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EVALUATION OF *IN VITRO/IN VIVO* CORRELATION UTILIZING A ROTATING BASKET-PADDLE DISSOLUTION APPARATUS

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ABSTRACT

The desirability of good correlations of parameters derived from *in vitro* dissolution study with parameters derived from *in vivo* bioavailability study is well established in biopharmaceutics. Reports on several *in vitro* dissolution apparatus, including the two official USP/NF methods, have appeared in the literature over the years. However, none have been accepted as universal because each apparatus is useful only for the dissolution testing of a specific group of drugs or dosage forms. Comparative dissolution testing was performed using the rotating basket-paddle apparatus and the two official USP/NF apparatus.

A comparative bioavailability study was carried out on four batches of rapidly disintegrating tablets (Formulations A to D) of nitrofurantoin and perphenazine using rabbit as an animal model. Excellent rank order (qualitative) correlations were observed among all combinations of *in vitro* and *in vivo* parameters. With the drug nitrofurantoin, an excellent quantitative correlation was found between the dissolution halftime and Cmax or Tmax or AUC. Yet, a repeated run with perphenazine yielded excellent correlation between dissolution halftime and Cmax or Tmax, but poor correlation between dissolution halftime and AUC.

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INTRODUCTION

The ultimate challenge for any dissolution apparatus is its ability to reflect the *in vivo* behavior of the dosage form during the absorptive phase following oral administration (1). This *in vivo* behavior of the dosage form can be explained more precisely by its bioavailability. Considerable attention was given to the correlation of the *in vitro* dissolution rate with bioavailability after regulations concerning bioavailability were implemented by the FDA in 1977 (2). The desirability of good correlations of variables derived from *in vitro* dissolution tests with variables derived from *in vivo* bioavailability is well established in biopharmaceutics (3,4). Two model drugs, nitrofurantoin and perphenazine were selected to evaluate the performance of the rotating basket-paddle apparatus against the USP/NF apparatus. One of these model drugs required the rotating basket (nitrofurantoin) and the other the rotating paddle (perphenazine) for official dissolution testing. Nitrofurantoin was also selected because of its poor water solubility and bioavailability problems (5-9). Furthermore, perphenazine was selected because of its high water solubility and the possible effect of formulation factors (i.e., binding, disintegrating, and lubricating agents) on its plasma concentrations (10).

The objective of this project was to study the correlation between the dissolution data (dissolution halftime, $DT_{50\%}$) obtained using the rotating basket-paddle apparatus and selected bioavailability parameters (Cmax, Tmax, and AUC) using nitrofurantoin and perphenazine tablets.

MATERIALS AND METHODS

Rotating basket-paddle apparatus

The design and preliminary evaluation of this apparatus is described earlier (11). In short, this apparatus is essentially a combination of the USP/NF apparatus 1 (rotating basket) and apparatus 2 (rotating paddle). The paddle is attached to the basket by means of a nichrome wire.

The following formulations of nitrofurantoin and perphenazine were designed to provide relatively slow to rapid drug release.

Nitrofurantoin tablets

Four different batches of rapidly disintegrating tablets (Formulations A-D) were prepared using 50 mg nitrofurantoin (< 20 μm particle size)/tablet (Table 1).

Perphenazine tablets

Four different batches of rapidly disintegrating tablets (Formulations A-D) were prepared using 8 mg perphenazine (< 30 μm particle size)/tablet (Table 2).

TABLE 1
Composition of Nitrofurantoin Tablets.

Formulation A	
Each tablet contains:	
Nitrofurantoin[a]	50.0 mg
Explotab[b]	7.2 mg
Lubritab[b]	2.4 mg
Di-Pac[c]	60.4 mg

Formulation B	
Each tablet contains:	
Nitrofurantoin	50.0 mg
Explotab	4.8 mg
Lubritab	2.4 mg
Di-Pac	62.8 mg

Formulation C	
Each tablet contains:	
Nitrofurantoin	50.0 mg
Explotab	2.4 mg
Lubritab	2.4 mg
Avicel[d]	65.2 mg

Formulation D	
Each tablet contains:	
Nitrofurantoin	50.0 mg
Starch-1500[e]	6.0 mg
Lubritab	2.4 mg
Di-Pac	61.6 mg

[a] Aldrich Chemical Co., WI;
[b] Edward Mendell Co., NY;
[c] Amstar Corporations, NY;
[d] FMC Corporation, PA;
[e] Staley and Co., IL;

TABLE 2
Composition of Perphenazine Tablets.

Formulation A	
Each tablet contains:	
Perphenazine[a]	8.0 mg
Explotab[b]	3.6 mg
Lubritab[b]	2.4 mg
Di-Pac[c]	106.0 mg

Formulation B	
Each tablet contains:	
Perphenazine	8.0 mg
Starch-1500[d]	3.6 mg
Lubritab	2.4 mg
Di-Pac	106.0 mg

Formulation C	
Each tablet contains:	
Perphenazine	8.0 mg
Avicel[e]	3.6 mg
Magnesium stearate	2.4 mg
Di-Pac	106.0 mg

Formulation D	
Each tablet contains:	
Perphenazine	8.0 mg
Avicel	36.0 mg
Lubritab	2.4 mg
Di-Pac	73.6 mg

[a] Sigma Chemical Co., MO;
[b] Edward Mendell Co., NY;
[c] Amstar Corporations, NY;
[d] Staley and Co., IL;
[e] FMC Corporation, PA;

Preparation of tablets

The following procedure was used for the preparation of 100 tablets of each formulation: the active and inactive (diluent, disintegrant, and lubricant) ingredients were weighed and mixed sequentially using a laboratory tumble mixer (Chemical and Pharmaceutical Industry Co., NY) for twenty minutes. Then, 120 mg of the mixture was weighed and compressed into tablets at 3000 lb force on a hydraulic press (Carver, Inc., NJ) using a 0.66 cm diameter tablet punch and die with a flat steel plate as the lower retainer.

Dissolution rate determination of nitrofurantoin tablets

The rotating basket is the official apparatus for dissolution testing of nitrofurantoin formulations (12). Dissolution rates were determined using the rotating basket-paddle apparatus and the rotating basket apparatus. Stirring speed was set at 100 rpm according to compendial specifications. The dissolution medium consisted of 900 ml pH 7.2 phosphate buffer, USP.

Analysis

The amount of nitrofurantoin dissolved at any time was measured at 375 nm using a spectrophotometer (Gilford Systems, OH).

Dissolution rate determination for perphenazine tablets

The rotating paddle is the official apparatus for dissolution testing of perphenazine formulations (13). Dissolution rates were determined using the rotating basket-paddle apparatus and the rotating paddle apparatus. Stirring speed was set at 50 rpm according to compendial specifications. The dissolution medium consisted of 900 ml 0.1 N HCl.

Analysis

The amount of perphenazine dissolved at any time was measured at 257 nm using a spectrophotometer (Gilford Systems, OH).

Animal model

Bioavailability studies were performed using New Zealand albino rabbits. Rabbits have often been used (14-17) as a tool in the evaluation of oral drug absorption because of their low cost and ease of handling.

Bioavailability study

A comparative bioavailability study was carried out on four batches of rapidly disintegrating tablets (Formulations A-D) for each of the two model drugs.

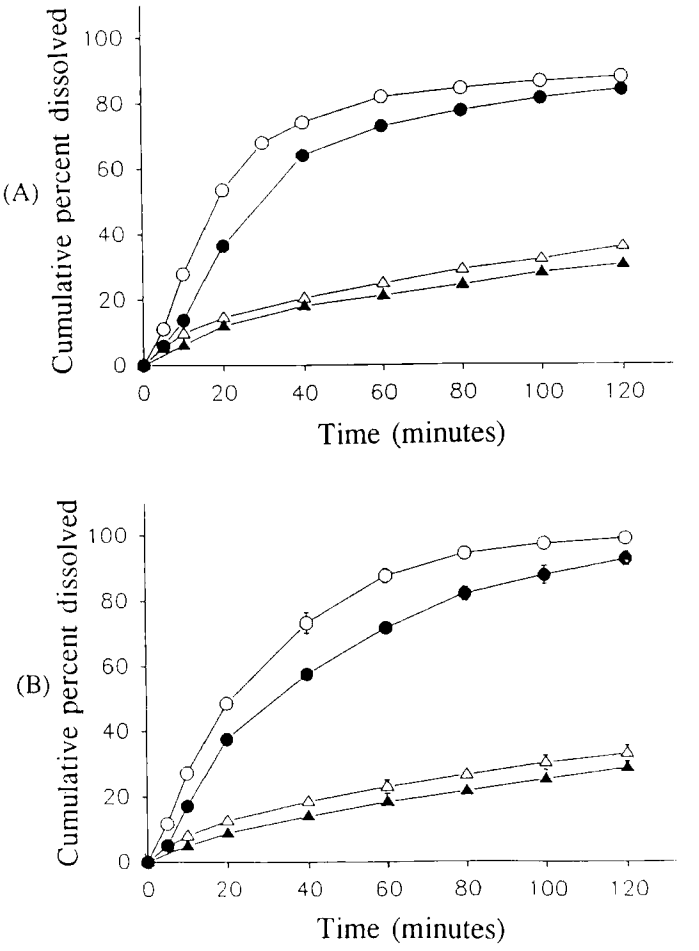

Figure 1-Dissolution profiles of four different tablet formulations of nitrofurantoin: (○) A, (●) B, (△) C, and (▲)D; using (A) rotating basket-paddle and (B) rotating basket. Each point represents average of six observations.

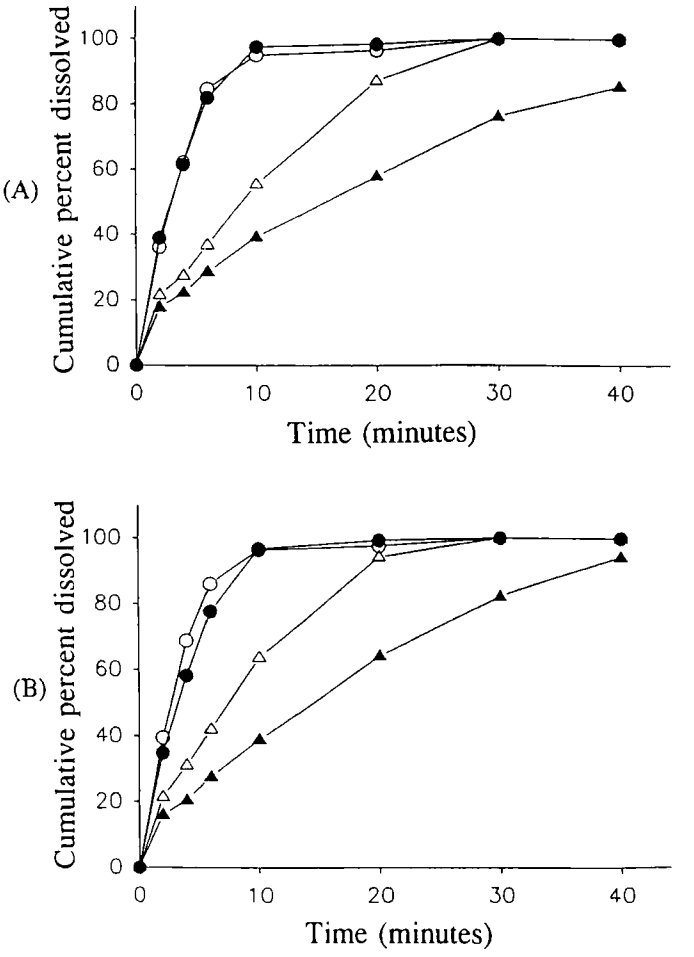

Figure 2-Dissolution profiles of four different tablet formulations of perphenazine: (o) A, (●) B, (Δ) C, and (▲)D; using (A) rotating basket-paddle and (B) rotating paddle. Each point represents average of six observations.

TABLE 3
Comparison of Average Dissolution Halftimes for Various Nitrofurantoin Formulations Using the Rotating Basket-Paddle and Rotating Basket.

Method	Dissolution halftime, ($DT_{50\%}$), min				Results of ANOVA	SNK[a] group
	Formulation					
	A	B	C	D		
Rotating basket-paddle	20.41 (1.01)[b]	34.95 (1.32)	250.77 (22.09)	345.83 (20.95)	$p < 0.0001$	D > C > B > A
Rotating basket	21.07 (1.22)	33.25 (1.72)	267.75 (25.30)	333.61 (20.39)	$p < 0.0001$	D > C > B > A

[a] Student-Newman-Keul's multiple range test ($\alpha = 0.05$)
[b] Standard error, n = 6

TABLE 4
Comparison of Average Dissolution Halftimes for Various Perphenazine Formulations Using the Rotating Basket-Paddle and Rotating Paddle.

Method	Dissolution halftime, ($DT_{50\%}$), min				Results of ANOVA	SNK[a] group
	Formulation					
	A	B	C	D		
Rotating basket-paddle	2.85 (0.09)[b]	3.08 (0.06)	8.80 (0.35)	13.95 (0.35)	$p < 0.0001$	D > C > B = A
Rotating paddle	2.54 (0.20)	3.14 (0.11)	7.25 (0.61)	11.80 (0.45)	$p < 0.0001$	D > C > B = A

[a] Student-Newman-Keul's multiple range test ($\alpha = 0.05$)
[b] Standard error, n = 6

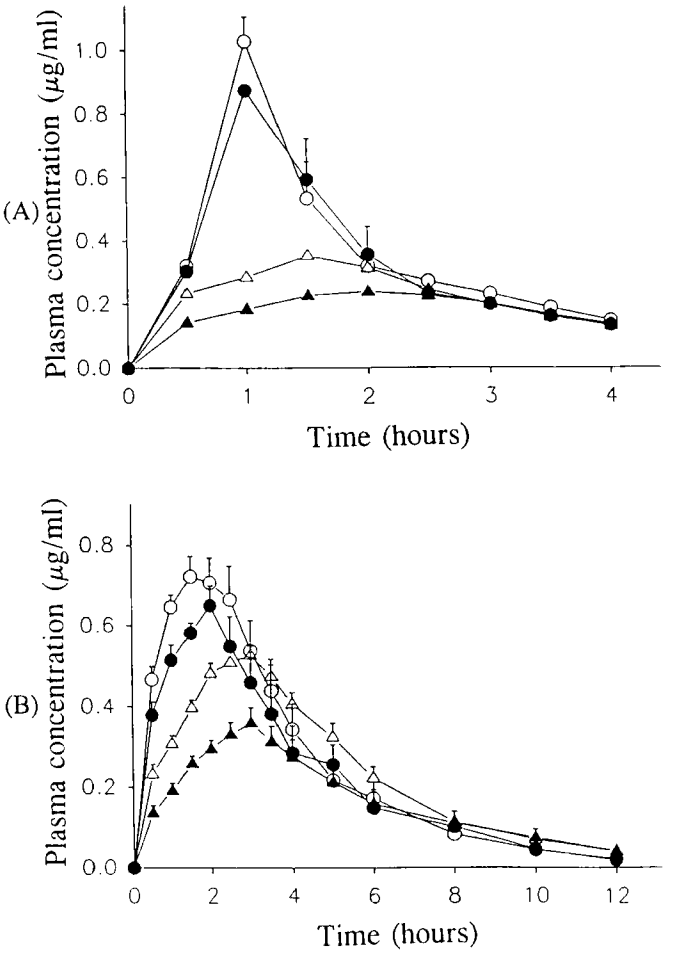

Figure 3-Mean plasma concentration versus time profiles in rabbits following separate administration of four different tablet formulations: (○) A, (●) B, (△) C, and (▲)D of (A) nitrofurantoin and (B) perphenazine. Each point represents average of eight observations.

TABLE 5
Bioavailability Parameters Following Oral Administration of Various Nitrofurantoin Formulations in Rabbits (n = 8).

Parameter	Formulation				Results of ANOVA	SNK[a] group
	A	B	C	D		
Cmax, μg/ml	1.10 (0.16)[b]	0.95 (0.14)	0.37 (0.12)	0.24 (0.11)	$p < 0.0001$	D < C < B < A
Tmax, hr	1.13 (0.18)	1.31 (0.13)	1.69 (0.19)	1.81 (0.19)	$p < 0.0050$	D = C > B = A
AUC (0- ∞), μg.hr/ml	1.83 (0.27)	1.82 (0.18)	1.28 (0.15)	1.16 (0.16)	$p < 0.0001$	D = C < B = A

[a] Student-Newman-Keul's multiple range test ($\alpha = 0.05$)
[b] Standard error, n = 8

TABLE 6
Bioavailability Parameters Following Oral Administration of Various Perphenazine Formulations in Rabbits (n = 8).

Parameter	Formulation				Results of ANOVA	SNK[a] group
	A	B	C	D		
Cmax, μg/ml	0.80 (0.16)[b]	0.70 (0.13)	0.56 (0.12)	0.40 (0.12)	$p < 0.0001$	D < C < B < A
Tmax, hr	1.56 (0.22)	2.00 (0.21)	2.75 (0.19)	2.94 (0.26)	$p < 0.0050$	D = C > B = A
AUC (0- ∞), μg.hr/ml	3.19 (0.34)	2.71 (0.35)	3.02 (0.19)	2.23 (0.28)	NS[c]	D = C < B = A

[a] Student-Newman-Keul's multiple range test ($\alpha = 0.05$)
[b] Standard error, n = 8
[c] NS = not significant

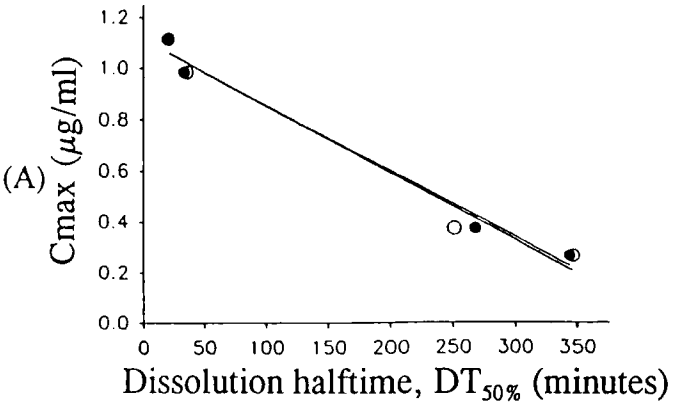

Figure 4-Correlation between (A) maximum plasma concentrations (Cmax) or (B) time to maximum plasma concentrations (Tmax) or (C) area under the plasma concentration-time curves (AUC) and *in vitro* dissolution rates ($DT_{50\%}$) for nitrofurantoin tablets using (o) rotating basket-paddle, B-P or (●) rotating basket, B. Each point represents average of 6-8 observations. r: Cmax vs B-P 0.99 ($p < 0.0001$), Cmax vs B 0.99 ($p < 0.0001$), Tmax vs B-P 0.98 ($p < 0.05$), Tmax vs B 0.98 ($p < 0.05$), AUC vs B-P 0.99 ($p < 0.001$), AUC vs B 0.99 ($p < 0.0001$).

(a) Nitrofurantoin tablets-Rabbits (n = 8) were fasted overnight before oral administration of the tablets. Oral doses were administered through a stomach tube. Dosing followed a randomized crossover design (18). Blood samples were collected from the marginal ear vein before medication and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 hours postdose. No food was permitted during the experiment. Water was available *ad libitum*.

Analysis

Plasma was separated from the blood samples by centrifuging at 3500 x g. Plasma samples were analyzed for nitrofurantoin using a sensitive HPLC method (19).

(b) Perphenazine tablets-The same protocol was followed as described for the nitrofurantoin tablets. Blood samples were collected before medication and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 12 hours postdose.

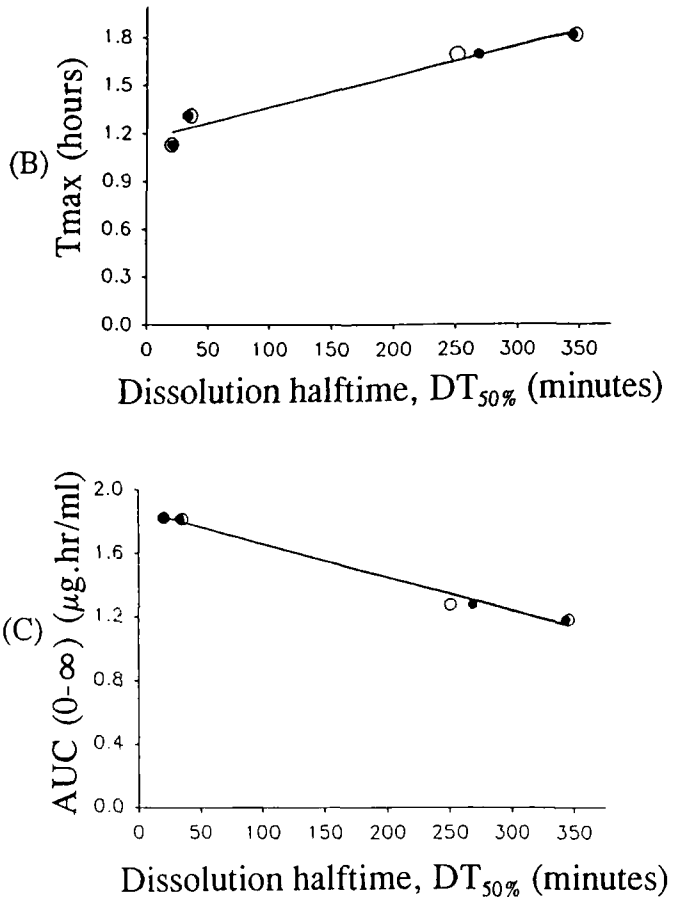

Figure 4. Continued

Analysis

Plasma was separated from the blood samples by centrifuging at 3500 x g. Plasma samples were analyzed for perphenazine using a sensitive HPLC method (20).

In vitro/in vivo correlation

Several bioavailability parameters (Cmax, Tmax, and AUC) were calculated using standard methods (21) and correlated with *in vitro* dissolution halftimes ($DT_{50\%}$).

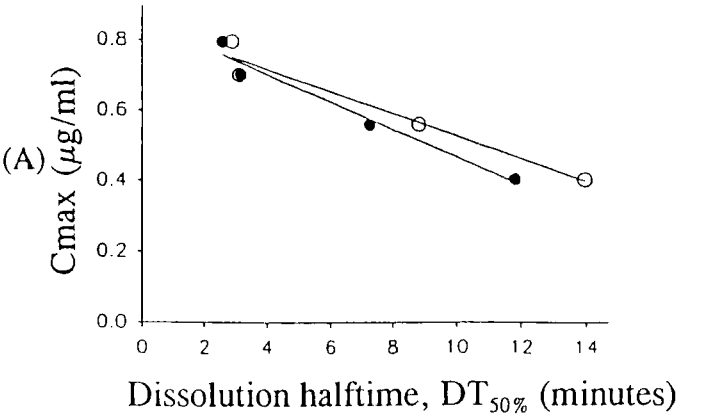

Figure 5-Correlation between (A) maximum plasma concentrations (Cmax) or (B) time to maximum plasma concentrations (Tmax) or (C) area under the plasma concentration-time curves (AUC) and *in vitro* dissolution rates ($DT_{50\%}$) for perphenazine tablets using (○) rotating basket-paddle or (●) rotating paddle. Each point represents average of 6-8 observations. r: Cmax vs B-P 0.98 ($p < 0.05$), Cmax vs B 0.98 ($p < 0.05$), Tmax vs B-P 0.93 ($p < 0.05$), Tmax vs B 0.93 ($p < 0.05$), AUC vs B-P 0.73 ($p > 0.05$), AUC vs B 0.76 ($p > 0.05$).

RESULTS AND DISCUSSION

Dissolution profiles of nitrofurantoin tablets (Formulations A-D) using the rotating basket-paddle apparatus and the USP/NF apparatus are shown in Figure 1. Dissolution profiles obtained from similar studies with perphenazine tablets are shown in Figure 2. Dissolution halftimes were calculated from these profiles and are presented in Tables 3 and 4.

Two-way analysis of variance (ANOVA) was performed to compare the dissolution halftimes of four different nitrofurantoin and perphenazine formulations, respectively. Statistical inferences and rank order relationship for the dissolution halftimes are presented in Tables 3 and 4. A significant difference ($p < 0.0001$) was observed among tablets of different formulations, whereas, the differences among tablets of the same formulation were not statistically significant.

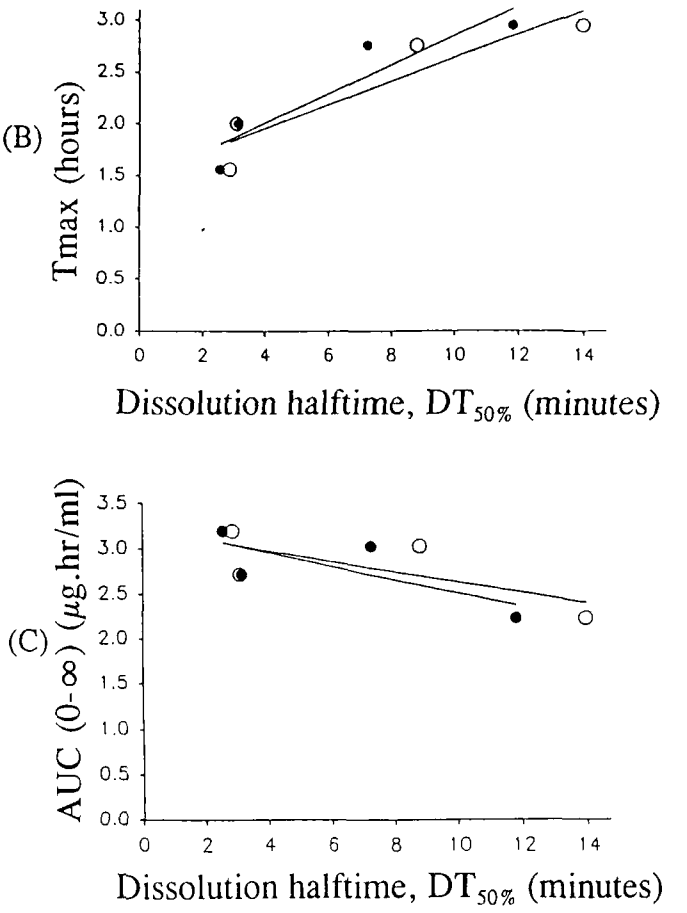

Figure 5. Continued

The mean cumulative plasma concentrations observed at various time periods after oral administration in rabbits are shown in Figure 3. Bioavailability parameters (Cmax, Tmax, and AUC) were calculated from these plasma level versus time curves and are presented in Tables 5 and 6.

Two-way analysis of variance (ANOVA) was performed to compare the bioavailability parameters of the four different nitrofurantoin and perphenazine formulations, respectively. Statistical inferences and rank order relationship for the bioavailability parameters are presented in Tables 5 and 6.

Comparison of the plasma concentration-time profiles (Figure 3) with the dissolution profiles (Tables 3-6; Figures 1 and 2) suggests a rank order (qualitative) *in vitro/in vivo* correlation.

Excellent quantitative correlation coefficients were observed between dissolution rates and: (a) maximum plasma concentration (Cmax), $r = 0.99$ ($p < 0.0001$), (b) time to maximum plasma concentration (Tmax), $r = 0.98$ ($p < 0.05$), and (c) area under the plasma concentration time curve (AUC), $r = 0.99$ ($p < 0.0001$) for the nitrofurantoin formulations (Figure 4). These results suggest that the dissolution halftime obtained using the rotating basket-paddle apparatus is a good predictor of Cmax, Tmax, and AUC for these formulations.

An excellent quantitative correlation coefficient was also observed between dissolution rate and (a) maximum plasma concentration (Cmax), $r = 0.98$ ($p < 0.05$) or (b) time to maximum plasma concentration (Tmax), $r = 0.93$ ($p < 0.05$) for the perphenazine formulations (Figure 5A-B). However, a poor correlation coefficient was observed between dissolution rates and area under the plasma concentration time curve (AUC), $r = 0.73$ ($p > 0.05$) for these formulations (Figures 5C). These results suggest that the dissolution halftime obtained using the rotating basket-paddle apparatus is only a good predictor of Cmax and Tmax but fails to predict the AUC for these formulations.

These relationships observed with the rotating basket-paddle apparatus approximate the relationships obtained with the USP/NF apparatus (Figures 4 and 5). It can be then concluded from these results that the rotating basket-paddle apparatus is an effective alternative to the two official USP/NF apparatus.

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