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CORRELATION OF URINARY EXCRETION WITH IN VITRO DISSOLUTION USING FOUR DISSOLUTION METHODS FOR AMPICILLIN CAPSULES

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ABSTRACT

The in vitro release of ampicillin from 8 brands of ampicillin capsules, using four dissolution apparatus, was determined. These apparatus were the USP dissolution apparatus, the USP paddle stirrer apparatus, the USP disintegration apparatus and the spiral-stirrer apparatus. Significance of the differences in dissolution between brands and between methods were tested. Analysis of variance of the dissolution data showed statistically significant differences between brands and between methods at selected time. The paddle method showed superior discriminating capacity than the other methods. Correlation between the present in vitro data and the previously reported in vivo data, in order to find the apparatus capable to mimic in vivo release of ampicillin from capsules, was also determined.

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INTRODUCTION

Bioavailability evaluation of pharmaceutical dosage forms requires a large number of human volunteers and highly trained clinical investigators. However, *in vitro* testing, as a valid predictor of bioequivalence, can greatly reduce human subject risk and cost involved with *in vivo* testing (1). In recent years, considerable interest has been focused on the development of a reliable *in vitro* dissolution test method which can truely mimic the *in vivo* dissolution rate-controlled absorption of drugs administered in solid dosage forms. Attempts to simulate the extremely complex GIT by various *in vitro* means was not always fruitful in obtaining data which will correlate with *in vivo* results. Finding a universal dissolution rate test, although desirable, is largely impractical. Each drug and its dosage forms have to be studied individually, and usually after *in vivo* data are available, to determine satisfactory dissolution rate procedures. Suggestions were made that research in dissolution rate testing should be directed mainly toward establishing correlations of the *in vitro* data with *in vivo* data obtained in man (2). If such a correlation could be established with an individual drug, an *in vitro* dissolution test may evolve which can serve not only as a guide to formulation development, but also as a reliable predictor of drug absorption.

Capsules were considered as a dosage form with minimum risk of drug release failure. Recently, it was demonstra-

ted that capsules suffer from problems as do other solid oral dosage forms. Formulation of the capsule content (3-11) is the main controlling factor affecting capsule burst and deaggregation of the content. In addition, the gelatin shell may act as a significant obstacle for the content release when subjected to certain storage conditions (12,13) and/or when probable dye gelatin interaction occurs (14).

The majority of capsule dissolution rate studies were done on apparatus specific for tablets with slight modifications for capsules. Lin et al (15) carried out an intensive work on apparatus available to determine the dissolution rate of capsules. Seven dissolution methods for release of drugs from hard gelatin capsules were screened. However, further studies are still needed in order to find a dissolution apparatus specific for capsules.

The availability of in vivo excretion data (16) for eight brands of ampicillin capsules initiated and encouraged the assessement of four dissolution rate apparatus for the in vitro evaluation of this drug from these different brands. The selection of the most suitable and discriminating apparatus that can correlate with the in vivo data was another objective of the present work.

EXPERIMENTAL

Materials

Hydrochloric acid, citric acid, disodium hydrogen phosphate, and copper sulphate of analytical grade were

used. Pure anhydrous ampicillin BP[1] was also used. The same brands and batches of the eight ampicillin capsules previously tested *in vivo* were used (16).

Dissolution Rate Study

Four dissolution apparatus were used to determine the dissolution of the various ampicillin capsules, namely; the official U.S.P. rotating basket dissolution apparatus (17) (the basket method); the official U.S.P. paddle stirrer apparatus (17) (the paddle method); the official U.S.P. disintegration apparatus (17) (the disintegration method) and the spiral-stirrer apparatus (18) (the spiral method). In this method, the dissolution medium was stirred by a glass spiral paddle attatched to an overhead constant speed motor. The paddle made 30 up-down movements per minute accompanied by 2 horizontal rotations through an arc of 45 degree with every up-down movement.

Dissolution Procedure

All dissolution studies were carried out at 37±0.5°C in 800 ml of 0.1N HCl. At zero time, one capsule (500 mg) or two capsules (250 mg) were placed in the apparatus. On using the disintegration method, each capsule (250 mg) was placed in a separate tube of the basket-rack assembly. On using the paddle method or the spiral method, the capsule was allowed to sink to the bottom of the vessel before starting rotation. A small, loose piece of stain-

1. Courtesy of the Alexandria Co. for Pharmaceutical and Chemical Industries, Alexandria, Egypt.

less steel wire was attached to the capsule to prevent its floating. At various time intervals, samples (1 ml) were withdrawn using a glass pipette fitted with cotton wool. Fresh volumes of the dissolution medium at 37°C±0.5 were immediately added to compensate the sample withdrawn.

Method of Analysis

The amount of ampicillin in the dissolution samples was determined chemically, according to Angelucci and Baldiere (19). Three standard concentrations of ampicillin (20, 30 and 40 ug/ml) were assayed side by side with the samples to overcome the effect of any fluctuation in the condition of the assay. At least three determinations of each brand were performed for each dissolution method investigated in this study, and their results averaged. Neither the gelatin shell nor the other ingredients in the diluted sample aliquots were found to interfere with the spectrophotometric assay.

Results and Discussion

The dissolution rate patterns for the different ampicillin capsules using the four dissolution methods in 0.1N HCl are shown in Figs. 1-4. It is evident from the previous plots that a lag period does exist prior to the dissolution of the drug from its encapsulated form into the dissolution medium. The lag period is the time required for the dissolution of gelatin shell prior to the leaching or releasing of the drug from the capsule. In general, the capsule opens initially from both ends and

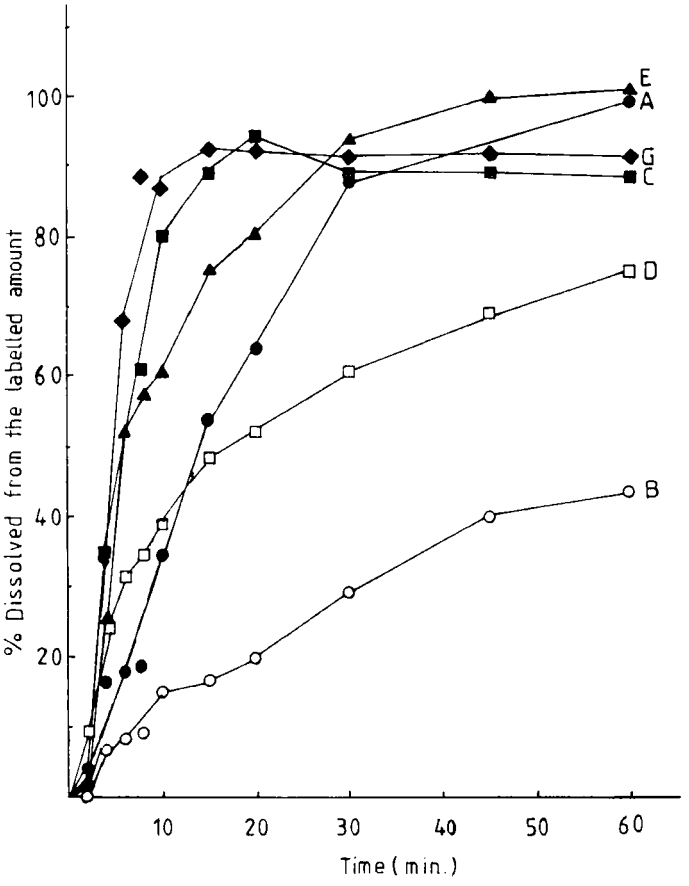

Fig. 1. Dissolution rate profiles of different brands of ampicillin capsules in O.1N HCl, at 37°C, using the rotating basket method (50 r.p.m).

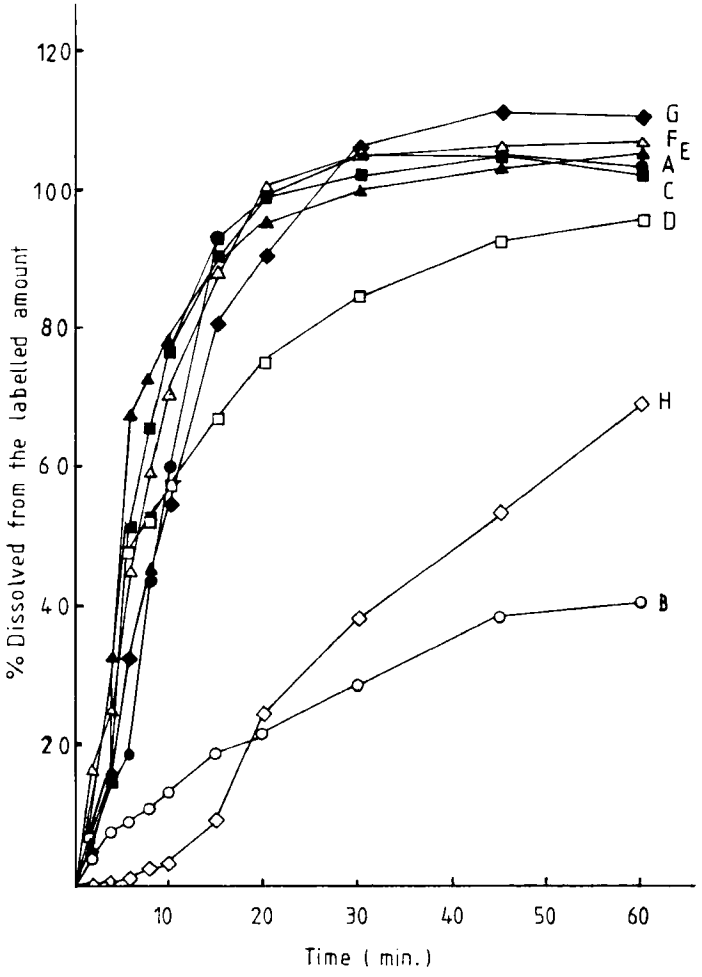

Fig. 2. Dissolution rate profiles of different brands of ampicillin capsules in 0.1N HCl, at 37°C, using the paddle method (25 r.p.m).

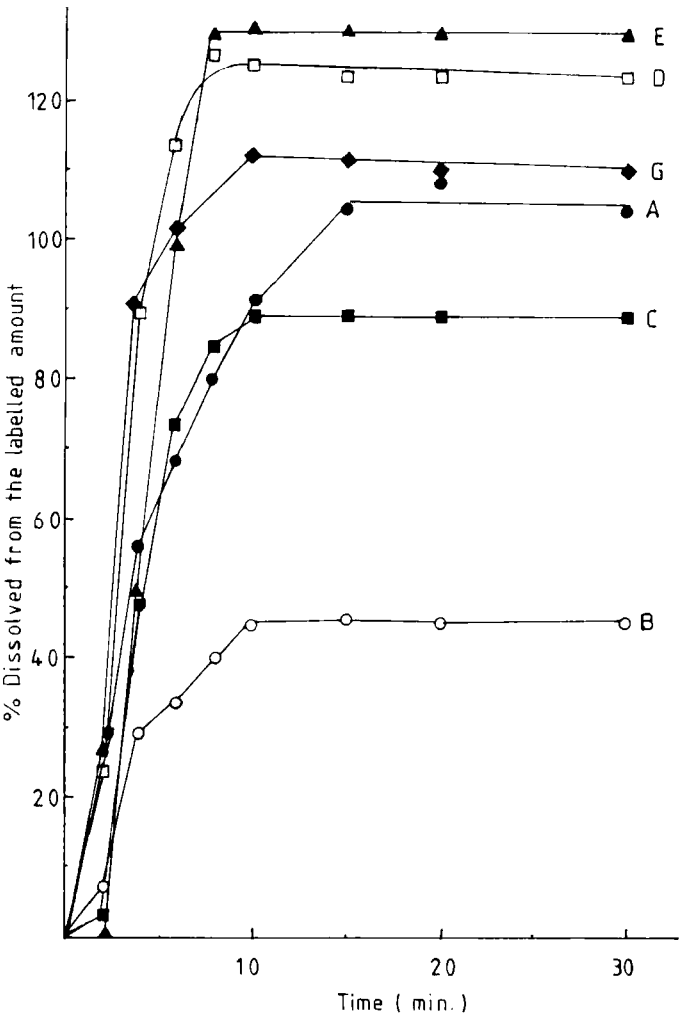

Fig. 3. Dissolution rate profiles of different brands of ampicillin capsules in 0.1N HCl, at 37°C, using the disintegration method (30 strokes/min).

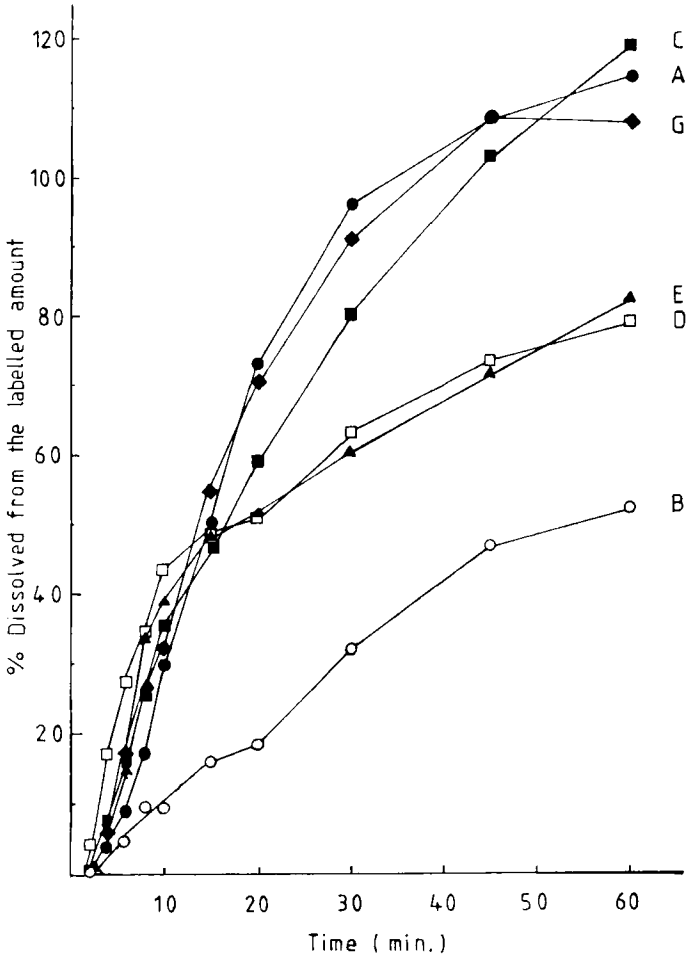

Fig. 4. Dissolution rate profiles of different brands of ampicillin capsules in 0.1N HCl, at 37°C, using the spiral method (30 strokes/min).

this is soon followed by the dissolution of gelatin from the middle portion of the capsule.

Comparison between Brands

The dissolution rates of the different brands of ampicillin capsules at some selected times are shown in

Table 1. The data represented in this table show that capsule B is the slowest dissolving brand on using any dissolution method. Brands A, C, E and G, on the ohter hand, exhibited high dissolution rate behaviours. In addition, the other brands showed intermediate dissolution rates. Table 2 shows the calculated values for $t_{50\%}$ and $t_{90\%}$ (times necessary for 50% and 90% dissolution) for the different brands of ampicillin capsules using the four dissolution methods. The average $t_{50\%}$ and $t_{90\%}$ for brand-B using the various apparatus, is more than one hour which again emphasizes the slow dissolution rate of this brand. Rapid release of ampicillin is also observed from brands A, C, E and G as indicated by their $t_{50\%}$ and $t_{90\%}$.

Although brands C, E and G showed relatively higher initial dissolution rate after 5 minutes(Table 1) compared to brand A, all these previous brands exhibited almost similar dissolution rate patterns with time. As capsule A was found to be the most biologically available brand as indicated by the *in vivo* data (16), it was selected for comparison with brand-B the slowest dissolving and the least biologically available brand. The dissolution profiles for both brands using the four dissolution methods are illustrated in Fig. 5. It is evident from this figure that after 5 minutes, in all the dissolution methods tested, there was about twofold increase in drug release from capsule A compared to B. However, after 20 minutes, the release of ampicillin from capsule A is about five-fold greater than

Table 1 - Percent drug dissolved* from various brands of ampicillin capsules using the different dissolution methods at selected times in 0.1N HCl at 37° .

Brand	Rotating basket			Paddle			Disintegration			Spiral		
	Time in minutes											
	5	15	30	5	15	30	5	15	30	5	15	30
A	19.0	54.4	88.0	17.0	93.0	>100	62.5	>100	>100	6.0	50.9	96.4
B	7.0	16.0	28.8	9.0	19.2	28.3	31.5	45.0	44.8	2.5	16.0	32.5
C	45.0	89.6	89.0	36.0	90.4	>100	62.5	88.0	88.0	13.5	46.6	80.5
D	27.0	48.0	60.8	43.0	67.1	84.1	100	>100	>100	23.0	49.5	63.8
E	40.0	75.2	93.6	50.0	87.5	99.9	76.0	>100	>100	11.5	48.6	60.3
F	-	-	-	33.0	87.1	>100	-	-	-	-	-	-
G	53.0	92.8	92.0	26.0	80.5	>100	97.5	>100	>100	13.5	54.4	91.6
H	-	-	-	1.0	9.6	38.7	-	-	-	-	-	-

* Each value is the average of three capsules.

Table 2 - Times (in min) for 50% and 90% dissolution of of the different brands of ampicillin capsules using the four dissolution methods in 0.1N HCl at 37° .

Brands	Rotating basket		Paddle		Disintegration		Spiral	
	$t_{50\%}$	$t_{90\%}$	$t_{50\%}$	$t_{90\%}$	$t_{50\%}$	$t_{90\%}$	$t_{50\%}$	$t_{90\%}$
A	13.0	34.5	8.5	14.5	3.8	10.0	15.0	27.0
B	>60	>60	>60	>60	>60	>60	51.0	>60
C	6.0	16.0	5.5	16.0	4.3	10.0	16.0	36.5
D	16.5	>60	6.0	48.0	2.8	4.0	15.0	>60
E	6.0	27.0	5.0	16.5	4.0	5.5	15.0	>60
F	-	-	6.5	16.0	-	-	-	-
G	4.5	11.5	8.5	20.0	2.5	5.0	14.0	30.0
H	-	-	40.5	>60	-	-	-	-

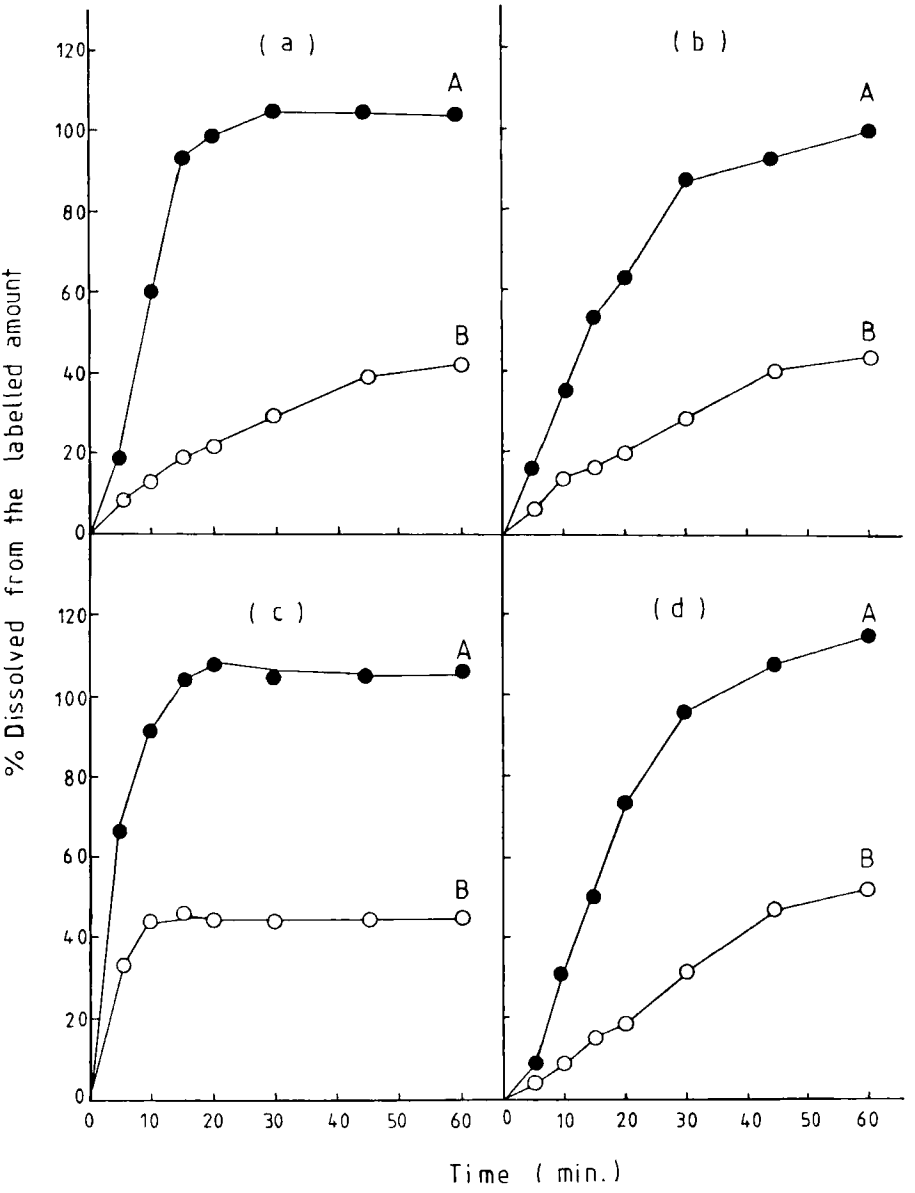

Fig. 5. Dissolution rate profiles of ampicillin capsules (O) brand A, (O) brand B using (a) paddle, (b) rotating basket, (c) disintegration and (d) spiral methods.

that of capsule B on using the paddle method(Fig. 5-a). This increase in drug release was only about three-fold on using both the rotating basket and the spiral methods(Fig. 5-b and d) and about two-fold on using the disintegration apparatus (Fig. 5-c) within this period of time. These results showed the better discriminating capacity of the paddle method than the other devices.

The observed reduction in drug release from capsule B may be attributed to several factors; variation in the hydration state of ampicillin; variation in the storage conditions; variation in the manufacturing procedures and/or variation in the type and concentration of the additives.

A possible explanation to the decreased dissolution of brand B may be due to the state of hydration of the drug. Ampicillin in brand A is in the anhydrous form and exhibited high dissolution rate, while that in brand B is in the trihydrate form and exhibited low dissolution rate. It has been reported (20,21) that suspension and capsule formulations containing anhydrous ampicillin exhibited superior bioavailability compared to formulations containing the trihydrate. The results of the present work are in good agreement with these findings but in contrast to others (22-24) who reported that capsules containing either form of ampicillin yield essentially identical bioavailability.

In the present work, excess improper additives in brand B compared to brand A may be the most possible explana-

tion for this observed reduction in drug release. The average weight of the powder content per capsule for both brands were determined experimentally. The content weights for capsule A (labelled amount 500 mg) and B (labelled amount 250 mg) were found to be 540 and 340 mg respectively. Since the sizes of capsule A and B were equal (zero size), compaction of capsule A content will be greater than that of capsule B content. It seems reasonable to expect that the release of ampicillin from capsule B (340 mg content), due to lower compaction, is greater than that from capsule A (540 mg content). However, contrary to what was expected, capsule B released ampicillin much more slower than capsule A. In addition, brand B yielded after rapid dissolution of the gelatin shell (about 2 min), a compact mass which remained till the end of the dissolution run indicating poor formulation. Improper storage conditions (e.g high relative humidity) may induce cementing of the powder content and consequent slug formation. In addition, manufacturing procedure (capsule filling pattern) may be responsible for slugging of the encapsulated powder in brand B. Therefore, the release of ampicillin from capsule B was slowed down due to the limited area of contact between the wet powder mass and the dissolution fluid. On the other hand, rapid deaggregation of capsule A content, after the dissolution of its gelatin shell, resulted in a large exposed surface of capsule A content and caused it to dissolve rapidly than B. It was also noticed that the rapid dissolution of capsule A correlates well with the rapid absorp-

tion and high biological availability observed with this brand (16). Also, the slow rate of dissolution of capsule B correlates well with the slow absorption and low biological availability of this brand (16).

Comparison between Methods

Comparison between the various dissolution methods was done to determine which apparatus is the most suitable for *in vitro* evaluation of ampicillin capsules. The dissolution profiles of each ampicillin brand using the four dissolution methods are shown in Fig. 6. Although the up and down movement was kept constant at 30 strokes per minutes on using both the disintegration apparatus and the spiral method, the release of the drug was more rapid on using the first method(Fig. 6). This finding was also reported by Lin and coworkers (15) who screened seven dissolution methods for release of drugs from hard gelatin capsules and found that the disintegration method provided the fastest rate and the greatest extent of dissolution. The increased dissolution rate observed on using this method could be attributed to the large diameter of the basket-rack assembly. Therefore, a stronger turbulent flow and a greater impacting force were exerted on the capsule by the disintegration method than that obtained by other devices. Therefore, this device has the disadvantage that mild differences in formulation characteristics may not be revealed (15). With the spiral method, the majority of the capsule contents was not well dispersed in the testing fluid at 30 strokes per minutes.

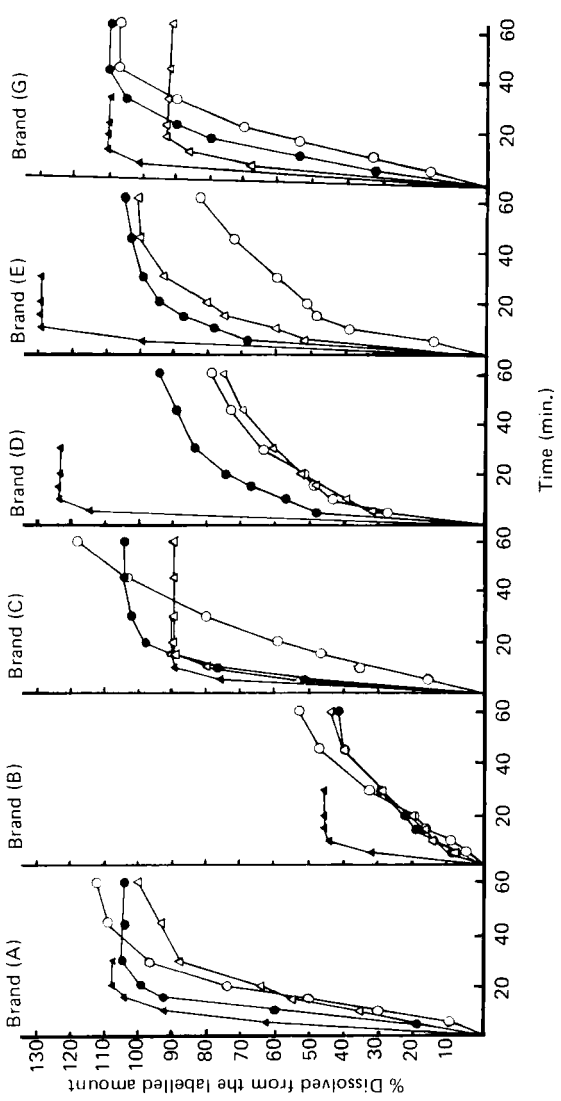

Fig. 6. Dissolution rate profiles for the different brands of ampicillin capsules, using the four dissolution apparatus. (▲) disintegration (●) paddle, (O)spiral, (△) rotating basket.

A fraction of the drug fell to the bottom of the dissolution vessel and consequently was not exposed to good agitation condition. Thus, the initial release of the drug was less rapid than the other methods(Fig. 6).

On using the paddle method, it was observed that the capsule content always comes to lie centrally under the stirrer, and in this way is subjected to a consistent effect of the stirring movements. In the rotating basket method, on the other hand, the particles pass through the mesh of the wire netting and settle on the base of the vessel and thus be outside the direct influence of the revolving basket. In addition, the wire mesh of the basket (40 mesh size) was easily fouled by the dissolving gelatin shell or other insoluble or gummy components, resulting in a nonuniform solvent flow arround the capsule inside the basket.

Table 3 shows the calculated ratios of the area under dissolution curves for the fastest and the slowest dissolving brands (capsules A and B respectively). It can be seen from this table that the paddle method gave the highest ratio (3.0) compared to the disintegration method which gave the lowest ratio (2.2). Ranking of the apparatus according to their discriminating capacity using this parameter was as follows:

Paddle > rotating basket > spiral > disintegration apparatus.

This finding agrees with those of Rothe and Schellhorn(25) concerning the usefulness of the paddle method.

TABLE 3

Ratio of the Area Under the Dissolution Curves for Capsule A and B on Using the Various Dissolution Methods.

Dissolution method	Area under the dissolution curve of capsules A/B
U.S.P disintegration	2.20
Spiral-stirrer	2.55
U.S.P rotating basket	2.56
U.S.P paddle-stirrer	3.00

Analysis of variance (26) is performed in order to determine if the methods themselves actually produce significantly different dissolution profiles or if the variations seen in the dissolution apparatus are of such magnitude as to produce overlapping curves that are essentially similar. Using the dissolution data (Table 1) in the form of percent drug released, from the six different brands of ampicillin capsules tested by each of the four dissolution methods, analysis of variance was performed at 5, 15 and 30 minutes. These times were selected to represent the entire dissolution profile. Table 4 shows that at all the times studied the calculated F value for the different dissolution methods exceeds the tabular F value at 5 percent level of confidence. It can be seen from this analysis that ampicillin capsules from the same brand and the same batch will release a significantly different amount of drug at various times when different dissolution apparatus are used.

TABLE 4

Analysis of Variance for Percent Drug Dissolved of the Various Ampicillin Brands at Selected Time.

Source of variance	% Diss. after 5 min			% Diss. after 15 min			% Diss. after 30 min		
	df[a]	M.S[b]	F[c]	df	M.S	F	df	M.S	F
Between methods	3	3831.5	27	3	1406.7	9.2	3	421.4	4.6
Between brands	5	814.5	5.7	5	1864.1	12.2	5	2259.3	24.7
Error	15	141.7	-	15	152.6	-	15	91.6	-

a. Degree of freedom

b. Mean of squares

c. F ratio

Table $F_{3,15} = 3.29$, $F_{5,15} = 2.9$ at $p = 0.05$

Correlation Between Dissolution Rate and Bioavailability

Rank order correlation of the sequence of dissolution data as it relates to the order of urinary excretion for the different brands of ampicillin capsules can be used to give a preliminary indication of in vivo-in-vitro correlation. The cumulative mg excreted during 8 hr after oral administration of 6 brands of ampicillin capsules (16) and the percent drug dissolved after 5, 15 and 30 minutes, for each of the four dissolution methods were ranked according to their increasing values. Brand B showed the lowest order on using both in-vivo and in-vitro parameters. However, it is difficult to conclude from these data which brand exhibits the highest order.

Brands A, B, C, D, E and G of ampicillin capsules were selected for the correlation study since they were evaluated in-vivo and in-vitro using the four dissolution apparatus. As brands F and H were studied in-vitro by means of the paddle apparatus only, they were included in correlation with in-vivo data using this apparatus.

Table 5 summarizes the rank order correlation coefficients for the in-vivo excretion data and the in-vitro dissolution data for six brands of ampicillin capsules as determined by applying the method of rank order correlation (27). Significant rank order correlation was observed between the cumulative mg excreted after 2 hr and the percent dissolved after 15 minutes using the paddle method (Table 5). In addition, significant rank order correla-

Table 5 - Rank order correlation coefficients for *in vivo* excretion and *in vitro* dissolution for six brands of ampicillin capsules at selected time.

In vivo time, hr	In vitro dissolution method											
	Rotating basket			Paddle			Disintegration			Spiral		
	5 min	15 min	30 min	5 min	15 min	30 min	5 min	15 min	30 min	5 min	15 min	30 min
1	0.086	0.143	0.486	0.486	0.657	0.314	0.186	0.086	0.086	0.071	0.371	0.029
2	0.029	0.200	0.571	0.371	0.886(S)*	0.543	0.043	0.571	0.571	0.271	0.257	0.486
3	-0.086	0.029	0.200	0.429	0.714	0.371	0.271	0.714	0.714	0.071	0.371	0.429
4	-0.143	0.257	0.086	0.371	0.600	0.314	0.357	0.714	0.714	0.243	0.429	0.486
6	-0.143	0.257	0.086	0.371	0.600	0.314	0.357	0.714	0.714	0.243	0.429	0.486
8	-0.086	-0.114	-0.029	0.257	0.657	0.429	0.271	0.571	0.571	0.214	0.371	0.600

* S= Significant rank order correlation

Tabulated value for rank order correlation coefficient (n= 6)= 0.829 at p= 0.05

TABLE 6

Correlation of In Vivo Excretion Data and In Vitro Dissolution Data for Eight Brands of Ampicillin Capsules Using the Paddle Method.

In Vivo data	In Vitro data	Rank order correlation coefficient r`	Regression analysis coefficient r
C.M.E[a](1hr)	% dissolved after 5 min	0.714 (S)[c]	0.624 (N.S)[d]
C.M.E (1hr)	% dissolved after 15 min	0.708 (S)	0.610 (N.S)
C.M.E (2hr)	% dissolved after 15 min	0.887 (S)	0.740 (S)
P.E.T[b]	$t_{50\%}$	0.571 (N.S)	-
P.E.T	$t_{90\%}$	0.685 (S)	0.870 (S)
P.E.T	% dissolved after 5 min	0.530 (N.S)	-
P.E.T	% dissolved after 15 min	0.440 (N.S)	-
Urinary Conc. after 2 hr	% dissolved after 15 min	0.448 (N.S)	-
Maximum peak value	% dissolved after 15 min	0.390 (N.S)	-

a. Cumulative mg excreted.

b. Peak excretion time.

c. Significant at p= 0.05

d. Insignificant at p= 0.05

Tabular r` (n= 8) = 0.643 at p= 0.05

Tabular r (n= 8) = 0.700 at p= 0.05

tions were obtained, for the eight brands, between early excretion data (cumulative mg excreted after 1 or 2 hr) and initial dissolution rates (percent dissolved after 5 or 15 minutes) using the paddle method (Table 6).

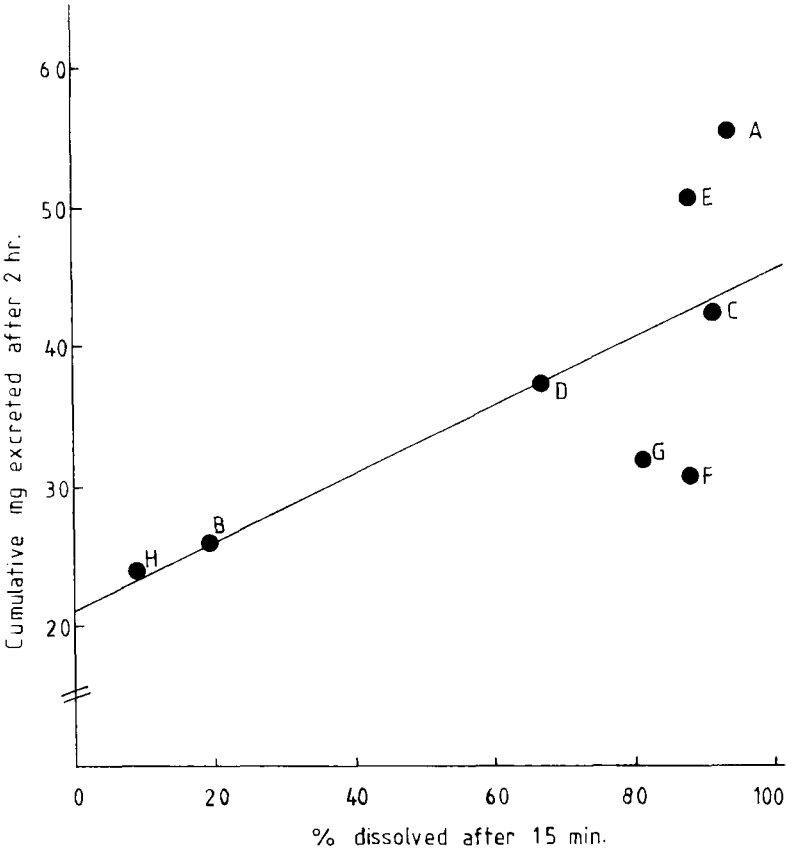

Fig. 7. Correlation between the average cumulative mg ampicillin excreted after 2 hr, following the oral administration of 8 brands of ampicillin capsules to six subjects, and the percent ampicillin dissolved after 15 minutes using the paddle method.

Using regression analysis (28), a significant quantitative correlation was observed ($p = 0.05$) between cumulative mg excreted of ampicillin after 2 hr and percent dissolved after 15 minutes using the paddle method (Table 6 and Fig. 7). However, regression analysis showed no significant correlations between cumulative mg excreted

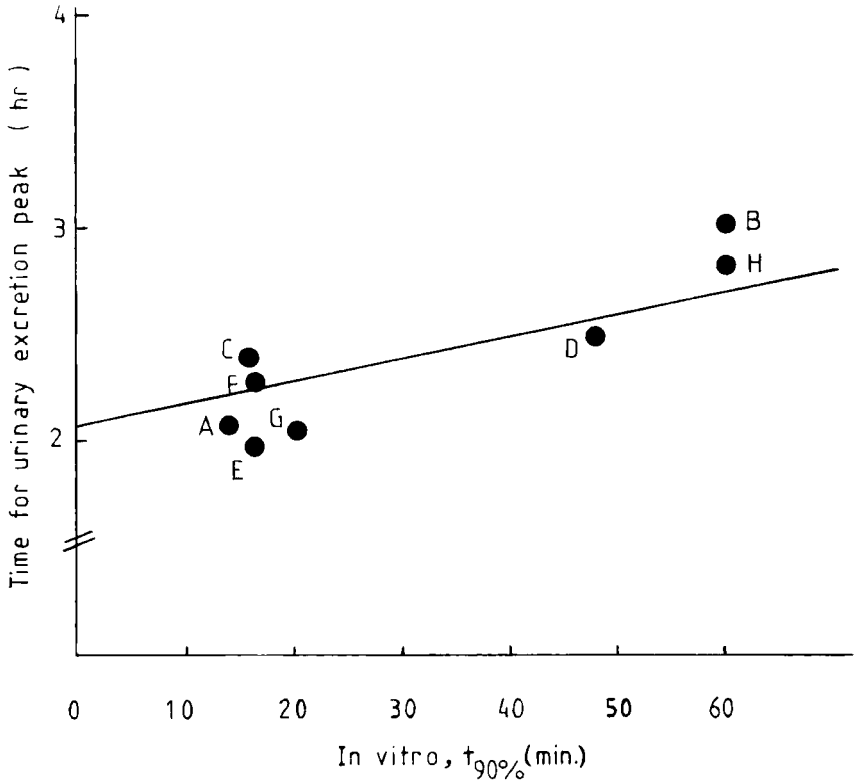

Fig. 8. Correlation between the average time necessary to reach the urinary excretion peak, following the oral administration of 8 brands of ampicillin capsules to six subjects, and the in vitro $t_{90\%}$ using the paddle method.

after 1 hr and percent dissolved after 5 or 15 minutes (Table 6). Furthermore, significant rank order correlation was obtained between the time of urinary excretion peaking and the time needed for 90% dissolution ($t_{90\%}$) (Table 6). Regression analysis also showed significant quantitative correlation between these two parameters (Fig. 8).

From the preceding results, it can be observed that data obtained from the urinary excretion of ampicillin capsules gave, in most cases, poor correlations with the *in vitro* data. However, on using the paddle method, significant correlations were obtained between the cumulative amount excreted after 2 hours and the percent drug dissolved after 15 minutes, as well as between the *in vivo* time of peaking and the time necessary for 90% dissolution ($t_{90\%}$) (Table 6). The usefulness of the paddle method, over the other three dissolution methods, in its capacity to correlate with *in vivo* data, was obvious in the present study. However, these findings were not in agreement with those of Gröning (29) who found no correlation between *in vitro* and *in vivo* results of nitrofurantoin tablets and capsules using the paddle method. Rothe and Schellhorn (25), on the other hand, demonstrated that the paddle method provides good *in vitro*-*in vivo* correlation for various active substances and formulations. In addition, dissolution profiles of prednisone and prednisolone tablets (30) using both the paddle apparatus and the spin filter apparatus were also successfully correlated with *in vivo* data. These findings support the selection of the paddle method, as an *in vitro* bioequivalence standard, by the official compendia.

CONCLUSIONS

From the preceding results and discussion, it can be concluded that, *in vitro* evaluation of the different brands

of ampicillin capsules using the four previously described dissolution apparatus showed significant differences between brands and between apparatus. Brand A exhibited high dissolution behaviour, while brand B showed the lowest dissolution characteristics on using any of the four dissolution apparatus. These findings are in agreement with the results of the bioavailability study (16) concerning these two brands.

On comparing the different dissolution methods, the paddle method showed the highest discriminating capacity while the U.S.P disintegration apparatus showed the lowest discrimination. However, we can not say that this in vitro method can replace the necessity of the biological evaluation of new formulation of this dosage form. The paddle method could successfully be used in routine batch-to batch quality control analysis for testing ampicillin capsules. Generally, dissolution testing is considered as a valuable adjunct to good formulation development as well as an excellent tool capable to monitor the consistency of drug delivery from a product. However, the development of meaningful and universal dissolution apparatus is still complicated and difficult to achieve. This desirable goal, stimulates scientists for further research to find the ideal predictive dissolution apparatus.

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