Studies on the Photolysis and Hydrolysis of Furosemide in Aqueous Solution¹⁾

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Photolysis and hydrolysis of furosemide were studied in buffer solutions (pH between 1.2 and 12.0). Photolysis experiments were made at room temperature under a Daylight lamp[®] or fluorescent lamps as artificial light sources and direct and indirect sunlight as natural light sources by changing the strength of illumination. The photolysis of furosemide was found to follow apparent first-order kinetics under both artificial and natural light sources. In acidic media, the rate of photolysis of furosemide was larger than those in neutral or alkaline media. The pH-rate profile for the photolysis of furosemide indicated that the rate of photolysis of unionized furosemide was much larger than that of ionized furosemide. A hydrolysis experiment of furosemide was made at 37 °C in the darkened room. The hydrolysis of furosemide was also found to follow apparent first-order kinetics. Furosemide was stable in neutral or alkaline solutions. In acidic media below pH 3, hydrolysis of furosemide was found to be catalyzed by hydrogen ion with an acid catalyzed rate constant of $0.893 \text{ M}^{-1} \cdot \text{h}^{-1}$.

Keywords furosemide; photolysis; photodegradation; hydrolysis; pH-rate profile; HPLC assay

Furosemide, 4-chloro-*N*-furfuryl-5-sulfamoylanthranilic acid, is a potent diuretic agent of pharmacokinetic/pharmacodynamic interest in humans.^{2,3)} Pharmacokinetic parameters in the literatures have varied much in different groups in spite of the development of high performance liquid chromatography (HPLC) methods for furosemide assay.^{3,4)} A difference in assay methods of furosemide in biological specimens might be one of the reasons for variations, as some of the groups did not notice that furosemide was an unstable compound. Furosemide is hydrolyzed in acidic media to form 4-chloro-5-sulfamoylanthranilic acid (CSA).⁵⁾ Extraction of furosemide into organic solvents from strongly acidified biological specimens prior to the assay was reported in several papers. In such cases, the concentration of furosemide extracted might be lower than the actual concentration. Further, CSA has been misunderstood as the metabolite of furosemide in plasma or urine. Although furosemide is recognized as a photosensitive drug, protection of the biological specimens from light has not been taken into consideration in several reports. In these cases, the concentration of furosemide might also be lower. Sekikawa *et al.*⁶⁾ reported on the photodegradation of furosemide and furosemide glucuronide in aqueous solution under direct or indirect sunlight as light sources.

In this study, the authors made comparative studies of photodegradation and hydrolysis of furosemide in aqueous solution. Photodegradation of furosemide was made under several conditions, such as light sources, strengths of illumination, or pHs, to obtain information prior to the kinetic studies in furosemide assays.

Experimental

Materials Furosemide (lot. No. 263 D210) was supplied by Hoechst Japan Ltd., Tokyo. CSA was obtained from U.S. Pharmacopeia (Rockville, MD). Acetonitrile was HPLC grade from Wako Pure Chemicals Ind., Ltd., Osaka. Piretanide (lot No. 700 L023), supplied by Hoechst Japan, was dissolved in phosphate buffer at pH 7.4 and used as an internal standard for furosemide assay. All other chemicals were of reagent grade.

Photodegradation Studies Irradiative sources employed were as follows; direct (illumination; between 10000 and 30000 lux) and indirect sunlight (illumination; between 75 and 8000 lux) were used as natural light sources. As an artificial light source, Daylight lamp[®] D400⁷⁾ with the stabilizer 4MT-105H-AE (400 W, Toshiba Electric Co., Ltd., Tokyo) was used in the darkened room. The maximum relative intensity was found to be around 500 nm. The strength of the illumination was controlled by changing the

distances of the samples from the lamp. The Daylight lamp[®] was stabilized enough prior to the study. "Fluorescent lightings" referred to setting the test solutions on a bench in the dark about 1.4 m below naked fluorescent lamps (40 W × 2, 340 ± 20 lux, white type, Mitsubishi, Tokyo) as artificial light sources. The strengths of illumination were measured using a lux meter of Maris[®] (A-BS type, Tokyo Maekawa Science Co., Japan).

Furosemide was dissolved in redistilled water containing a small volume of 0.1 N NaOH, and the solution was used as a stock solution (100 µg/ml). A test solution (10 µg/ml) was made by dilution with buffer solutions of various pHs (pH 1.2–12.0). The buffer systems used ($\mu=0.2$) were pH 1.2–2, 0.2 M KCl–0.2 M HCl; pH 3–5, 0.1 M CH₃COOH–0.1 M CH₃COONa; pH 6–9, 0.1 M KH₂PO₄–0.05 M Na₂B₄O₇; pH 10–12, 0.1 M NaOH–0.1 M Na₂B₄O₇. The test solutions were put into test tubes (15 cm × 15 mm i.d.) and light sources described above were irradiated. At appropriate time intervals, portions of the test solutions were removed and put into the test tubes, mixed with the internal standard solution, and transferred into glass inserts and assayed by HPLC for automatic assay. Temperatures were not controlled and varied between 24 ± 3 °C. All of the analytical conditions were carefully protected from light.

Hydrolysis Studies The solutions were put into light-resistant flasks, and were shaken (50 strokes/min) at 37 ± 0.1 °C in an incubator. Portions of the solutions were removed at the appropriate time and assayed as described in photodegradation studies.

Assay Procedure for Furosemide The assay procedure for furosemide was based on the previous study.⁶⁾

HPLC was performed on a Shim-pack CLC-ODS, with a 5 µm reversed-phase column (150 × 6 mm i.d.) and a Shim-pack CLC-ODS, with 30 µm guard column (50 × 2.1 mm i.d.) from Shimadzu Co., Kyoto. The columns were maintained at 40 ± 0.5 °C in the column oven (CTO-6A, Shimadzu). A fluorescence spectrophotometer Model RF-535 (Shimadzu) was used as a detector. Excitation and emission wave lengths, respectively, were 345 and 415 nm. The mobile phase used was 40% acetonitrile containing phosphoric acid (0.1%, pH 3.5). The flow rate was 1.0 ml/min. Samples were automatically injected onto HPLC using a SIL-6A auto injector (Shimadzu) and a LC-6A pump (Shimadzu) with a run time of 12 min using a SCL-6A system controller (Shimadzu). A C-R6A computing injector (Shimadzu) was used to obtain the area of the peaks of the chromatogram. In the chromatogram, peaks of furosemide and an internal standard were observed at 6.0 and 9.6 min, respectively.

Identification of Photodegradation Product After the irradiation of indirect sunlight to furosemide aqueous suspension, the major photodegradation product was separated using an Intersil PREP-ODS, 10 µm column (20.0 × 250 mm i.d.) from Gasukuro Kogyo Inc. The mobile phase was 40% acetonitrile containing acetic acid (0.3%, pH 4.0). The flow rate was 3.0 ml/min. The elute of the fraction was collected. Acetonitrile in the elute was removed *in vacuo*. The organic phase was washed with water after the solution was extracted into ethylacetate. Then ethylacetate was evaporated *in vacuo* using a rotary evaporator at 40 °C. The molecular weight of the residue was determined by a GCMS 9100-MK (Shimadzu) and a GCMS-PAC 1500 (Shimadzu) mass data system. Proton nuclear magnetic resonance (¹H-NMR) spectrum [CD₃OD + CD₃COCD₃ (δ)]: 8.37 (1H, s), 7.49 (1H, m), 6.32–6.42 (3H, m), 4.48 (2H, s). FAB-MS

m/z : 313 ($M^+ + 1$).

Results and Discussion

Photodegradation Studies Figure 1 shows the time course of the photodegradation of furosemide with different lights at pH 5. The degradation of furosemide was found to follow apparent first-order kinetics. Under the direct sunlight (20000 lux) and indirect sunlight (1600 lux), half-lives ($t_{1/2}$) were about 18 and 290 min, respectively. These results indicate the possible loss of furosemide in solution if the sample is not protected from natural light sources in daytime.

The use of curtains or window shades may be one of the ways to protect the sample solutions from natural light sources. Figure 1 also shows the effects of plastic window shades on the photodegradation of furosemide. Using plastic window shades on a sunny day (220 ± 30 lux) and a cloudy day (75 ± 25 lux), the apparent first-order rate constants for the degradation were 3.60×10^{-2} and $3.20 \times 10^{-3} \text{ h}^{-1}$, respectively. The results show that the apparent reductions in the degradation of furosemide were made by the window shades, however, significant decreases of the concentration of furosemide were observed within the experimental period. The apparent degradation rate constant was $5.12 \times 10^{-3} \text{ h}^{-1}$ when fluorescent lightings (340 ± 20 lux) were used. The half-life of the degradation was 135 h. This value was much larger than that reported

Fig. 1. Apparent First-Order Plots for the Photolysis of Furosemide at pH 5.0 with Different Light Sources

○, direct sunlight (20000 $\pm$ 1000 lux); ●, indirect sunlight (1600 $\pm$ 200 lux); △, indirect sunlight shut off by a plastic window shade on a sunny day (220 $\pm$ 30 lux); ▲, indirect sunlight shut off by a plastic window shade on a cloudy day (75 $\pm$ 25 lux); □, fluorescent lamp (340 $\pm$ 20 lux). Each point represents the mean of four determinations. All standard deviations were within 3%.

Fig. 2. Apparent First-Order Plots for the Photolysis of Furosemide under Indirect Sunlight (3500 $\pm$ 300 lux)

○, pH 1.2; ●, pH 4.0; △, pH 6.0; ▲, pH 12.0.

by Kerremans *et al.* (5 h)⁸) though the experimental conditions were different. Sekikawa *et al.*⁶) found no degradation of furosemide at pH 5 by irradiation of fluorescent lights covered with a translucent plastic frame (260 lux) for 4 h. The plastic frame might cut the light at wave lengths that caused the photodegradation of furosemide. The results suggested that the effect of irradiation on the photodegradation was not due to the illumination but to the wave lengths contained in the light sources.

Figure 2 shows the time course of the photodegradation of furosemide in solutions with different pHs (pH 1.2–12.0) under indirect sunlight (3500 $\pm$ 300 lux). The photodegradation of furosemide in all solutions was found to follow apparent first-order kinetics. Degradation rates of furosemide in acidic regions were much larger than those in neutral and alkaline solutions. This tendency was in agreement with the result by Bundgaard *et al.*⁹) although they did not indicate the illumination value when furosemide solutions were exposed to normal laboratory light (furosemide solutions in test tubes were placed beside the laboratory window) or diffused daylight (furosemide solutions in test tubes were placed on a laboratory table in normal laboratory light and with diffused daylight entering the room from the window). The half-lives of the degradation at pHs 5, 6 and 7 were 4.2, 6.8 and 11 h, respectively. The pH of urine from a normal subject ranges between 5 and 8. A range between 2000 and 4000 lux is considered to be the usual lighting conditions in the laboratory in daytime without any care taken to alter the lighting conditions. In these cases, 5% of the concentration will be lost within 20 min.

Furosemide glucuronide is the major metabolite excreted in human urine. For the assay of furosemide and furosemide glucuronide simultaneously, urine must be acidified appropriately to prevent the intramolecular acyl migration of furosemide glucuronide.¹⁰) In such cases, a rapid photodegradation of furosemide ($t_{1/2}$ was about 30 min at pH 3, 3500 $\pm$ 300 lux) might be noticed.

Using sunlight as the light source is important for photodegradation studies of drugs, because it provides information about the degradation rates of drugs naturally occurring in the laboratory, the rooms for collection of biological specimens or the rooms for analysis. However, interday and intraday variations of the illumination are considerably large, and the experimental time periods are limited. In studies of the photodegradation of drugs; fluorescent lights, mercury lamps or other artificial lamps were utilized as the light sources. In this study, the authors used a Daylight lamp[®] as the light source. The wave lengths of the light from the Daylight lamp[®] developed by Toshiba Electronics are similar to those of sunlight. The spectral radiation energy of the Daylight lamp[®] was reported by Matsuda and Masahara.⁷)

Figure 3 shows the time course the photodegradation of furosemide in solutions of different pHs under irradiation of the Daylight lamp[®] (10000 lux). The illumination was constant during the study. A similar tendency to those observed in the photodegradation study of furosemide under indirect sunlight (Fig. 2) was obtained. In the acidic region at pHs 1.2, 2 and 3, rapid degradation was obtained. In the neutral region at pHs 6, 7, 8 and 9, the degradation

Fig. 3. Apparent First-Order Plots for the Photolysis of Furosemide under the Daylight Lamp® (10000 ± 500 lux)

Symbols are same as Fig. 2.

Fig. 4. pH-Rate Profile for the Photolysis of Furosemide under the Daylight Lamp® (10000 ± 500 lux)

rate constants were smaller than those obtained in the acidic region. The degradation rate constants obtained in the neutral region had similar values. The pH-rate profile is shown in Fig. 4. The pK_a of furosemide is 3.8.¹¹⁾ At lower pHs 1.2, 2 and 3, the molecule of furosemide exists mostly unionized. At pHs 6, 7, 8 and 9, the molecule of furosemide exists mostly ionized. The result indicates that the photodegradation rate constants of unionized furosemide are about ten times larger than those of the ionized form. At higher pHs (pH 10–12), photodegradation rate constants larger than those at neutral pHs were obtained. Rowbotham *et al.*⁵⁾ reported on the photodegradation of furosemide in solution after irradiation by ultraviolet light. They found a degradation product in alkaline solution as 4-chloro-5-sulphoanthranilic acid. Moore *et al.*^{12,13)} estimated a chlorine ion after irradiation of furosemide with ultraviolet light and some chlorine-containing drugs in aqueous or methanol solution. They suggested that these drugs correlated with their abilities to photosensitive oxidation by the free radical reaction. We ascertained the photodegradation product by fast atom bombardment mass spectrum (FAB-MS) and ¹H-NMR spectrum described above. From these results the major photodegradation product was determined to be 4-hydroxy-*N*-furfuryl-5-sulfamoylanthranilic acid (HFSA). Chart 1 shows the structure of the photodegradation product. Sekikawa *et al.*⁶⁾ reported on the photodegradation of furosemide in aqueous solution. By the photodegradation of furosemide, the first product (HFSA) was found. After that, several peaks in the

Chart 1. Structure of Photodegradation Product of Furosemide

Fig. 5. Apparent First-Order Rate Constant of the Photodegradation of Furosemide and Illumination by the Daylight Lamp®

Fig. 6. pH-Rate Profile for the Hydrolysis of Furosemide at 37 °C

HPLC chromatogram were observed after the photodegradation of HFSA. Further study concerning the identification of these photodegradation products may clarify the total degradation process of furosemide in a solution with different pHs.

Figure 5 shows the relation between the rate constants of the photodegradation of furosemide at pH 5 and the strength of illumination by the Daylight lamp®. Good correlation was observed between the illumination and the rate constants of photodegradation with $Y = 7.20 \times 10^{-5} X - 0.027$, $r = 0.9989$. The result indicated that the rate constants of photodegradation were proportional to the strength of illumination, if the light source was the same.

When the light source was sunlight, however, such a proportional relation was not observed. Distribution of the wave lengths contained in direct or indirect sunlight might be different with different conditions.

Hydrolysis Studies Hydrolysis of furosemide was found to follow apparent first-order kinetics. Figure 6 shows the pH-rate profile for the hydrolysis of furosemide at 37 °C. Similar studies were reported by Cruz *et al.*¹⁴⁾ or Bundgaard *et al.*⁹⁾ From Fig. 6, the apparent rate constant of hydrolysis may be described by:

$$k_{\text{obs}} = k_{\text{H}^+}[\text{H}^+] + k_{\text{H}_2\text{O}} \quad (1)$$

where k_{obs} is the apparent degradation rate constant, k_{H^+} is the specific acid catalytic rate constant and $k_{\text{H}_2\text{O}}$ is the rate constant by water molecule. In the acidic pH region below 3, where the furosemide molecule is mostly unionized, the contribution of $k_{\text{H}_2\text{O}}$ is considerably small, and the slope of the pH-rate profile indicated specific hydrogen ion catalysis. In this region, the pH-rate profile may be described approximately by:

$$k_{\text{obs}} = k_{\text{H}^+}[\text{H}^+] \quad (2)$$

or

$$\log k_{\text{obs}} = \log k_{\text{H}^+} - \text{pH} \quad (3)$$

According to Eq. 3, the slope was -1.15 with an intercept ($\log k_{\text{H}^+}$) of 1.33 ($k_{\text{H}^+} = 0.893 \text{ M}^{-1} \cdot \text{h}^{-1}$).

By these parameters, the half-life of furosemide at pH 1.0 and 37°C may be calculated as 7.76 h. Cruz *et al.*¹⁴⁾ and Bundgaard *et al.*⁹⁾ calculated the half-life at pH 1.0 and 37°C as 2.97 and 17.2 h, respectively. The value of our result was almost intermediate compared to these workers. Cruz *et al.*¹⁴⁾ calculated this half-life by extrapolation from the rate constants of hydrolysis obtained between 60 and 80°C . Probably, at those temperatures, actual concentrations of hydrogen ion presented in the solution might be different from those at room temperature at which buffer solutions were prepared.

Under those conditions, furosemide would hydrolyze to an extent of approximately 4, 0.4 and 0.04% if it remained at pHs 1.2, 2 and 3 for 1 h at 37°C , respectively. Sekikawa *et al.*⁶⁾ found furosemide and furosemide glucuronide in urine and plasma after the oral administration of furosemide tablets and intravenous administration of furosemide in ten male subjects. They also found no detectable CSA in any of the urine or plasma samples. Furosemide is practically insoluble in water. After the disintegration of the tablet in the stomach, furosemide may not dissolve rapidly. The furosemide fraction that would hydrolyze during passing through the stomach may be very little. Thus, the hydrolysis of furosemide would be negligible under physiological conditions in the stomach.

Between pHs 3 and 6, the slope was not -1 because the molecule of furosemide is partially ionized. Above pH 6, the furosemide molecule present may be mostly ionized. In this region, the contribution of k_{H^+} is negligible and the rate constant of the hydrolysis of ionized furosemide may be described approximately as:

$$k_{\text{obs}} = k_{\text{H}_2\text{O}} \quad (4)$$

Due to the difference of the component of the buffer solution, the values of $k_{\text{H}_2\text{O}}$ were between $2.5 \times 10^{-3} \text{ d}^{-1}$ ($\text{KH}_2\text{PO}_4\text{--Na}_2\text{B}_4\text{O}_7$ system) and $3.1 \times 10^{-3} \text{ d}^{-1}$ ($\text{NaOH--Na}_2\text{B}_4\text{O}_7$ system).

In some reports,¹⁵⁻¹⁸⁾ CSA was considered to be one of the photodegradation products. During the photodegradation study, CSA was not detectable in the HPLC chromatogram even when the pH of the solution was 1.2 under indirect sunlight (3200 to 3800 lux) or direct sunlight (20000 to 25000 lux). The rate constants of photodegradation were much larger than that of hydrolysis at room temperature ($k_{\text{obs}} = 174 \times 10^{-3} \text{ d}^{-1}$, pH 1.2, and $23 \pm 0.5^\circ\text{C}$).

Concentrated hydrochloric acid was used to acidify the biological specimen prior to the organic solvent extraction. In such a case, the pH of the solution might decrease below 1. CSA might be produced as the artifact during the assay.¹⁹⁾ However, CSA could not be the photodegradation product.

For the assay of furosemide in biological specimens, sufficient care must be taken during the collection, storage or analysis of biological specimens to protect the photodegradation of furosemide and its metabolite, glucuronic acid conjugate, which was more photosensitive than furosemide.^{3,6)}

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