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Correlation of Urinary Excretion with *In Vitro* Dissolution Using Several Dissolution Methods for Hydrochlorothiazide Formulations

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Abstract □ Four different hydrochlorothiazide formulations were prepared, and cumulative urinary hydrochlorothiazide excretion was determined in a crossover study using six volunteers. A comparison of *in vivo* results showed that one formulation (Formulation D) was significantly different from the others at 2, 3, 4, 5, 8, and 14 hr. A dissolution study was conducted on each formulation using the flask, USP basket, and magnetic basket methods at agitation speeds of 50, 100, and 150 rpm. Formulation D was significantly different from other formulations when determined using the USP basket method at 150 rpm and a sampling time of 10 min; the USP basket method at 100 rpm and a sampling time of 100 min; the flask method at 100 rpm and sampling times of 30, 40, 60, and 120 min; and the flask method at 150 rpm and sampling times of 30 and 40 min. Significant *in vitro* and *in vivo* correlations were found using a regression analysis and *F* test. With a correlation coefficient and 95% confidence intervals, it was established that the USP basket method at 150 rpm was the best predictor of urinary hydrochlorothiazide excretion among the dissolution methods tested.

Keyphrases □ Hydrochlorothiazide—urinary excretion correlated with *in vitro* dissolution, various methods, bioavailability □ Diuretic agents—hydrochlorothiazide, urinary excretion correlated with *in vitro* dissolution, various methods, bioavailability □ Dissolution testing—hydrochlorothiazide, urinary excretion correlated with *in vitro* dissolution

Hydrochlorothiazide is a widely used diuretic and antihypertensive agent. Due to its limited aqueous solubility, this drug has a potential for poor absorption from the GI tract. In January 1977, the Food and Drug Administration (FDA) issued final regulations on bioequivalency and bioavailability (1). Hydrochlorothiazide was included in a list of drug entities described as having "known or potential bioequivalency or bioavailability problems."

In this publication, FDA also reported that: "A bioequivalence requirement for the majority of products should be an *in vitro* test in which the drug product is compared to a reference material. Preferably, the *in vitro* test should be an *in vitro* bioequivalence standard, *i.e.*, a test that has been correlated with human *in vivo* data. In most instances, the *in vitro* test should be a dissolution test" (1). FDA further stated that since *in vivo* testing requires an enormous number of human subjects and clinical investigators, the *in vitro* test as a valid predictor of bioequivalency can greatly reduce human subject risk and cost involved with *in vivo* testing. With these regulations, correlation between *in vitro* dissolution and bioavailability for drugs having bioavailability problems becomes more important.

McGilveray *et al.* (2) studied hydrochlorothiazide tablets manufactured by 39 Canadian companies. *In vitro* dissolution times using the USP method and bioavailability using urinary excretion were measured. Very poor correlation was found between these two parameters.

Meyer *et al.* (3) studied 14 different commercial hydrochlorothiazide formulations marketed in the United States. This work also did not reveal any apparent rela-

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tionship between bioavailability and *in vitro* dissolution. These workers measured dissolution times using the USP apparatus and bioavailability using urinary excretion data.

A literature review revealed that only the USP dissolution method had been used in the search for a correlation between *in vitro* dissolution and *in vivo* bioavailability for hydrochlorothiazide formulations. The purposes of this study were to investigate several different dissolution methods and to determine conditions and parameters for predicting the bioavailability of hydrochlorothiazide formulations.

EXPERIMENTAL

Materials—Hydrochlorothiazide powder was obtained from a commercial source[1]. Microcrystalline cellulose pH 101[2], magnesium stearate[3], lactose[4], corn starch[5], and gelatin[3] were purchased.

Tablet Formulations—Two basic formulations were designed for this investigation. Formula I (direct compression) contained (in milligrams per 150-mg tablet): hydrochlorothiazide, 25.0; microcrystalline cellulose, 124.0; and magnesium stearate, 1.0. Formula II (wet granulation) contained (in milligrams per 142.5-mg tablet): hydrochlorothiazide, 25.0; lactose, 95.5; corn starch, 20.0; gelatin (as 5% solution), 1.5; and magnesium stearate, 0.5.

Tablets were compressed on a single-punch tablet machine[6] using a 0.64-cm (0.25-in.) flat face punch and die set. Tablets were compressed to produce two different hardnesses from each formula and were tested using a motor-driven hardness tester[7]. Formulations A and C were produced from the wet granulation at hardnesses of 2.15 ± 0.98 and 7.85 ± 0.80, respectively. Formulations B and D were produced by direct compression at hardnesses of 5.35 ± 0.34 and 10.85 ± 0.63, respectively. The formulations also were subjected to weight variation and content uniformity testing, and they conformed to the USP requirements.

***In Vitro* Studies**—The three dissolution methods were the USP method (4), the flask method (5), and the magnetic basket method (6). All dissolution studies were carried out at 37 ± 0.5° in 500 ml of 0.1 *N* HCl. At various times, 1.0-ml samples were pipetted through a glass wool plug; an equal amount of 0.1 *N* HCl was added to the dissolution medium to replace each sample. The rotational agitation speeds used were 50, 100, and 150 rpm. An electronically controlled stirring motor[8] was used in each experiment, and an appropriate propeller or basket was mounted on the stirrer.

***In Vivo* Studies**—Six male volunteers, 21–30 years old and within 90–110% of their ideal body weight, were within normal limits after undergoing a chest X-ray, an ECG, and a normal blood profile. Each subject gave written informed consent before participation. All volunteers were asked to refrain from any drugs or alcohol for 72 hr prior to and during the experiment. A 25-mg dose of hydrochlorothiazide tablet or powder was taken with 200 ml of water in the morning following overnight fasting. The powder was suspended in water before administration. No food or liquid other than water was permitted until 4 hr after drug ingestion.

Cumulative urine samples were collected at 0, 1, 2, 3, 4, 5, 8, 11, 14, and 24 hr. The various formulations were administered to six volunteers at weekly intervals according to a 5 (formulations) × 6 (subjects) × 9 (sampling times) repeated measures design. Urine samples were immediately refrigerated until analysis.

Analytical Methods—In Vitro *Assay*—Hydrochlorothiazide concentration was determined using a spectrophotometer[9] at 273 nm against a blank of 0.1 *N* HCl.

In Vivo *Assay*—The method of Rehm and Smith (7) was used with some modifications. Four milliliters of urine was pipetted into a 50-ml separator and extracted with 10 ml of ethyl acetate. The ethyl acetate portion was separated, and the urine portion was reextracted with an additional 3 ml of ethyl acetate. Both ethyl acetate portions were combined and washed with 10 ml of 0.2 *N* NaOH on a mechanical shaker[10] for at least 6 hr. The ethyl acetate portion was separated from the alkali.

Two 5-ml portions of the ethyl acetate solution were transferred into separate 50-ml volumetric flasks. The volumetric flasks were transferred to a boiling water bath, and solutions were evaporated. The residue was hydrolyzed by addition of 1 ml of 0.7% hydroxylamine hydrochloride and immersion in a boiling water bath for 30 min. Both flasks were cooled, and 2 ml of 1% $NaNO_3$ and 5 ml of 1 *N* HCl were added to each. Both flasks were allowed to stand for 2 min, and 4 ml of 2% ammonium sulfate was added.

To one flask, 1 ml of 0.5% chromotropic acid was added. Finally, 10 ml of 1 *N* sodium acetate was added to both flasks and the volume was adjusted to 50 ml with distilled water. The absorbance was determined at 500 nm. The absorbance of the solution without chromotropic acid was subtracted from the value of the solution with chromotropic acid. The concentration of each sample was determined by comparison of absorbance values with those obtained from a standard curve. The color produced by hydrochlorothiazide in amounts of 12.5–62.5 μg when carried through the procedure was reproducible and followed Beer's law (r = 0.9979).

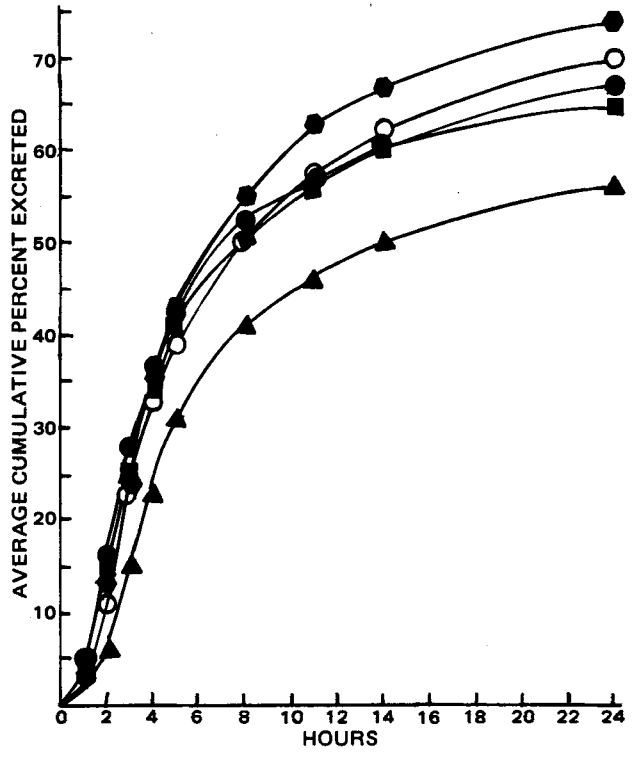

Figure 1—*Average cumulative percent of hydrochlorothiazide excreted following the administration of five formulations to each of six subjects. Key: ■, Formulation A; ●, Formulation B; ●, Formulation C; ▲, Formulation D; and ○, powder.*

RESULTS AND DISCUSSION

***In Vivo* Data**—The mean cumulative percentages of hydrochlorothiazide excreted by six subjects receiving each of the four tablet formulations (A–D) and powder (E) are shown in Fig. 1. The powder was included so that results could be compared with the other dosage forms as well as with a previously reported study (3). The average cumulative percent excretion of the powdered drug in 24 hr was 69.0 ± 10.5 (*SD*). The calculated excretion half-lives for the powdered drug administered in this study and of that reported previously (3) were 6.8 and 7.4 hr, respectively.

All data as percent excreted were transformed using an arc sine transformation to conform to the assumption of normal distribution and

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[2] FMC Corp., Newark, Del.
[3] Fisher Chemicals, Fair Lawn, N.J.
[4] Mallinckrodt Chemical Works, St. Louis, Mo.
[5] Roger Chemical Co., Irving, N.J.
[6] Model F, F. J. Stokes Machine Co., Philadelphia, Pa.
[7] Erweka Chemical and Pharmaceutical Industry Co., New York, N.Y.
[8] GT21 laboratory stirrer, Gerald K. Heller Co., Long Island, N.Y.
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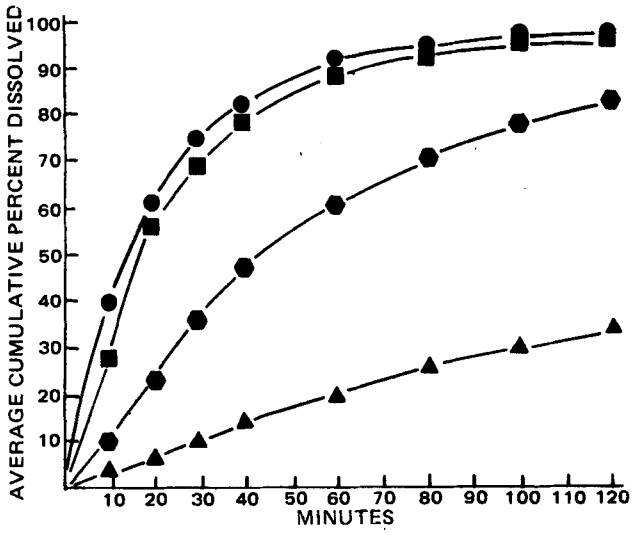

Figure 2—*Comparison of the dissolution profiles of hydrochlorothiazide formulations found using the USP basket method at 50 rpm. Each data point is the mean of five determinations. Key:* ■, *Formulation A;* ●, *Formulation B;* ●, *Formulation C; and* ▲, *Formulation D.*

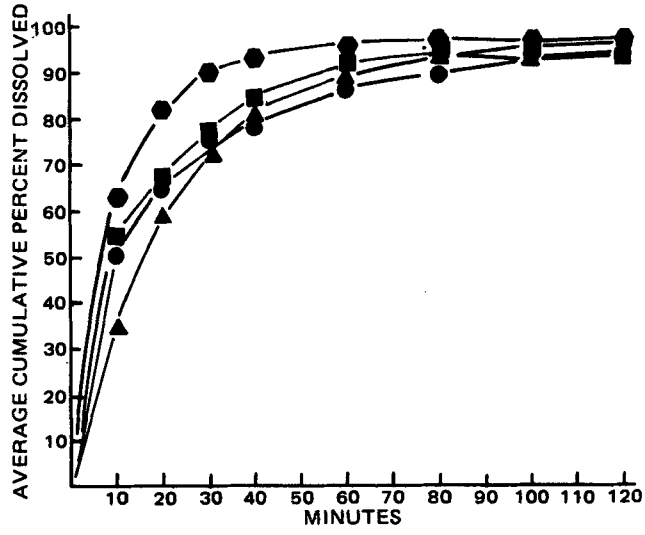

Figure 4—*Comparison of the dissolution profiles of hydrochlorothiazide formulations found using the USP basket method at 150 rpm. Each data point is the mean of five determinations. Key:* ■, *Formulation A;* ●, *Formulation B;* ●, *Formulation C; and* ▲, *Formulation D.*

equal variance (8). A two-way classification of an analysis of variance was used to compare the urinary hydrochlorothiazide excretion from the four tablet formulations and the powder at selected sampling times. The calculated F value of 4.72 was significantly higher than the tabulated $F_{0.95}$ value of 2.7, suggesting that there were significant differences ($p = 0.05$) among the formulations.

The calculated F value of 908.49 for the time factor was significantly higher than the tabulated $F_{0.95}$ value of 2.25, because cumulative data were used for this factor. Significant interactions between sampling times and formulations were found also. Thus, a one-way analysis of variance at each sampling time was used to determine the times at which significant differences between formulations existed. At all sample times studied except 1 hr, the calculated F exceeded the tabular $F_{0.95}$ value of 2.76 and confirmed that significant differences in excretion existed at each sample time. A Newman–Keuls multiple-range test (8) was performed to determine which formulations were significantly different. Only Formulation D was significantly different from all other formulations at 2, 3, 4, 5, 8, and 14 hr.

***In Vitro* Data**—Each formulation administered *in vivo* was dissolved using the USP basket method (4), the flask method (5), and the magnetic basket method (6) to determine any significant differences in drug release as a function of the dissolution method. In all cases, the percent of drug dissolved was transformed using an arc sine transformation to conform to the assumption of normal distribution and equal variance (8).

Comparison of the individual dissolution methods at agitation speeds of 50, 100, and 150 rpm showed that Formulation D dissolved slower at all agitation speeds. For each method, the gradual grouping of dissolution profiles was observed as the agitation speed was increased. This finding illustrates the difficulty in distinguishing the difference between formulations at higher agitation speeds. Examples of this behavior are shown in Figs. 2–4 for the USP method. The other two methods showed similar behavior.

Statistically significant differences ($p = 0.05$) between and within formulations were found using a repeated measures, two-way analysis of variance to compare hydrochlorothiazide formulations over time for the USP basket, flask, and magnetic basket methods at each agitation speed. Again, as with the *in vivo* analysis, the interactions between the sampling times and formulations were significant at all agitation speeds for each dissolution method. Therefore, a one-way analysis of variance was performed at each sampling time to determine if any significant differences between formulations could be detected. The calculated F value for the different formulations exceeded the tabular F value of 3.24 at the 0.95 level. To find which formulations were significantly different, a Newman–Keuls multiple-range test (8) was performed (Table I).

Correlation of *In Vitro* with *In Vivo* Results—Hydrochlorothiazide is a potential candidate for bioavailability problems because of its limited aqueous solubility. Previous studies (2, 3) showed very poor correlation between *in vivo* urinary excretion and the *in vitro* dissolution procedure for various formulations of this drug. It was postulated that comparison of the dissolution profiles from several methods using different agitation rates with urinary excretion data in which a significant difference in formulations had been found could help determine the correlation between these two parameters. Therefore, rank-order correlations were determined using cumulative excretion sample times of 1, 2, 3, 4, 5, 8, 11, 14, and 24 hr and dissolution times of 10, 20, 30, 40, 60, 80, 100, and 120 min for the USP basket, flask, and magnetic basket methods at 50-, 100-, and 150-rpm agitation speeds.

The sequential order correlation of the *in vivo* and *in vitro* results produced very few simple correlations between these two parameters. Most of these correlations were present between urinary excretion of 1 hr and only a few *in vitro* dissolution sample times. The magnetic basket method at 50 rpm failed to produce any rank-order correlation; the USP basket method at 150 rpm produced a greater number of correlations than any other combination of method and agitation speed.

To determine if a quantitative correlation existed between the transformed data of percent cumulative excretion and the cumulative percent of drug dissolved for each of the four formulations, a regression analysis was performed at each *in vivo* and *in vitro* sampling times. The F ratio was used to determine significance ($\alpha = 0.99$) (Table II). The correlation

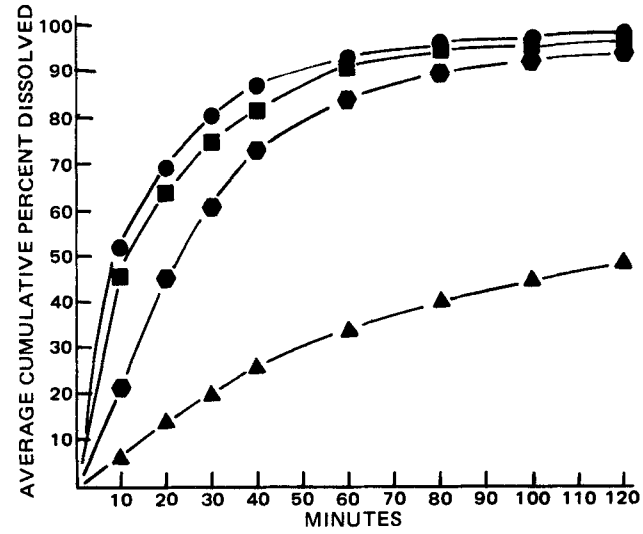

Figure 3—*Comparison of the dissolution profiles of hydrochlorothiazide formulations found using the USP basket method at 100 rpm. Each data point is the mean of five determinations. Key:* ■, *Formulation A;* ●, *Formulation B;* ●, *Formulation C; and* ▲, *Formulation D.*

Table I—Newman–Keuls Multiple-Range Test Comparing *In Vitro* Dissolution of Hydrochlorothiazide Formulations Using USP Basket, Flask, and Magnetic Basket Methods at Different Agitation Speeds and Sampling Times

Dissolution Method	Agitation Speed, rpm	Sampling Time, min 10	20	30	40	60	80	100	120
USP basket	50	CABD[a]	CABD	CABD	CABD	CABD	CABD	CABD	CABD
	100	CABD	ACBD	CABD	CABD	CABD	CABD	CABD	BADC
	150	BACD	BACD	BACD	BADC	BADC	BADC	BADC	ABCD
Flask	50	CABD	CABD	CABD	DBAD	CBAD	CBAD	BCAD	CBAD
	100	CABD	CABD	CBAD	CABD	ABCD	ACBD	ACBD	ACBD
	150	CABD	CBAD	CBAD	BCAD	ABCD	ABDC	ABCD	ACBD
Magnetic basket	50	ACBD	ACBD	ACBD	ACBD	ACBD	ACBD	ACBD	ACBD
	100	CABD	CABD	CABD	CABD	CABD	CABD	CABD	CABD
	150	CABD	CABD	CABD	CABD	CABD	CABD	CABD	CABD

[a] Products underlined by a common line indicate statistical similarity. Product rankings are from highest to lowest.

Table II—Correlation[a] of *In Vivo* Excretion and *In Vitro* Dissolution Found Using Different Dissolution Methods[b] for Four Hydrochlorothiazide Formulations

In Vivo Excretion Time, hr	50-rpm Agitation Speed 10 min	20 min	30 min	40 min	60 min	80 min	100 min	120 min	100-rpm Agitation Speed 10 min	20 min	30 min	40 min	60 min	80 min	100 min	120 min	150-rpm Agitation Speed 10 min	20 min	30 min	40 min	60 min	80 min	100 min	120 min
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	M	—	—	—	—	F	U F	M F	M U F	M U	M U	M U	—	—	—	—	M F	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	F	U	U	U	U	—	—	—	—	F	—	—	—
4	—	—	—	—	F	F	F	F	—	F	F	F	U	M U	U	U	U	—	—	M	M	—	—	—
5	—	—	—	—	—	—	F	F	—	F	—	F	—	—	—	—	U	—	—	—	—	—	—	—
8	—	—	F	F	F	F	F	F	—	F	F	F	U F	U	M U	U	U	U F	U F	U F	M	M	M	—
11	—	—	F	F	F	F	F	F	—	F	F	—	—	U	M U	U	U	U F	U F	U F	—	M	—	—
14	—	—	F	F	F	F	F	F	F	F	F	F	M U F	M U	M U	U	U	U F	U F	M F	M	M	M	—
24	—	—	F	F	F	F	F	F	—	F	F	M F	M U F	M U	M U	U	U F	U F	F	M F	M	M	—	M

[a] Correlation was determined using regression analysis and *F* test. [b] U, F, and M indicates significant correlation ($\alpha = 0.99$) found using USP basket, flask, and magnetic basket methods, respectively.

coefficients were computed for each *in vivo* and *in vitro* sampling time for four hydrochlorothiazide formulations for each dissolution procedure. The correlation coefficient between the excretion at the sample time of 14 hr and the *in vitro* dissolution at the sample time of 80 min for the flask method at 50 rpm was the highest correlation coefficient ($r = 0.748$). When the same apparatus was used with an agitation speed of 100 rpm, the highest correlation was obtained between the urinary excretion at the sample time of 14 hr and an *in vitro* sample time of 30 min ($r = 0.698$). For the same method with an agitation speed of 150 rpm, the highest correlation was present between the *in vivo* sample time of 14 hr and the *in vitro* sample time of 20 min ($r = 0.644$). All of these correlation coefficient values were statistically significant ($p = 0.01$).

With the magnetic basket method, the highest significant correlations ($p = 0.01$) were found between the *in vivo* sample time of 2 hr and the *in vitro* sample time of 60 min for each agitation speed ($r = 0.630$, 50 rpm; $r = 0.672$, 100 rpm; and $r = 0.604$, 150 rpm).

For the USP basket method, the highest correlation was found between the urinary excretion at the sample time of 2 hr and the *in vitro* sample time of 60 min at 50- and 100-rpm agitation speeds ($r = 0.656$, 100 rpm; and $r = 0.609$, 50 rpm). With an agitation speed of 150 rpm, the highest correlation was between the excretion data obtained at the sample time of 8 hr and the *in vitro* data obtained at the sample time of 10 min ($r = 0.649$). All of these correlation coefficient values were statistically significant ($p = 0.01$).

The urinary hydrochlorothiazide excretion at the sample time of 14 hr and the dissolution data at the sample time of 80 min using the flask method at 50 rpm produced the highest correlation when compared with all other *in vivo–in vitro* correlation coefficients. However, the use of these

Table III—Regression Analysis of *In Vitro* Dissolution and *In Vivo* Excretion for *In Vitro–In Vivo* Times Where Highest Significant Correlation Was Found

Method	Agitation Speed, rpm	*In Vivo* Time, hr	*In Vitro* Time, min	Slope	Intercept	Correlation Coefficient[a]
Flask	50	14	80	2.29	−1.90	0.748
Flask	100	14	30	2.07	−1.54	0.698
Flask	150	14	20	1.33	−0.21	0.644
Magnetic basket	50	2	60	1.88	−1.09	0.630
Magnetic basket	100	2	60	2.02	−0.48	0.672
Magnetic basket	150	2	60	2.13	−0.17	0.604
USP basket	50	2	60	2.36	−0.33	0.609
USP basket	100	2	60	2.18	−0.69	0.656
USP basket	150	8	10	1.18	−0.25	0.649

[a] Significant at 0.99 level.

Table IV—Prediction of Average Percent Hydrochlorothiazide Excretion at Sample Time Where Highest Significant Correlation Was Found

Method	Agitation Speed, rpm	*In Vivo* Time, hr	*In Vitro* Time, min	Mean *In Vitro* Dissolution[a], %	Mean *In Vivo* Prediction[a], %
Flask	50	14	80	77	60 ± 0.8
Flask	100	14	30	77	60 ± 0.9
Flask	150	14	20	77	60 ± 0.5
Magnetic basket	50	2	60	52	2 ± 0.6
Magnetic basket	100	2	60	64	11 ± 0.1
Magnetic basket	150	2	60	86	25 ± 0.1
USP basket	50	2	60	68	11 ± 1.0
USP basket	100	2	60	79	11 ± 0.4
USP basket	150	8	10	50	49 ± 0.3

[a] Values were converted to percent from arc sine transformation.

two parameters for prediction seems to be limited since the *in vitro* time was not found on the rate-limiting portion of the curve and the analysis of variance and Newman–Keuls multiple-range test for this particular *in vitro* time could not differentiate among Formulations A–D (Table I). Thus, selection of the best dissolution procedure probably should be based on something more than a correlation coefficient and should include considerations of the: (*a*) use of a correlation coefficient that is statistically significant, (*b*) use of an *in vitro* time that is on the rate-limiting portion of the dissolution–time curve, and (*c*) use of analysis of variance and Newman–Keuls multiple-range data that show significant differences between formulations within the experimental design.

Table III shows the slope, intercept, and *in vitro–in vivo* parameters where the highest correlation coefficients were obtained for each dissolution method. The regression equation that produced a significant correlation for each method can be used for urinary excretion prediction. The following equation was used:

$$UE = \frac{(\textit{in vitro}\text{ dissolution at time } T) - \text{intercept}}{\text{slope}} \quad \text{(Eq. 1)}$$

where UE is the prediction of *in vivo* urinary hydrochlorothiazide excretion at the sample time where the highest significant correlation was obtained. *In vitro* dissolution data as percent dissolved should be transformed using an arc sine transformation before insertion into Eq. 1.

Table IV shows the prediction of mean hydrochlorothiazide excretion along with confidence intervals. The confidence limits can be calculated using this formula (9):

$$\text{prediction of urinary hydrochlorothiazide excretion at time } T \pm t_{0.95} = (\text{residual mean square})\left(\frac{1}{n}\right) \quad \text{(Eq. 2)}$$

where n is the sample size used for regression and the residual mean square was calculated from analysis of variance for regression.

The percent of *in vitro* dissolution for each dissolution procedure that produced the highest significant correlation with urinary excretion is shown in Table IV. Comparison of these values with those from the previous dissolution profiles (Figs. 2–4) showed that all predicted values for percent of *in vitro* dissolution that were used for prediction of *in vivo* excretion lay on the plateau region of the curve, except the values obtained for the USP basket method and for the flask method at 150 rpm. These values fell within that portion of the profile that could be considered to be rate limited. Thus, it seems logical to select either the USP basket or the flask method at an agitation speed of 150 rpm for the prediction of urinary hydrochlorothiazide excretion.

Formulations A–C were statistically similar and significantly different from Formulation D when *in vivo* data at 8 hr and *in vitro* data at 10 min were compared after using the USP basket. However, the urinary hydrochlorothiazide excretion at 14 hr and *in vitro* dissolution at 20 min using the flask method at 150 rpm produced different results when Formulations A–D were compared (Table I).

This approach has good utility for establishing a standard for *in vitro* dissolution. Once correlation is established between *in vitro* and *in vivo* parameters and it is found that the regression equation can be utilized for predicting *in vivo* activity, interlot or intralot variations can be determined for hydrochlorothiazide formulations. In this case, the standard to produce an acceptable formulation should be that at least 50% hydrochlorothiazide is dissolved in 10 min using the USP basket method at the 150-rpm agitation speed. As shown in Fig. 4, Formulation D had only 35% dissolution at the 10-min sample time. Formulations A, B, and C had 54, 63, and 50% dissolution, respectively. Thus, Formulation D will have urinary excretion less than 49% at a sample time of 8 hr when administered orally. Moreover, a statistical comparison using a Newman–Keuls multiple-range test (8) for the USP basket method showed that Formulations A–C were statistically the same and that Formulation D was statistically different from the other three formulations.

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