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Effect of Formulation and Process Variables on Bioequivalency of Nitrofurantoin I: Preliminary Studies

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Abstract □ Fifty-two combinations of nitrofurantoin were developed to assess the effect of dosage form type, particle size, diluent, and process on *in vitro* availability. With the official procedure and conditions, dissolution rates fell in a 66-fold range. Statistical analysis of the dissolution rates indicated no significant differences as a result of particle size, processing method, or compression force. The diluent choice and dosage form type significantly influenced the dissolution rate. Based on *in vitro* screening, six formulations presenting a broad range of dissolution rates were selected for further study relating to human bioavailability and bioequivalence.

Keyphrases □ Nitrofurantoin—*in vitro* dissolution rate, effect of formulation and process variables □ Dissolution rate, *in vitro*—nitrofurantoin, effect of formulation and process variables □ Antibacterials, urinary—nitrofurantoin, *in vitro* dissolution rate, effect of formulation and process variables

Nitrofurantoin, an antibacterial agent used in urinary tract infections, was included by the Food and Drug Administration on its list of drugs requiring bioavailability/bioequivalence testing (1). Because of its physicochemical properties, dissolution testing might be used as a means for eliciting inferences concerning its bioavailability. Formulation and manufacturing processes may affect its dissolution and ultimate bioavailability (2), and dissolution is particle size dependent (3–5).

No useful correlation was observed between the extent of urinary excretion and either the disintegration or dissolution characteristics of nitrofurantoin tablets (6, 7). However, the third supplement of USP XIX (8) requires that not less than 25% of the labeled amount of nitrofurantoin must dissolve in 60 min. Since the bioavailability characteristics of commercially produced nitrofurantoin tablets were examined in previous investigations without regard to specific formulation and process variability information, a study was undertaken utilizing predetermined variables to assess their effect on bioequivalence.

EXPERIMENTAL

Particle-Size Reduction and Analysis—To determine the effect of particle size, crystalline nitrofurantoin USP[1] was used both in its commercially available form and in micronized form prepared by processing the commercial material through a fluid energy jet mill[2]. A differential pressure of 30% was used between the inlet and opposing jets.

The original and micronized crystals were then suspended in peanut oil, and the particle size was determined by microscopy (9).

Formulations and Processing—Fifty-two formulations were prepared, incorporating the following variables:

1. Three types of dosage forms (chewable tablets, swallow tablets, and hard gelatin capsules).
2. Two particle sizes of nitrofurantoin crystals.
3. Two processes of incorporation (wet granulation and direct blending–compression).
4. Two diluents [compressible sugar[3] and mannitol[4]–lactose[5] (2:1)].
5. Three levels of hardness or compression force for the tablet formulations.

The formulations are summarized in Tables I–III.

For wet granulation, all ingredients except the lubricant were blended in a cuboidal blender[6] for 10 min at 35 rpm. Granulation then was accomplished in a planetary mixer[7] at 120 rpm for 10 min, using sufficient distilled water to produce the proper consistency. This mixture was granulated through a 12-mesh screen, dried overnight at 48°, and sized through a 16-mesh screen. The granulation was blended with the lubricant in the cuboidal blender for 10 min at 35 rpm. Compression was accomplished on an instrumented rotary tablet press[8] at three levels of force.

For direct compression, all ingredients were blended in the cuboidal blender for 10 min at 35 rpm and compressed as described.

For the hard gelatin capsules, the blends were prepared as for direct compression. The blend was filled into hard gelatin capsules[9] (size 0) using a hand-operated capsule-filling machine[10].

The tablets were physically evaluated on the basis of weight variation, hardness variation, friability, and disintegration. The capsules were physically evaluated on the basis of weight variation only. All dosage forms were evaluated chemically on the basis of composite average assay and content uniformity using a slight modification of the method originally proposed by Conklin and Hollifield (10). An accurate sample equivalent to approximately 100 mg of nitrofurantoin was mixed with approximately 10 ml of dimethylformamide, filtered, quantitatively transferred with adequate rinsing, and diluted to 50 ml with dimethylformamide. A 1.0-ml aliquot of this solution was diluted to 50.0 ml with 10% dimethylformamide. A 1.0-ml aliquot of this dilution was acidified with 2.0 ml of 0.2 *M* HCl and extracted with 5.0 ml of nitromethane. Then 3.0 ml of the nitromethane layer was combined with 0.5 ml of 0.04 *M*

[1] Lot 12060, Berry and Withington Co., Cambridge, Mass.

[2] Gem-T research model, Trost Air Mill Department, Newton, Pa.
[3] Nu-Tab, lot DB917M, Specialty Products by SuCrest, Pennsauken, N.J.
[4] Granular, lot 1219, ICI America, Wilmington, Del.
[5] Anhydrous, lot 4NM10, Sheffield Chemical Co., Union, N.J.
[6] Model KB-15, Erweka-G.m.b.H., Frankfurt, West Germany.
[7] Model N-50, Hobart Manufacturing Co., Troy, Ohio.
[8] Model B-2, Stokes Division, Pennwalt Corp., Warminster, Pa.
[9] Elanco Products Co., Indianapolis, Ind.
[10] Model SGR-O Capsulator, Spielman and Co., Clifton, N.J.

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Table I—Preliminary Formulation of Nitrofurantoin Chewable Tablets

Formulation	Ingredients[a], %							
	X_1	X_2	Li	Ca	Sa	Mg	D_1	D_2
IA, IB, IC	6.67	—	0.67	1.67	1.00	0.50	89.50	—
ID, IE, IF	—	6.67	0.67	1.67	1.00	0.50	89.50	—
IG, IH, I-I	6.67	—	0.67	1.67	1.00	0.50	—	89.50
IJ, IK, IL	—	6.67	0.67	1.67	1.00	0.50	—	89.50
IM, IN, IO	6.67	—	0.67	1.67	1.00	0.50	89.50	—
IP, IQ, IR	—	6.67	0.67	1.67	1.00	0.50	89.50	—
IS, IT, IU	6.67	—	0.67	1.67	1.00	0.50	—	89.50
IV, IW, IX	—	6.67	0.67	1.67	1.00	0.50	—	89.50

[a] X_1 = nitrofurantoin crystal USP, X_2 = nitrofurantoin micronized crystals, Li = lime flavor, Ca = citric acid, Sa = saccharin sodium powder, Mg = magnesium stearate USP, D_1 = compressible sugar, and D_2 = mannitol–lactose (2:1).

quaternary ammonium hydroxide[11] in absolute methanol. The absorbance was determined spectrophotometrically[12] at 400 nm against a blank sample prepared by carrying 1.0 ml of 10% dimethylformamide through the extraction procedure. The resulting absorbance then was compared to a previously constructed standard curve to determine concentration.

All dosage forms were evaluated for dissolution rate using the USP XIX procedure (11). The dissolution medium consisted of 900 ml of pH 7.2 phosphate buffer maintained at 37° in a constant-temperature bath, and the basket was rotated at 100 rpm. Samples of 2.5 ml were collected at 5, 10, 15, 20, 30, 40, 50, 60, 75, and 90 min. Each sample withdrawal was replaced by an equivalent amount of dissolution medium.

The samples were assayed by diluting 1.0 ml of each sample to 10.0 ml with buffer and determining the absorbance at 380 nm against a blank consisting of the buffer. The dissolution rate was then calculated for each formulation. All resulting evaluation data were subjected to a factorial design analysis of variance to determine which variables affected the dissolution rate. Ultimately, six formulations were selected for Phase II of the study.

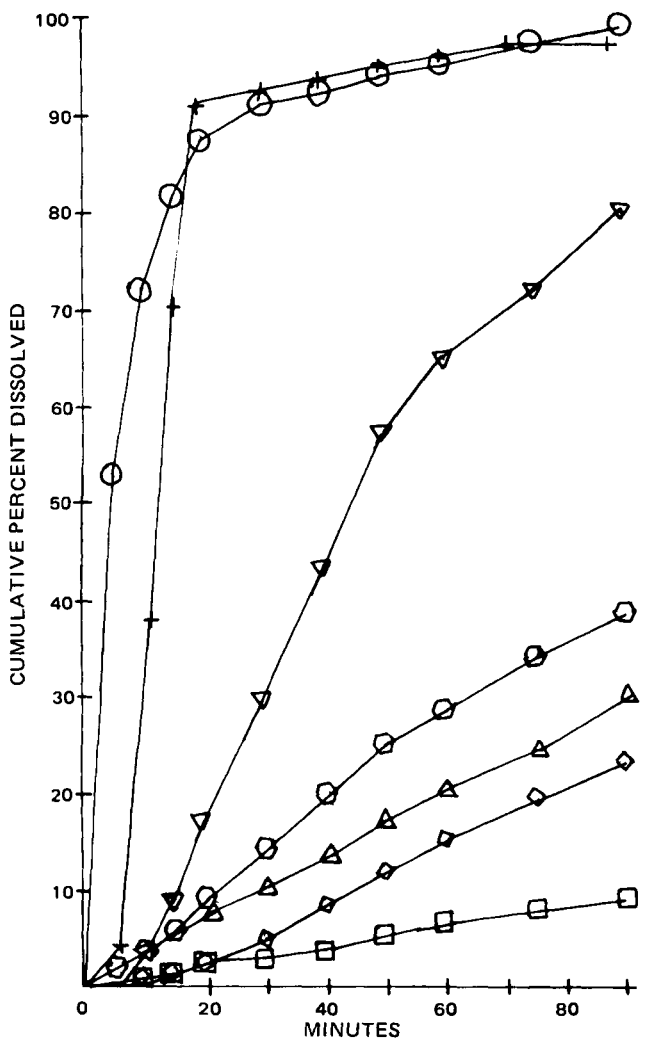

Figure 1—*Mean cumulative percent of nitrofurantoin dissolved in phosphate buffer. Each data point is the mean of three determinations. Key: ○, Formulation IIIC; +, Formulation IG; ○, Formulation CTL; □, Formulation IIA; ◇, Formulation IIM; △, Formulation IA; and ▽, Formulation IIIA.*

[11] Hyamine.
[12] Model DB-GT, Beckman Instrument Co., Fullerton, Calif.

RESULTS AND DISCUSSION

The microscopic particle-size analysis, based on the examination of 100 particles, revealed a geometric mean diameter for the commercial material of 3.0 μm with a standard deviation of 1.66. The micronized product had a geometric mean diameter of 0.75 μm with a standard deviation of 2.0. There was, therefore, a fourfold difference in the average particle sizes of the two forms of nitrofurantoin.

All tablet formulations were produced without difficulty to contain 100 mg of active ingredient. Because of bulk density and a desire for reasonable size, the capsules were produced to contain 50 mg each; two capsules were then used as one unit dose.

The dosage form evaluation data are summarized categorically by dosage form type and processing method in Tables IV–VIII.

The weight variation for all formulations was well within compendial limits, *i.e.*, ±5% for tablets and ±10% for capsules. In general, the weight variation for formulations produced with compressible sugar was better than for those produced with the mannitol–lactose combination. Presumably, this result is due to the better flowability of the former excipient.

Table II—Preliminary Formulation of Nitrofurantoin Swallow Tablets

Formulation	Ingredients[a], %					
	X_1	X_2	Mg	Vg	D_1	D_2
IIA, IIB, IIC	16.67	—	0.50	—	82.83	—
IID, IIE, IIF	—	16.67	0.50	—	82.83	—
IIG, IIH, II-I	16.67	—	0.50	5.00	77.83	—
IIJ, IIK, IIL	—	16.67	0.50	5.00	77.83	—
IIM, IIN, IIO	16.67	—	0.50	—	—	82.83
IIP, IIQ, IIR	—	16.67	0.50	—	—	82.83
IIS, IIT, IIU	16.67	—	0.50	5.00	—	77.83
IIV, IIW, IIX	—	16.67	0.50	5.00	—	77.83

[a] X_1 = nitrofurantoin crystal USP, X_2 = nitrofurantoin micronized, Mg = magnesium stearate, Vg = aluminum magnesium silicate (Veegum) medium fine, D_1 = compressible sugar, and D_2 = mannitol–lactose (2:1).

Table III—Preliminary Formulation of Nitrofurantoin Capsules

Formulation	Ingredients[a], %				
	X_1	X_2	Mg	D_1	D_2
IIIA	9.52	—	1.00	89.48	—
IIIB	—	9.52	1.00	89.48	—
IIIC	11.36	—	1.00	—	87.89
IIID	—	11.36	1.00	—	87.89

[a] X_1 = nitrofurantoin crystal USP, X_2 = nitrofurantoin micronized, Mg = magnesium stearate, D_1 = compressible sugar, and D_2 = mannitol–lactose (2:1).

Table IV—Evaluation Summary for 12 Nitrofurantoin Chewable Tablet Formulations Produced by Direct Compression

Diluent	Particle Size of Nitrofurantoin, μm	Compression Force, kg	Hardness, SCU	Disintegration, min	Dissolution Rate, mg/min
Compressible sugar	USP, 3.0	1571–2200	8–11	8–12	0.0021–0.0026
	Micronized, 0.75	1571–2200	9–12	8–12	0.0021–0.0036
Mannitol–lactose	USP, 3.0	1257–1885	9–14	6–10	0.0300–0.0418
	Micronized, 0.75	1257–1885	12–17	8–10	0.0141–0.0529

Table V—Evaluation Summary for 12 Nitrofurantoin Chewable Tablet Formulations Produced by Wet Granulation

Diluent	Particle Size of Nitrofurantoin, μm	Compression Force, kg	Hardness, SCU	Disintegration, min	Dissolution Rate, mg/min
Compressible sugar	USP, 3.0	1571–2200	12–15	8–12	0.0022–0.0024
	Micronized, 0.75	1571–2200	12–17	8–12	0.0014–0.0019
Mannitol–lactose	USP, 3.0	1257–1414	15–18	8–10	0.0140–0.0209
	Micronized, 0.75	1257–1414	15–18	7–10	0.0166–0.0213

Table VI—Evaluation Summary for 12 Nitrofurantoin Swallow Tablet Formulations Produced by Direct Compression

Diluent	Particle Size of Nitrofurantoin, μm	Compression Force, kg	Hardness, SCU	Disintegration, min	Dissolution Rate, mg/min
Compressible sugar	USP, 3.0	943–1257	8–10	14–16	0.0012
	Micronized, 0.75	1100–1414	9–11	14–16	0.0013
Mannitol–lactose	USP, 3.0	629–943	9–14	8–15	0.0022–0.0029
	Micronized, 0.75	471–943	8–14	6–12	0.0023–0.0041

Table VII—Evaluation Summary for 12 Nitrofurantoin Swallow Tablet Formulations Produced by Wet Granulation

Diluent	Particle Size of Nitrofurantoin, μm	Compression Force, kg	Hardness, SCU	Disintegration, min	Dissolution Rate, mg/min
Compressible sugar	USP, 3.0	707–1021	9–13	14–16	0.0090–0.0091
	Micronized, 0.75	707–1021	10–14	14–16	0.0080–0.0090
Mannitol–lactose	USP, 3.0	550–943	9–13	2–4	0.0018–0.0048
	Micronized, 0.75	550–943	9–13	2–5	0.0020–0.0043

Tablet hardness fell generally in a range of 8–18 Strong–Cobb units (SCU) with a standard deviation of approximately ±1.0.

Friability for the tablet formulations fell within a range of 0.12–2.03%. In general, the friability of formulations produced with mannitol–lactose was higher than for formulations produced with compressible sugar. The friability of the chewable tablets was generally greater than that of the swallow tablets, no doubt because of their greater size, weight, and surface area. The disintegration times for the tablets fell within a range of 2–16 min. In general, disintegration was more rapid for formulations made with mannitol–lactose than for those made with compressible sugar. The composite assays for all formulations fell within the compendial limits of 95–105% of the labeled amount. Content uniformity testing also fell within the compendial limits of 85–115%.

The dissolution rates for the formulations ranged from 0.0008 to 0.0529 mg/min (Fig. 1). Statistical analysis indicated that the difference in the two particle sizes of nitrofurantoin did not significantly affect dissolution. Apparently, the particle size of commercially available nitrofurantoin is sufficiently small to elicit the maximum dissolution rate for this material. Surprisingly, neither process method (wet granulation *versus* dry blending–compression) nor compression force–hardness significantly affected the dissolution rate of the tablets. This result indicates that the intrinsic solubility of the drug is sufficient to overcome the binding of individual particles through both wet granulation and void space reduction as a result of applied compression force.

The diluent choice produced significantly ($p < 0.05$) different dissolution rates. Dissolution of the formulations made with mannitol–lactose was significantly faster than that of formulations made with compressible sugar. A viable explanation for this difference is not apparent. It appears that the presence of compressible sugar definitely depressed the nitrofurantoin dissolution rate. This effect could be due to competitive solubility, gelatinization–encapsulation, or chemical complexation.

Dosage form type also produced a significant ($p < 0.05$) difference in dissolution rate. The hard gelatin capsules produced the most rapid dissolution, followed by the chewable tablets and, finally, by the swallow tablets. The rapid dissolution of the capsules was, of course, anticipated since the disintegration of a capsule shell is generally faster than the disintegration of tablets and since no binding force is applied during encapsulation. The percentage concentration of active ingredient in the chewable tablets was considerably lower (6.67%) than in the swallow tablets (16.67%). This concentration difference could have led to more rapid dissolution as disintegration took place since greater surface area would be provided to the dissolution medium by the physically larger chewable tablets.

Based on the foregoing results, six formulations were selected for Phase II (bioequivalence) of the study. Two capsules (IIIA and IIIC), two chewable tablets (IA and IG), and two swallow tablets (IIA and IIM) were chosen to provide a broad range of dissolution rates, the objective being to conduct the bioequivalence study with a group of formulations that could be expected to produce diverse bioavailability.

Table VIII—Evaluation Summary for 12 Nitrofurantoin Capsule Formulations

Diluent	Particle Size of Nitrofurantoin, μm	Dissolution Rate, mg/min
Compressible sugar	USP, 3.0	0.0176
	Micronized, 0.75	0.0143
Mannitol–lactose	USP, 3.0	0.0460
	Micronized, 0.75	0.0518

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Effect of Formulation and Process Variables on Bioequivalency of Nitrofurantoin II: *In Vivo–In Vitro* Correlation

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Abstract □ Based on preliminary *in vitro* evaluation, six formulations presenting a broad range of dissolution rates were selected for bioequivalency determination in a randomized complete block crossover. *In vitro–in vivo* correlations were developed relating cumulative percent dissolved to cumulative percent excreted. These correlations appear to be useful for comparing different formulations as well as different batches of the same formulation.

Keyphrases □ Nitrofurantoin—various formulations, bioavailability in humans correlated to dissolution rate *in vitro* □ Bioavailability—various formulations of nitrofurantoin in humans, correlated to dissolution rate *in vitro* □ Dissolution rate, *in vitro*—various formulations of nitrofurantoin correlated with bioavailability in humans □ Antibacterials, urinary—nitrofurantoin, various formulations, bioavailability in humans correlated to dissolution rate *in vitro*

Numerous reports (1–5) provided support for the contention that not all commercially available products meeting compendial requirements necessarily exhibit equivalent bioavailability. Nitrofurantoin exhibits this problem (3–5). The Food and Drug Administration included nitrofurantoin on its list (6) of drugs requiring bioavailability testing for market preclearance, and the American Pharmaceutical Association included it in their bioavailability monograph project (7).

Previous studies on the bioinequivalence of nitrofurantoin utilized commercially available products for testing without regard to formulation and process variables that might affect bioequivalency. A preliminary study (8) concerned the development and screening of 52 nitrofurantoin products having controlled variables in formulation and processing. This screening on the basis of *in vitro* test procedures led to the selection of six final formulations for bioequivalency testing and attempts at correlation with *in vitro* test results.

Table I—Final Formulations of Nitrofurantoin Tablets and Capsules

Formulation[a]	IA	IG	IIA	IIM	IIIA	IIIC
Nitrofurantoin crystals USP, %	6.67	6.67	16.67	16.67	9.52	11.36
Lime flavor, %	0.67	0.67	—	—	—	—
Citric acid monohydrate, powdered USP, %	1.67	1.67	—	—	—	—
Saccharin sodium USP, %	1.00	1.00	—	—	—	—
Magnesium stearate USP, %	0.50	0.50	0.50	0.50	1.00	1.00
Compressible sugar, %	89.5	—	82.83	—	89.48	—
Lactose, anhydrous USP, %	—	30.0	—	27.67	—	29.30
Mannitol, granular USP, %	—	59.5	—	55.16	—	58.59

[a] IA = nitrofurantoin chewable tablets, IG = nitrofurantoin chewable tablets, IIA = nitrofurantoin swallow tablets, IIM = nitrofurantoin swallow tablets, IIIA = nitrofurantoin swallow capsules, and IIIC = nitrofurantoin swallow capsules.

Table II—Experimental Design for Nitrofurantoin Bioavailability Evaluation [a]

Subject	Day 1	4	7	10	13	16	19
1	IG	CTL	IIA	IIM	IIIA	IIIC	IA
2	CTL	IIA	IIM	IIIA	IIIC	IA	IG
3	IIA	IIM	IIIA	IIIC	IA	IG	CTL
4	IIM	IIIA	IIIC	IA	IG	CTL	IIA
5	IIIC	IA	IG	CTL	IIA	IIM	IIIA

[a] Each item within the matrix corresponds to a specific formulation as described in Table I; CTL = control.

EXPERIMENTAL

Formulations—Based on preliminary dissolution data (8), six formulations (Table I) were selected to provide a broad range of dissolution rates with the expectation that this range would lead to a wide variation in bioavailability. The six formulations consisted of three dosage forms

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