Correlation of Aspirin Excretion with Parameters from Different Dissolution Methods

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Received July 5, 1977, from the *School of Pharmacy, University of Georgia, Athens, GA 30602.* Accepted for publication November 18, 1977. *Present address: University of Isfahan, Isfahan, Iran.

Abstract □ The cumulative urinary excretion of four different aspirin products (two tablets, a capsule, and a timed-release tablet) was determined in a crossover study using five subjects. Comparison of *in vivo* results showed a significant difference in cumulative urinary excretion levels at only 1 hr. The excretion from the two regular tablets was significantly different from the timed-release tablet, but the capsule showed no significant difference from the other three products. Each product was tested in the USP, Levy beaker, and the regular and large magnetic basket dissolution apparatus. Analysis of variance of the *in vitro* results showed a significant difference between the aspirin products and the dissolution methods at selected times. *In vitro* comparison with *in vivo* results for the four products showed that a regression analysis can be used to determine which dissolution methods produce a significant correlation with urinary excretion.

Keyphrases □ Aspirin—excretion in humans correlated with parameters from various *in vitro* dissolution methods □ Excretion—aspirin in humans, correlated with parameters from various *in vitro* dissolution methods □ Dissolution methods, various—*in vitro* parameters correlated with human excretion of aspirin □ Analgesics—aspirin, excretion in humans correlated with parameters from various *in vitro* dissolution methods

The evolution of *in vitro* dissolution testing has introduced a myriad of methods useful mainly for quality control. The acceptance of this testing technique can be measured by its use in quality control and the adoption of an official dissolution apparatus as well as dissolution standards for some USP XVIII (1) drugs. Controversy has marked the official USP test, with suggestions ranging from continued acceptance to replacement by other dissolution methods. The 1980 USP XX is expected to require dissolution standards for all official drugs. The Food and Drug Administration also has reconfirmed the importance of dissolution with the acceptance of this type of *in vitro* testing for demonstrating bioequivalence (2).

With these regulations, comparisons of the different methods for monitoring dissolution become more important. Use of different dissolution methods produced significantly different amounts of drug dissolved at specified times in a dissolution profile (3). The variance produced by these different dissolution methods was not significantly different and was of the same magnitude (3).

The availability of aspirin from different oral brands and formulations was studied (4–8), and bioavailability comparisons were made. Dissolution of various aspirin dosage forms was compared with their *in vivo* absorption or availability (4–7). However, a comparison of different dissolution methods for several types of oral aspirin formulations and their correlation with *in vivo* excretion should provide a means of determining if the selection of an official (and required) dissolution method is necessary or if several methods allow a measure of bioequivalence.

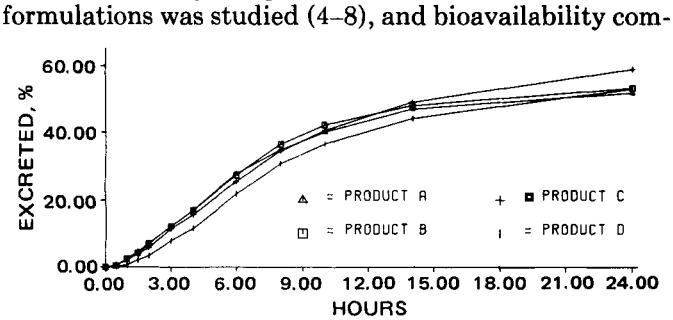

Figure 1—*Cumulative urinary excretion of aspirin from four products.*

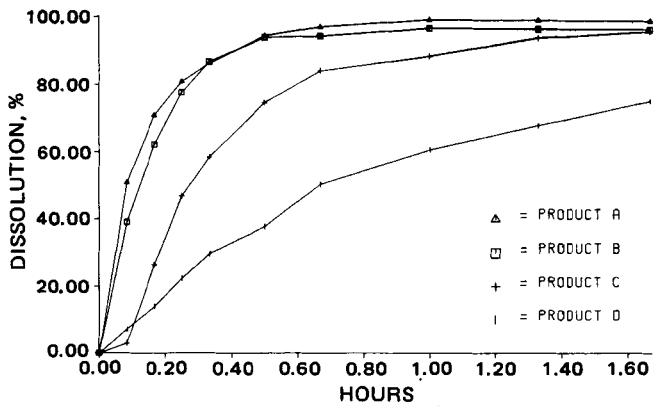

Figure 2—*Comparison of the dissolution profiles of four aspirin products using the USP method.*

EXPERIMENTAL

Aspirin Dosage Forms—Four different dosage forms of aspirin were purchased through local retail outlets. Products A[1] and B[2] were 325-mg tablets, Product C[3] was a 325-mg capsule, and Product D[4] was a 648-mg timed-release tablet.

***In Vitro* Studies**—Four dissolution apparatus were used including: the USP (U) method (1), the modified Levy (L) beaker method (9–11),

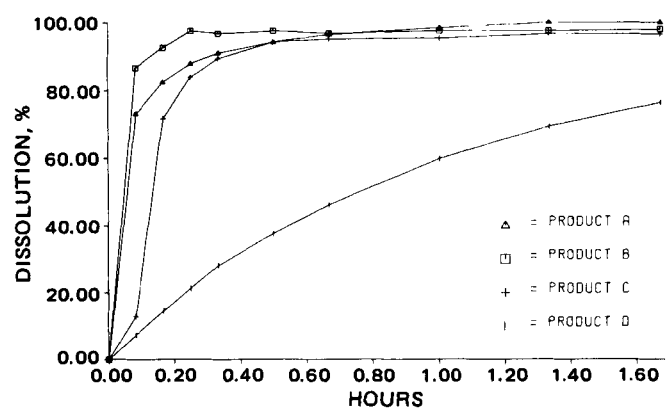

Figure 3—*Comparison of the dissolution profiles of four aspirin products using the modified Levy beaker method.*

[1] Lot 3F10G, Bayer Co., Division of Sterling Drug, New York, NY 10016.
[2] Product 10407-B1 (Lot 694997), Eckerd Drugs, Charlotte, N.C.
[3] Lot 7FF98A, Eli Lilly & Co., Indianapolis, IN 46206.
[4] Lot F3223, Bayer Co., Glenbrook Laboratories, Division of Sterling Drug, New York, NY 10016.

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Table I—Cumulative Percent Urinary Excretion of Four Brands of Aspirin as a Function of Time

Hours	Brands[a] A	B	C	D
0.5	0.53 (0.41)	0.43 (0.28)	0.71 (0.62)	0.22 (0.21)
1.0	2.42 (0.83)	2.45 (1.13)	2.08 (1.13)	0.63 (0.63)
1.5	4.62 (1.51)	4.25 (2.20)	4.02 (1.28)	2.11 (1.56)
2.0	7.20 (2.31)	7.06 (2.66)	5.88 (1.11)	3.44 (1.97)
3.0	12.13 (4.03)	12.24 (5.58)	11.40 (1.98)	7.84 (1.61)
4.0	17.08 (4.97)	16.98 (6.53)	15.74 (1.84)	11.56 (2.41)
6.0	27.71 (5.34)	27.37 (7.59)	25.49 (2.84)	21.84 (4.37)
8.0	34.81 (6.86)	36.52 (7.42)	34.46 (5.04)	30.58 (6.63)
10.0	39.99 (7.67)	41.14 (6.84)	40.53 (5.37)	36.48 (6.05)
14.0	46.93 (7.72)	48.07 (6.69)	49.10 (5.48)	44.12 (4.16)
24.0	51.82 (9.73)	53.26 (5.75)	58.96 (6.28)	55.76 (5.36)

[a] Average percent excreted for five subjects (*SD*).

the regular magnetic basket (R) method (12), and the large magnetic basket (B) method (13). A stirring rate of 75 rpm, a temperature of 37 ± 0.5°, and 600 ml of 0.1 *N* HCl were used. At appropriate times, 1-ml samples were withdrawn and an equal volume of medium was added to the beaker. Samples were analyzed using Trinder's reagent method (14). Each dissolution profile is the average of five separate capsules or tablets.

***In Vivo* Studies**—Five healthy male subjects[5], 21–32 years old and within 90–110% of their ideal body weight, had no history of GI, liver, or kidney disease. Each volunteer was instructed to abstain from all medication, alcohol, and beverages or foods that might interfere with the drug for 1 week before each administration.

Following an overnight fast, each subject was instructed to void his bladder and ingest 250 ml of water. In 1 hr, the 0-hr urine sample was taken, and 650 mg of aspirin was ingested with 250 ml of water. Cumulative urine samples were taken at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 14, and 24 hr and refrigerated immediately. Each subject was instructed to drink 250 ml of water after each urine collection for the first 2 hr. Subjects were allowed to eat after the 2-hr sample.

The four different dosage forms were given to each subject, using a random crossover design with 7 days between administrations. The urine samples were assayed using the described *in vitro* procedure with no variation in urine blanks noted. While the percent of drug excreted in the urine was less than some previous reports (4, 6) because of the inability of Trinder's reagent to react completely with some metabolites of salicylic acid (15), these differences did not affect the interbrand comparisons.

RESULTS AND DISCUSSION

Analysis of *In Vivo* Drug Excretion—Figure 1 and Table I illustrate the cumulative percent of drug excreted in the urine for each dosage form as a function of time. The cumulative percent of drug excreted was transformed using a log arc sine transformation to give homogeneity of variance and additivity of effects (16). These data were analyzed using a 4 (brands) × 5 (subjects) × 11 (sample times) repeated measures ANOV (17).

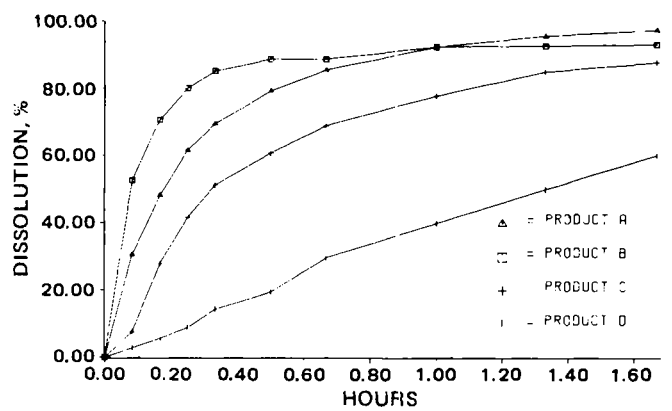

Figure 4—*Comparison of the dissolution profiles of four aspirin products using the regular magnetic basket method.*

[5] Informed consent was obtained from each subject.

Table II shows that the calculated *F* value for subjects of 0.42 and for brands of 2.76 was not significant ($p = 0.05$) across the elapsed time of the experiment. The time–brand interaction was statistically significant. A visual inspection of Fig. 1 shows crossover of the dosage form excretion profiles. Therefore, an analysis of the data at specific sample times was undertaken. These subsequent ANOV's produced a statistically significant *F* value for the different dosage forms administered at only 1 ($F = 5.19$) and 2 ($F = 3.98$) hr, with all other times showing no significant difference (18). This result indicates that absorption is dissolution rate controlled and agrees with the suggestion of Levy and Yacobi (5) that urine collections must be made at times of less than 3 hr to detect meaningful differences.

The Neuman–Keuls (19) analysis of the cumulative amount of drug excreted from each of the different brands further substantiates these conclusions. At 1 hr, a significant difference was found between the two regular tablets (Products A and B) and the timed-release tablet (Product D). The capsule (Product C) showed no statistically significant difference from either the regular tablets or the timed-release tablets. At 2 hr, the Neuman–Keuls analysis showed no significant difference between any of the four brands. This result could be expected since the calculated *F* value is barely larger than the tabular *F* value of 3.24.

Analysis of *In Vitro* Drug Dissolution—The dissolution profiles for the aspirin dosage forms are shown in Figs. 2–5 for each of the four dissolution methods using the same medium and agitation rate. The timed-release tablet dissolved at a slower rate in each apparatus, and the remaining three brands showed a close grouping of their profiles.

The percent of drug dissolved was transformed using a log arc sine transformation to give homogeneity of variance and additivity of effects (16). A 4 (brands) × 4 (methods) × 9 (sample times) with five replications repeated measures analysis of variance (ANOV) was applied to the data and yielded significant differences ($p = 0.05$) among brands, methods, and times (Table III). However, all interaction effects were also significant at this level.

Reducing the analysis to a two-way ANOV at specific sample times presented a better illustration of the results. At the sample times of 5, 40, 60, 80, and 100 min, the calculated *F* values for brands, methods, and the brand–method interaction were all significant ($p = 0.05$). This interaction factor was not significant at sample times of 10, 15, 20, and 30 min, while the calculated *F* values for brands and methods were significant. These results indicate a statistically significant difference between each of the single factors with no crossover between them.

Comparison of the individual dissolution methods at 10, 15, 20, and 30 min to determine which apparatus produced significantly different drug release, as well as comparison of the different dosage forms, was accomplished using the Neuman–Keuls test (19). Table IV demonstrated that for the sample times 10 through 30 min the rank order of drug re-

Table II—Repeated Measures Analysis of Variance for the *In Vivo* Data

Source	*df*	Mean Square	*F*	Probability *F* Exceeded
Subjects	4	0.0456	0.42	0.791
Brands	3	0.2987	2.76	0.088
Error (between)	12	0.1084	—	—
Time	10	5.7079	1351.36	0.000
Time–subjects	40	0.0058	1.38	0.094
Time–brands	30	0.0068	1.60	0.040
Error (within)	120	0.0042	—	—

Table III—Repeated Measures Analysis of Variance of the *In Vitro* Dissolution Data

Source	*df*	Mean Square	*F*	Probability *F* Exceeded
Brands	3	45.67	127.27	0.000
Methods	3	13.62	37.98	0.000
Brands–methods	9	1.43	3.97	0.000
Error	64	0.36	—	—
Time	8	22.49	1860.39	0.000
Time–brands	24	0.75	61.61	0.000
Time–methods	24	0.32	26.64	0.000
Time–brands–methods	72	0.07	5.81	0.000
Error	512	0.01	—	—

leased was B > A > C > D, with significant differences between dosage forms B–C, B–D, A–C, A–D, and C–D.

Investigation of the drug release as a function of the dissolution methods showed more variability through the same timespan. The Levy beaker method showed a significant difference from all of the other methods at the first three times. The Levy method was also different from the two magnetic baskets at 30 min. The USP method showed no statistical difference from the regular magnetic basket at 15, 20, and 30 min and a difference from the large magnetic basket at 20 and 30 min. The two magnetic basket methods were not significantly different in drug released at any of the four times analyzed.

Correlation of *In Vitro* with *In Vivo* Results—Previously (4–7), it was shown that aspirin absorption is dissolution rate controlled. Quantification of dissolution behavior as a means of testing aspirin release from various dosage forms would seem an excellent initial step in assuring drug availability. The ability of a particular dissolution method to show correlation with the urinary excretion of aspirin is an important means of qualifying that procedure for use in quality control testing and/or as an indication of drug bioavailability.

Rank-order correlation of the sequence of dissolution as it relates to the order of urinary excretion for different dosage forms of aspirin can be used to give a preliminary indication of *in vivo–in vitro* correlation.

Figure 1 shows that the order of drug excretion after administration of the different types of aspirin products varied as a function of time. During the first 8 hr, the highest amount of urinary excretion was found for either Product A or B followed by C and then D. At 10 hr after drug administration, the excretion from the highest to the lowest cumulative amount found in the urine was in the order of B > C > A > D. At 14 hr, the order was C > B > A > D; and at the last sampled time, the order was C > D > B > A.

The *in vitro* drug release can be seen in Figs. 2–5. For each of the four dissolution methods, the exact order of drug release from each of the four

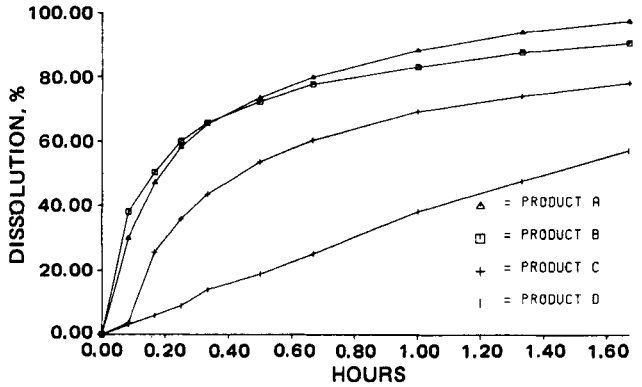

Figure 5—*Comparison of the dissolution profiles of four aspirin products using the large magnetic basket method.*

different dosage forms was somewhat different at the individual sampling times. However, the sequence of drug release varied in only two ways at the representative times of 10, 20, 30, and 40 min. In the order of the highest to the lowest rate of dissolution, B > A > C > D or A > B > C > D.

Table V shows a simple sequential order correlation of the *in vivo* and *in vitro* results. By using the urinary excretion sample times of 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 hr and the dissolution sample times of 10, 20, 30, and 40 min, many correlations were produced. Consideration of the effect of dissolution on urinary excretion does not seem to affect these correlations. In fact, if the number of simple correlations found for *in vivo* times less than 2 hr are compared to those for the same number of times greater than 2 hr, an equal number of correlations can be seen for each dissolution method. This result indicates that the importance of dissolution rate-controlled absorption is lost with this method of correlation.

To investigate a more quantitative measure of correlation between the transformed data of percent cumulative urinary excretion and the percent drug dissolved for each of the four dosage forms tested, a regression analysis was performed at each *in vivo–in vitro* time shown in Table V, and the *F*-ratio was used to determine significance. Table VI presents the results of the correlation using a linear regression analysis of the averaged transformed data and an *F*-test. The number of significant correlations found for the *in vivo* times of 1.0, 1.5, and 2.0 hr when absorption was dissolution rate controlled were definitely higher than the number found for the *in vivo* times greater than 2 hr for all dissolution methods except the large magnetic basket. The greatest number of correlations was found for the 1-hr urinary excretion and the representative dissolution times. The Levy method showed a significant correlation at all four

Table IV—Significant Differences[a] in *In Vitro* Drug Release Produced by the Products and the Dissolution Methods at Selected Times using a Neuman–Keuls Analysis

	Sample Times															
	10 min				15 min				20 min				30 min			
Products	B	A	C	D	B	A	C	D	B	A	C	D	B	A	C	D
B		—	*	*		—	*	*		—	*	*		—	*	*
A			•	*			*	*			*	*			*	*
C				*				*				*				*
Dissolution methods[b]	L	U	R	B	L	U	R	B	L	U	R	B	L	U	R	B
Levy		*	*	*		*	*	*		*	*	*		—	*	*
USP			—	—			—	*			*	*			*	*
Regular basket				—				—				—				—

[a] The symbol * indicates a significant difference between the brands or methods compared. [b] Four dissolution methods were used: Levy (L), USP (U), regular magnetic basket (R), and large magnetic basket (B).

Table V—Rank-Order Correlations (S) for *In Vivo* Excretion and *In Vitro* Dissolution for Four Brands of Aspirin

	In Vitro Methods															
	USP				Levy				Regular Basket				Large Basket			
In Vivo Time, hr	10 min	20 min	30 min	40 min	10 min	20 min	30 min	40 min	10 min	20 min	30 min	40 min	10 min	20 min	30 min	40 min
1.0	—	S	S	—	S	S	S	S	S	S	S	—	S	—	—	—
1.5	S	—	—	S	—	—	—	—	—	—	—	S	—	S	S	S
2.0	S	—	—	S	—	—	—	—	—	—	—	S	—	S	S	S
3.0	—	S	S	—	S	S	S	S	S	S	S	—	S	—	—	—
4.0	S	—	—	S	—	—	—	—	—	—	—	S	—	S	S	S
6.0	S	—	—	S	—	—	—	—	—	—	—	S	—	S	S	S

Table VI—Correlation [a] of *In Vivo* Excretion and *In Vitro* Dissolution for Four Brands of Aspirin Using Regression Analysis and the *F* Test

	In Vitro Methods															
	USP				Levy				Regular Basket				Large Basket			
In Vivo Time, hr	10 min	20 min	30 min	40 min	10 min	20 min	30 min	40 min	10 min	20 min	30 min	40 min	10 min	20 min	30 min	40 min
1.0	—	—	S	S	S	S	S	S	—	S	S	S	S	S	—	—
1.5	—	—	—	S	S	—	S	S	—	—	—	S	—	—	S	S
2.0	—	—	—	S	—	—	—	—	—	—	—	S	—	S	—	S
3.0	—	—	—	—	S	S	S	S	—	—	—	—	—	—	—	—
4.0	—	—	—	S	—	—	—	—	—	—	—	S	—	S	S	S
6.0	—	—	—	S	—	—	—	—	—	—	—	S	—	S	S	S

[a] S indicates significant correlation at $\alpha = 0.95$.

in vitro times, and the regular magnetic basket showed significant correlations at three of the four times.

In summary, significant differences in the cumulative urinary excretion of the four aspirin products could only be determined at the 1-hr excretion time, although the ANOV indicated a difference at the 2-hr sample time. Statistically significant differences also were found between these four products and between the four dissolution methods at selected times in the dissolution profiles. Attempts to use a simple sequential order correlation showed a random array of significant correlations between *in vitro* data and a number of excretion times that exceeded the limits of dissolution rate-controlled absorption. The regression analysis, on the other hand, showed the best correlation with *in vivo* 1-hr excretion times. This result indicates a need to analyze both *in vitro* and *in vivo* data statistically before selecting the parameters for data correlation. Adoption of this approach shows that, for aspirin, the Levy beaker and regular magnetic basket provide the best correlation with urinary excretion.

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Sensitive Electron-Capture GLC Determination of Metoclopramide in Biological Fluids

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Received October 3, 1977, from the *Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada.* Accepted for publication November 18, 1977.

Abstract □ A highly sensitive and specific electron-capture GLC assay capable of detecting picogram quantities of metoclopramide, a procaine derivative, in biological fluids was developed. This assay consisted of extracting metoclopramide from an alkalinized aqueous layer into benzene. A portion of the organic phase was derivatized with heptafluorobutyric anhydride. Quantitative estimation of the derivative was accomplished by adding diazepam, the internal standard, in benzene (750 ng/ml). A calibration curve was prepared for the plasma extracts. Linearity was observed in the range studied (91–825 ng/ml). No interference from endogenous substances was observed. The minimum detectable amount was 1 pg/injection. The structure of the derivative was confirmed by electron-impact and chemical-ionization mass spectrometry. The applicability of this method was shown by a preliminary study of the elimination kinetics of metoclopramide in rats after a 10-mg/kg iv dose.

Keyphrases □ Metoclopramide—electron-capture GLC analysis in biological fluids □ GLC, electron capture—analysis, metoclopramide in biological fluids □ Antiemetics—metoclopramide, electron-capture GLC analysis in biological fluids

Metoclopramide, 4-amino-5-chloro-2-methoxy-*N*-(2-diethylaminoethyl)benzamide, an antiemetic procaine derivative (1), is currently used in GI diagnostics (2, 3) and in the treatment of various GI disorders (4, 5). Metoclopramide increases the tone and peristalsis of the stomach and the duodenum, distends the duodenal bulb, and im-

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