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Notes

Degradation and gastrointestinal stability of nitrofurantoin in acidic and alkaline media

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Summary

The degradation of nitrofurantoin in acidic (pH 1.2) and alkaline (pH 10) solutions at 100°C was performed. Decomposition of the drug was faster under alkaline conditions than under acidic conditions. The major degradation product was isolated from the alkaline solution by column chromatographic separation using silica gel as adsorbant and was further purified by preparative thin-layer chromatography. The structure of this product was determined as 2-(5-nitro-2-furfurylidene) hydrazine acetate by the use of relevant UV, IR and ^{1}H-NMR spectra. Degradation kinetics were evaluated; the reaction rate constants (k day^{-1}) and chemical half-lives ($t_{1/2}$) were calculated depending on the first-order kinetics. Moreover, the stability of pure nitrofurantoin and of its commercial drug form in simulated gastric and intestinal media were investigated.

Nitrofurantoin (1-[(5-nitrofurfurylidene)amino]hydantoin) is an antibacterial agent which is used for the treatment of urinary tract infections (Cadwallader and Jun, 1976) and is preferred over sulfonamides and antibiotics (Ristuccia and Cohna, 1984: Merck, 1987; Martindale, 1989). It is reported that nitrofurantoin crystals and its solutions are discolored by alkali and exposure to light. Nitrofurantoin also decomposes upon contact with metals other than stainless steel and aluminum (Remington's Pharmaceutical Sciences, 1985; USP XXI, 1985). Vishnupad (1980) demonstrated that the decomposition half-life was 1 h at pH 1.5, 154 h at pH 4 and 14 h at pH 12 at 45°C. In addition, the activation energy was also found to be 11.4 kcal/M at pH 1.5, 7.8 kcal/M at pH 4 and 185 kcal/M at pH 11. It is known that at low pH, the hydrolytic cleavage of nitrofurantoin yields 5-nitro-2-furaldehyde and 3-aminohydantoin (Paul et al., 1960). Juenge et al. (1985) observed selective heterocyclic ring cleavage of nitrofurantoin to yield 3-(5-nitrofurfurylideneamino)hydantoic acid in citrate buffer. In a different study, gastric degradation was considered to be the main cause of the reduction in bioavailability of nitrofurantoin following oral administration due to the reversible hydrolysis to 5-nitro-2-furaldehyde and 1-amino hydantoin by azomethine bond cleavage (Inotsume and Nakano, 1981).

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However, Watari et al. (1983) claimed that this suggestion is invalid, or at least the in vivo effect is minor, because of the equilibrium of the hydrolytic reaction being markedly in the direction of hydrolysis. Buzard et al. (1961) reported that nitrofurantoin shows good absorption in the jejunum and in the colon. Nitrofurantoin is absorbed through the gastric mucosa in small quantities (Veronese et al., 1974). Based on these considerations, further work seems to be necessary to examine particularly the intestinal stability of the drug in order to evaluate its bioavailability. It is therefore the purpose of this study to investigate the gastrointestinal stability and degradation of nitrofurantoin in acidic and alkaline media to explain the reduction in the bioavailability of this drug following oral administration.

Materials: Nitrofurantoin was supplied by Eaton Co., Switzerland, and commercial hard gelatin capsules containing 50 mg nitrofurantoin were obtained from Biyofarma Co., Turkey. The other chemicals and solvents were purchased from Merck.

Hydrolysis of nitrofurantoin solutions: Clark and Lubs buffer system (pH 1.2) and Sorensen-borate buffer system (pH 10) were used as hydrolyzing solutions. Simulated gastric (SGM) and intestinal media (SIM) were prepared according to USP XXI.

Adsorbants: silica gel G 60 (70–230 mesh) was used for column chromatography. For TLC. (a) 40 g silica gel G 60 suspended in 100 ml water and (b) 40 g silica gel HF_{254}-G 60 (13 : 27) and 2.5 g 1% carboxymethylcellulose suspended in 100 ml water were used.

Solvent systems: acetone-benzene-water-glacial acetic acid (40 : 10 : 7 : 0.5) and acetone-benzene-*N*,*N*-dimethylformamide-glacial acetic acid (40 : 10 : 3 : 0.5) systems were used for TLC and column chromatography, respectively.

Acidic hydrolysis of nitrofurantoin: 5 mg of drug was dissolved in 10 ml of Clark and Lubs buffer and filtered through a quantitative filter paper (Sands Rundfilter/black ribbon). It was then kept at 100°C for 3 h. At given time intervals, 5 μl of this solution was withdrawn and applied on a chromatographic plate. After development, the spots were visualised under UV light. The areas of spots were measured at 357 nm on a TLC scanner (Shimadzu cs-920; D_2-lamp, $\lambda = 357$ nm, mode = ABS, AZS = 10, linearizer = 0).

Alkaline hydrolysis of nitrofurantoin: 40 mg of nitrofurantoin was dissolved in 10 ml of Sorensen-borate buffer and was filtered through a quantative filter paper. It was then kept at 100°C for 8 h. At certain time intervals, 1 μl of this solution was withdrawn and then processed according to the above-mentioned procedure.

Preparation and application of the degradation product on the column: 4 g of drug was dissolved in 10 ml of Sorensen-borate buffer and hydrolysed at 100°C for 45 min. The hydrolysis product was distilled and then applied on a silica gel column (3 × 50 cm), being eluted by solvent at 14–16 drops per min. 10–15 ml fractions were collected.

Isolation of the degradation product: Preparative thin-layer chromatography (TLC) was used for isolation of the main degradation product from the alkaline solution. The column chro-

TABLE 1

Decrease of nitrofurantoin in pH 1.2 and pH 10 at 100°C

		Time						
		0	15 min	30 min	45 min	1 h	2 h	3 h
pH 1.2	drug (%)	100	95.1	92.3	–	83.9	67.9	55.9
	loss (%)	–	4.9	7.7	–	16.1	32.1	44.1
	significance	$p < 0.05$						
pH 10	drug (%)	100	91.5	66.4	47.5	31.6	8.9	–
	loss (%)	–	8.5	33.6	52.5	68.4	91.1	100
	significance	$p < 0.01$						

matographic fractions collected were applied on plates of 20 × 20 cm. Following development the band corresponding to the degradation product located under UV light at 366 nm was scraped off into a column (2.5 × 25 cm) and eluted with chloroform-methanol (18:2). Distillation of the solvent in vacuo yielded the degradation product in the pure state.

Stability of pure nitrofurantoin and its commercial drug form in SGM and SIM: Both SGM and SIM were prepared with and without enzyme. They were kept at 37°C for 3 and 8 h with nitrofurantoin, respectively. At time intervals, samples of 5 μl were taken and the same procedure as above was applied.

The decrease of nitrofurantoin in hydrolyzing solutions at pH 1.2 and 10 vs time is given in Table 1. It is shown that the decrease in drug content of the alkaline medium is greater than that in the acidic medium. Nitrofurantoin decomposes completely in the alkaline medium within 3 h at 100°C.

The plot of concentration vs time is given in Fig. 1. A linear relation is obtained when the equation for a first-order reaction is applied.

The reaction rate constant and chemical half-lives were calculated (Table 2) using the following relationships:

$$\ln C = \ln C_0 - kt \quad (1)$$

$$t_{1/2} = \frac{0.693}{k} \quad (2)$$

One major degradation product was detected on TLC in the alkaline hydrolyzing solution (R_f 0.4). The increase of the main degradation product and decrease in nitrofurantoin vs time are shown in Fig. 2. As the amount of drug decreases, the main degradation product increases for the first hour in the alkaline hydrolyzing solution followed by a gradual decrease during the rest of the reaction.

It is known that the solubility of nitrofurantoin is highest at pH 10 (Vishnupad, 1980). For this reason, the drug solution was adjusted to pH 10 with Sorenson-borate buffer and allowed to hydrolyse at 100°C. The hydrolysis product was de-

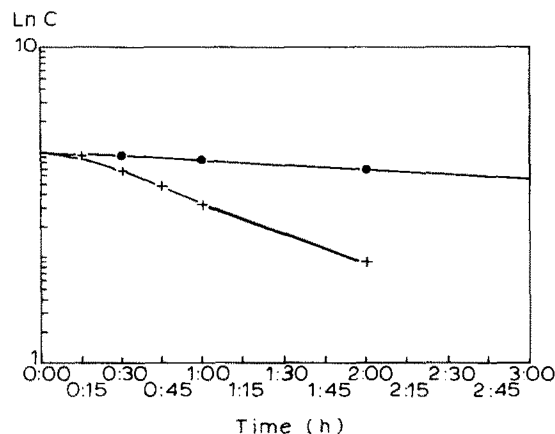


Fig. 1. Degradation curves for nitrofurantoin in acidic and alkaline media at 100°C: (+——+) in alkaline medium; (●——●) in acidic medium.

tected in the SIM both with and without enzyme. Stability studies showed that the drug is unstable in both SGM and SIM with or without enzyme. The amount of drug decreases by 31.1% in SGM without enzyme in 3 h, and by 28.7% in SGM with enzyme in 3 h; and 38.1% in SIM without enzyme in 8 h, and 36.3% in SIM with enzyme in 8 h. Table 3 shows the loss of nitrofurantoin in powder form as well as in its commercial preparation during the hydrolysis in SGM and SIM with and without enzyme. The enzyme added to the test solutions brought about an increase stability of nitrofurantoin in both the pure and commercial drug forms.

The major degradation product was analyzed by UV, IR and NMR spectroscopic methods. Spectral data were as follows: $C_7H_6N_3O_5$. UV λ_{max} (MeOH) 273, 370 nm. IR ν_{max} (KBr) 1560, 1530, 1440, 970 cm^{-1}. ^{1}H-NMR (200 MHz, DMSO-d_6) δ 4.24 (2H, S, $-CH_2-$), 7.29 (1H, S, -C=N-), 7.39 (1H, d, H-3, $J = 3.8$ Hz), 7.81 (1H, d, H-4, $J = 3.8$ Hz).

TABLE 2

Reaction rate constants and chemical half-lives of nitrofurantoin in solutions

	k (day^{-1})	$t_{1/2}$ (min)
pH 1.2	0.002	237.3
pH 10	0.019	36.3

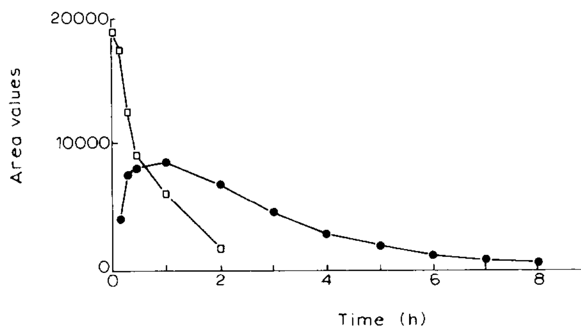


Fig. 2. Degradation curves of nitrofurantoin and its main product at pH 10 and 100°C: (□) nitrofurantoin; (■) product.

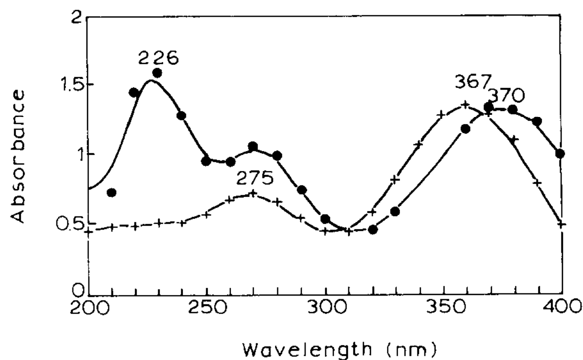


Fig. 3. Absorption spectra of nitrofurantoin and degradation product: (+ ——— +) nitrofurantoin; (● ——— ●) product.

In the IR spectrum, the absorption observed at 970 cm^{-1} indicates that it is a 2,5-disubstituted furan. The peaks observed at 1560 and 1440 cm^{-1} belong to the asymmetric and symmetric stretching vibrations of the carboxylate anion. The strong absorption signal observed at 1530 cm^{-1} belongs to the asymmetric stretching of the aromatic nitro group.

In the UV spectrum of the degradation product, the maxima observed at 226 and 370 nm are identical with those of nitrofurantoin, which displays corresponding maxima at 275 and 367 nm (see Fig. 3). The only noticeable difference is in their molar absorptivity values.

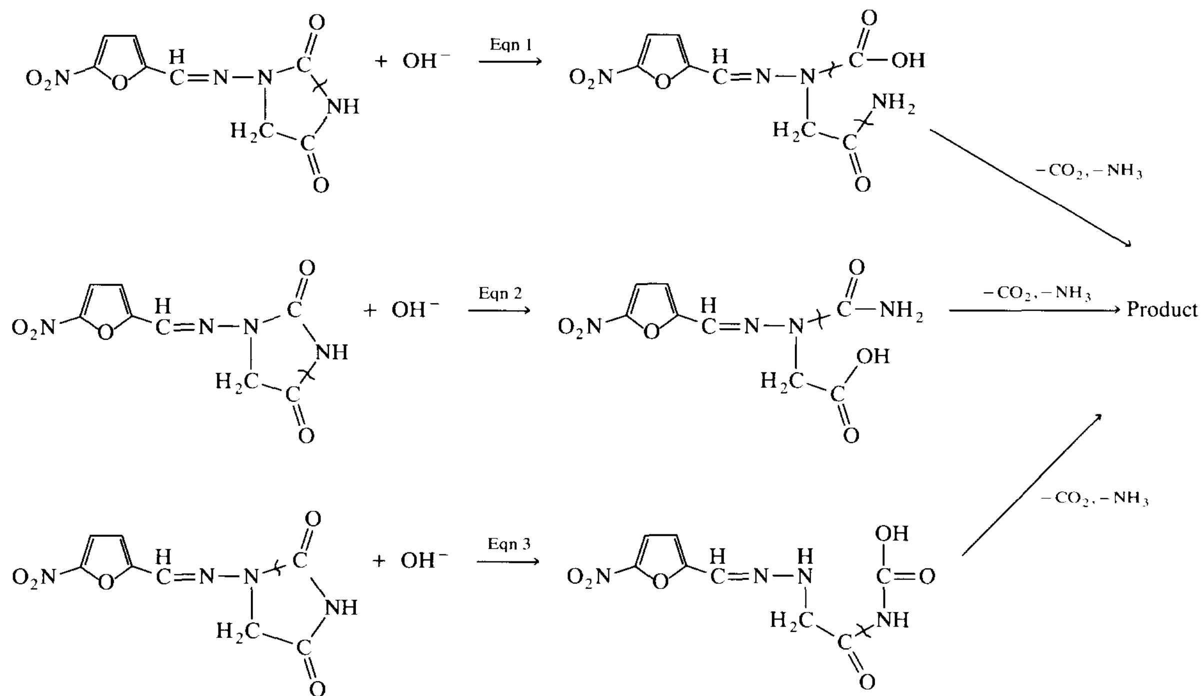
In the ^{1}H-NMR spectrum of the compound, two doublets of one proton each are observed at δ 7.39 and 7.81. These signals split with a coupling constant of 3.8 Hz. Both the chemical shifts

TABLE 3

Percentages of decrease of nitrofurantoin and commercial drug form in SGM and SIM at 37°C

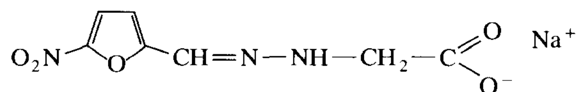
Time (h)	Nft in SGM		Nft in SIM		C-Nft in SGM		C-Nft in SIM	
	Without enzyme	With enzyme	Without enzyme	With enzyme	Without enzyme	With enzyme	Without enzyme	With enzyme
0 drug loss	100	100	100	100	100	100	100	100
1 drug	90	90.6	94.9	96.4	92.4	97.8	97.2	98
loss	10	9.4	5.1	3.6	7.6	2.2	2.8	2
2 drug	78.5	84.9	90	91.4	89.7	96	92	97.4
loss	21.5	15.1	10	8.6	10.3	4	8	2.6
3 drug	68.9	71.3	85.5	87.9	76.8	87	88.5	95.5
loss	31.1	28.7	14.5	12.1	23.2	13	11.5	4.5
4 drug			83.6	87.4			86.4	94.5
loss			16.4	12.6			13.6	5.5
5 drug			76.3	77.0			82.9	92.7
loss			23.7	23			17.1	7.3
6 drug			71.8	75.2			81.9	91.8
loss			28.2	24.8			18.1	8.2
7 drug			67.2	67.5			77	88.5
loss			32.8	32.5			23	11.5
8 drug			61.9	63.7			76.3	84.6
loss			38.1	36.3			23.7	15.4
Significance $p < 0.01$								

Nft, nitrofurantoin; C-Nft, commercial hard gelatin capsule of nitrofurantoin.



Scheme 1. Alkaline degradation of nitrofurantoin.

and the coupling constant values are characteristic for the H-3 and H-4 of the furan ring. The sharp singlet at δ 7.24 belongs to the proton of the azomethine group. The methylene protons on the carbon which is α both to the carboxylate and the hydrazine group display a resonance at δ 4.24 as a singlet of two hydrogens. In the light of the above-mentioned data, the chemical structure of our polar compound is suggested to be sodium 2-(5-nitro-2-furfurylidene)hydrazine acetate.



This product does not have any toxic effect on humans.

In a recent study, a theoretical mechanism for the degradation of nitrofurantoin in alkaline medium was suggested to be as shown above (Vishnupad, 1980). The degradation product detected in our work could be a further step in the proposed reactions.

In vitro gastrointestinal stability of nitrofurantoin was investigated for the first time and the drug was shown to be unstable in both simulated gastric and intestinal media. Therefore, it is suggested that the reason for the decrease in bioavailability following oral administration was due not only to the decomposition of the drug in gastric fluid, but also was a consequence of its decomposition in the intestinal fluid. In addition, a degradation product from alkaline medium was isolated and its structure was elucidated as 2-(5-nitro-2-furfurylidene)hydrazine acetate by using UV, IR and NMR spectroscopy.

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