide esters in aqueous solution.

the chlorine atom with a hydroxyl group derived from the solvent may also be imagined (Fig. 5).

In conclusion, furosemide esters and furosemide in its unionized form have been found to be extremely susceptible to undergo light-induced degradation in aqueous solution. Such quick degradation was also found to take place in water admixed with organic solvents like methanol and acetonitrile. It may be noted that these findings may have analytical implications. For example, in the analysis of furosemide in urine samples, which may be acidic, care should be taken to protect the samples from ordinary light. A major pathway for the biotransformation of furosemide is conjugation with glucuronic acid (Smith and Benet, 1983) and since such conjugates are esters of furosemide (Rachmel et al., 1985) adequate protection against light may also be important in the handling of such compounds.

Acid-catalyzed hydrolysis of furosemide

It is well-documented that furosemide shows an incomplete (40–60%) and highly variable absorption pattern following peroral administration (Kelly et al., 1973; Beerman et al., 1975; Tilstone and Fine, 1978; Waller et al., 1982; Hammarlund et al., 1984; Grahnén et al., 1984). An explanation for this could be acid-catalyzed hydrolysis of the drug in the stomach prior to absorption. Cruz et al. (1979) studied the hydrolysis of furosemide in buffer solutions of varying pH at 70 °C, using a direct ultraviolet spectrophotometric method. From temperature-accelerated runs in acidic solutions they estimated the half-life of hydrolysis to be 3.0 h at pH 1.0 and 37 °C. Thus, furosemide

TABLE 3

Rate data for the hydrolysis of furosemide in diluted hydrochloric acid solutions ($\mu = 0.5$) at various temperatures

Temperature (°C)	pH 1.0		k_H (M^{-1} min^{-1})
	k	$t_{1/2}$	
70	4.2×10^{-2} min^{-1}	16.5 min	0.42 M^{-1} min^{-1} *
60	1.2×10^{-2} min^{-1}	57.8 min	0.12 M^{-1} min^{-1}
37	6.7×10^{-4} min^{-1}	17.2 h	0.0067 M^{-1} min^{-1}

* At $\mu = 1.0$ a k_H value of 0.56 $M^{-1}\cdot min^{-1}$ was obtained (70 °C).

would hydrolyze to an extent of approximately 20% if it remained at pH 1 for 1 h at 37 °C and hence, hydrolysis of furosemide in the stomach was concluded to account in part for the reduced bioavailability. On the other hand, Andreasen et al. (1982) have found that only between 1.0% and 4.4% of furosemide were hydrolyzed after 1 h at 37 °C in gastric juice solutions of pH 1.2–2.3. The analytical method used was an HPLC procedure but only the product of hydrolysis (saluamine) was determined. In both of these studies the reaction solutions were protected from light.

Because of these conflicting data we have reinvestigated the kinetics of hydrolysis of furosemide in acidic aqueous solutions (pH 1–2) at 37–70 °C, using a specific HPLC procedure to quantitate remaining furosemide. All the solutions were kept in a water-bath and were well protected against light. At constant pH and temperature the hydrolysis followed strict first-order kinetics over several half-times. At pH 1–2 the observed pseudo-first-order rate constants (k) increased with increasing hydrogen ion activity according to Eqn. 1. The k_H values obtained at various temperatures are listed in Table 3 along with rate data at pH 1.0.

Our data show that furosemide is more stable in acidic solutions than previously reported by Cruz et al. (1979). These workers found a k_H value of 2.03 $M^{-1}\cdot min^{-1}$ at 70 °C and $\mu = 1.0$ whereas a value of 0.56 $M^{-1}\cdot min^{-1}$ at the same conditions was obtained in our study (Table 3). Also, our direct determination of the rate of hydrolysis at 37 °C showed that furosemide is considerably more stable ($t_{1/2}$ = 17.2 h at pH 1.0 and 37 °C) than predicted by Cruz and co-workers ($t_{1/2}$ of 3.0 h).

In the investigation by Cruz et al. the reaction progress was followed spectrophotometrically by measuring the absorbance of the solutions at 235 nm. In order to test the possible occurrence of light-induced degradation of furosemide under these assay conditions a 1-cm quartz cuvette containing a furosemide solution of pH 2.0 was placed in a spectrophotometer and exposed to 235 nm radiation. However, even after 2 h no significant degradation had taken place as determined by HPLC.

Our data on the acid-stability of furosemide, when protected against light, indicate that acid-catalyzed degradation in the stomach is not a significant factor for the incomplete peroral absorption of the drug in agreement with the conclusion reached by Andreasen et al. (1982). Assuming the gastric emptying process to be associated with a half-time of 50 min (Digenis et al., 1977) it can be calculated using the equation for two parallel first-order processes (Wassermann and Bundgaard, 1983) that at a gastric pH of 1.0 95% of an oral dose would leave the stomach in intact form.

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