AN EVALUATION OF SOME COMMERCIAL BRANDS OF AMPICILIN CAPSULES AVAILABLE IN NIGERIA

By Eseozobo, E. & Aloba, D. Dept. of Pharmaceutics and Pharm. Technology School of Pharmacy, University of Benin Benin.

SUMMARY

Ten commercial brands of ampicillin capsules available locally were subjected to weight variation, uniformity of content, disintergration time and dissolution rate tests. The methods used were those recommended in the United States Pharmacopoiea (U.S.P) and the British Phrmacopoiea (B.P) with slight modifications in some tests.

Considerable variations in these parameters were observed from brand to brand. Some brands failed some of the tests and passed others. A particular, brand was found to have an average ampicillin content of only 73.6% while, the 90% dissolution of the active drug from another brand was not achieved even after the end of an hour. However, a few of the brands passed in all the tests.

Statistically significant correlation was found between weight variation and content uniformity results. Also, a good rank order correlation between disintegration time in acid medium and time for 50% dissolution (i.e. T50%) was obtained.

No significant difference was observed between the dissolution rates of capsules containing the anhydrous ampicillin and those containing ampicillin tri-hydrate.

The results emphasizes the need for a stringent quality control on ampicillin capusules being imported into the country before they are made available to the Nigerian market.

INTRODUCTION

Capsules are among the most common oral unit dosage forms currently in use, being next only to tablets. Although their formulation requirements are minimal when compared to tablets, various studies have indicated that the ultimate properties of capsules are profoundly influenced by the ingredients and procedure employed in the formulations and the manufacturing process (1, 2, 3, 4, 5). Such formulation effects are manifested in-vivo as differences in physiological availabilities and therapeutic efficacies of generic brands of a given drug and this has been aptly demonstrated by various workers. In a review of these studies, Florence (6) pointed out that the use of in-vitro dissolution rate and disintegration time studies to predict such in-vivo behaviour of solid dosage foms is restricted by the frequent lack of correlation between in-vitro and in-vivo results. Nevertheless, an indication of such in-vivo behaviour can be obtained from in-vitro tets(7). In addition, they are less expensive and more easily performed than the in-vivo tests hence, they are employed for the routine quality control of capsule formulations, in conjunction with other tests, such as weight variation and content uniformity.

Ampicillin is an antibiotic powder used for the general treatment of microbial infections. Although, it is available as powder (which requires reconstitution with water into a suspension before administration) and as injections, the main form in which it is presented is as capsules. Its potential for formulation problems is as great as that for any other drug in capsule form, and this, coupled with its popularity as shown by various studies (8,9) have prompted some drug regulatory bodies to request that dissolution rate tests be performed as a routine quality control procedure on ampicillin capsules (10). In addition, various studies have produced conflicting results as to the differences in dissolution rates between commercial ampicillin capsules containing the anhydrous ampicillin and those containing the less soluble trihydrate.

Most drugs consumed in Nigeria today, including ampicillin capsules, are imported and the few studies carried out on some of these have indicated that many are of low standard in terms of quality and efficacy (11, 12). However, none of these studies have been carried out on ampicillin capsules. Besides, we have observed that of the numerous genric brands of ampicillin capsules available, any brand is dispensed in our hospital pharmacies for such reason as costs and shortage of drugs without any knowledge of the quality of these brands. It is for these reasons that the present study was undertaken to investigate the variations in average weight, uniformity of content, disintegration time and most importantly, dissolution rates among ten commercial brands of ampicillin capsules. An attempt was also made to show the differences, if any, between capsules containing anhydrous ampicillin and those containing the trihydrate form. The effects of temperature on the dissolution rate of some of the brands were also studied.

MATERIALS AND METHODS

Materials:

Ten different brands of ampicillin capsules, each labelled as containing the equivalent of 250mg. of ampicillin were purchased from local retail pharmacy shops. Their relevant informations and designations are shown in Table I.

Experimental Methods:

Weight Variation – The U.S.P. (13) method was used. Twenty capsules from each brand were weighed individually and the mean weight was determined. The standard deviation (6), and coefficient of variation (C.V.) of the weight were calculated.

TABLE 1

List of Commercial brands of Ampicillin capsules used in this study

Code	Form of Ampicillin	Country of Origin	Batch Number	Expiry Date
A	Trihydrate	India	ROC/-33-77	8/79
B	Trihydrate	Italy	1020	2/80
C	Trihydrate	W.Germany	40797/72315	6/81
D	Anhydrous	Yogoslavia	772445	4/80
E	Trihydrate	Italy	1264/1578/A	4/79
F	Trihydrate	W.Germany	196	9/80
G	Anhydrous	Italy	237	2/80
H	Anhydrous	Nigeria	22777	7/80
I	Anhydrous	U.K.	9972	2/80
J	Anhydrous	U.K.	1586	2/80

Note: All the capsules were inside their expiry date when this study was undertaken.

TABLE 2(a)

Results of the weight variations for the different brands of ampicillin capsules

Brand Code	Mean Weight (mg)	Weight Range as % of of mean weight	Standard deviation (mg)	Coefficient of variation (%)	Compliance with U.S.P.
A	410.3	93.55-102.85	10.73	2.62	Passed
B	394.2	84.68-109.66	25.59	6.49	Failed
C	381.1	96.12-105-04	8.99	2.36	Passed
D	392.9	62.71-109.14	37.61	9.57	Failed
E	377.7	90.23-107.55	17.82	4.72	Passed
F	447.2	96.13-102.01	7.22	1.62	Passed
G	367.1	97.98-102.04	4.59	1.25	Passed
H	359.7	91.58-103.64	10.48	2.91	Passed
I	375.6	91.69-107.51	17.44	4.64	Passed
J	371.9	96.86-102.75	5.50	1.48	Passed

TABLE 2(b)

Results of further weight variation tests on brands B and D

Brand	B	D
Mean weight of Capsule content (mg)	317.4	309.0
Range as % of mean net content	81.41-110.02	53.14-112.07
No. of capsules beyond the limits 90% – 110%	2	3
No. of Capsules beyond the limits 75% – 125%		1
Compliance with U.S.P. specification	Passed	Failed
Capsule Shell Weights		
Standard deviation (mg)	1.93	1.92
Coefficient of variation (%)	2.45	2.43

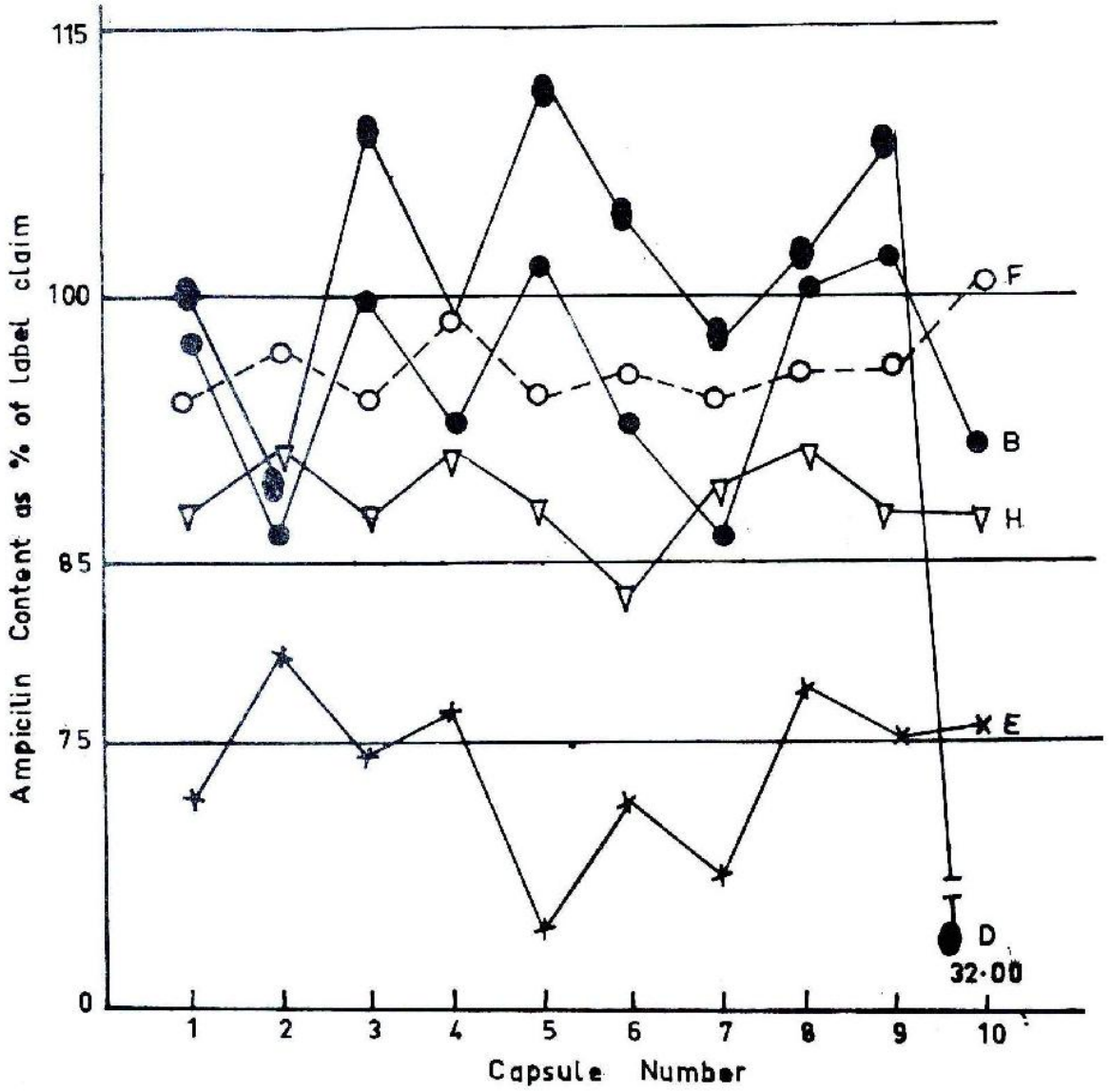


FIG. 1A. Variation in Uniformity of Contents of ampicilin per Capsule, Brands F, B, H, E, D

When the weight of any of the capsules fell outside the limits, 90-110% of the mean weight, each capsule was then emptied, the weight of its contents obtained and the mean calculated.

Content Uniformity:

Ten capsules were randomly selected from each batch and the contents of each capsule was determined by the B.P. (14) assay method for ampicillin capsules at an absorbance of 322 nm with a Unican SP 500 series 2 Spectrophotometer, and by referring to a calibration graph obtained from a solution of ampicillin trihydrate powder (Beecham Research International–Apapa).

Disintegration Time:

B.P. Disintegration method – The test was carried out in a Manesty Disintegration Tester using the B.P. method. Distilled water was used as the disintegrating fluid and the temperature was maintained at 37± 0.5°C. Five capsules were placed in each basket, the determination was done in triplicate and averaged.

The test was then repeated by placing only one capsule in each basket and noting the longest time for one of the capsules to completely disintegrate

Modified U.S.P. Disintegration Methods:

The procedure was the same as above but only one capsule was placed in each basket and the disintegration time was taken as the mean of ten runs.

Distintegration in Simulated Gastric Fluid:

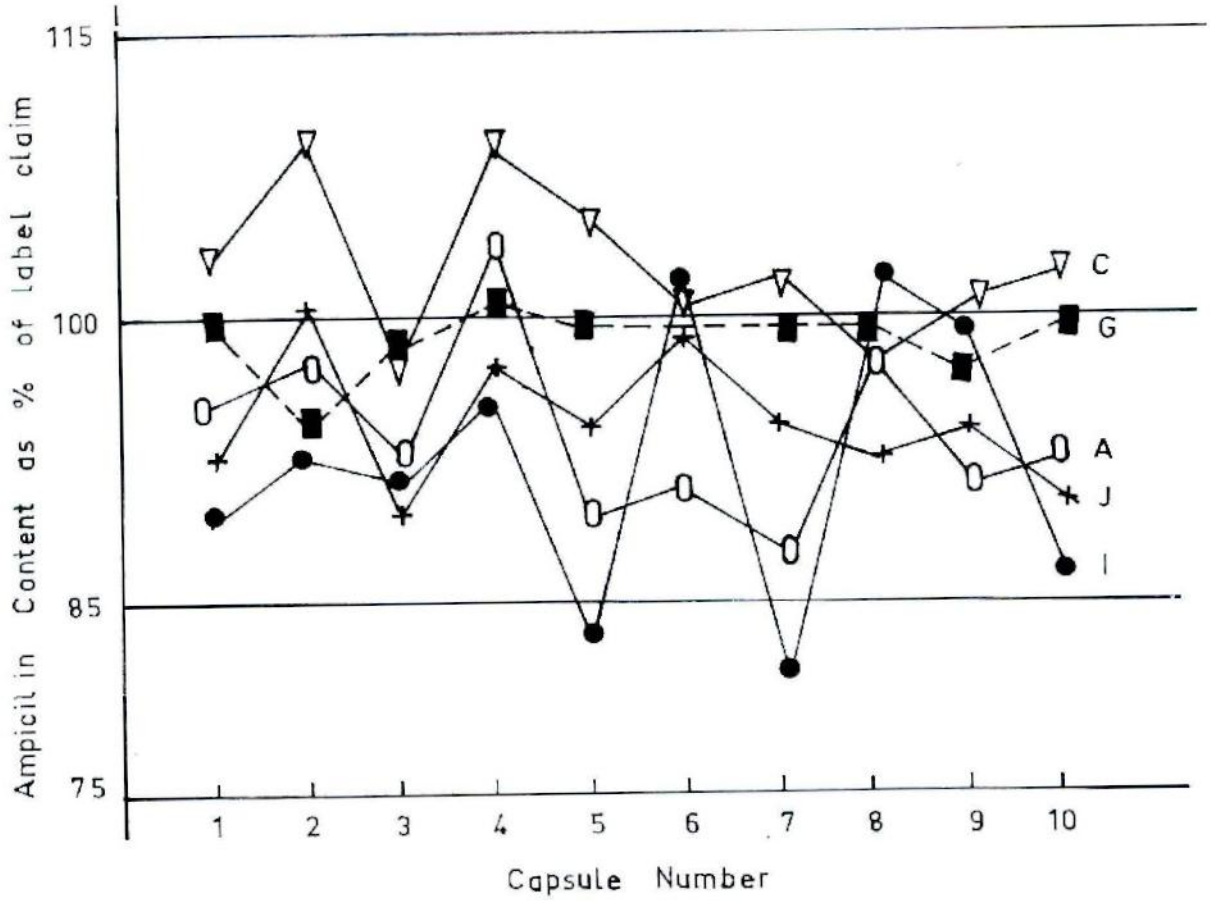
The test was repeated for individual capsules using instead of water, simulated gastric fluid T.S. (without pepsin) U.S.P. The mean was calculated from six runs.

Dissolution Rate:

A modified form of the U.S.P. dissolution rate apparatus was used. It consisted of a 1 litre beaker containing 900ml. of simulated gastric fluid T.S. (without pepsin) U.S.P., a doubly-wound basket made from a No. 16 mesh size wire gauze, attached to a rod connected to a variable speed motor and rotated at 120 r.p.m. The beaker was immersed in a water bath maintained at 37 + 0.5oC (unless otherwise stated).

1ml. samples were withdrawn at different time intervals and rapidly filtered. The concentration of ampicillin that had dissolved in the medium during the period concerned was determined in the same way as for the content uniformity test. The test was performed in triplicate for each brand except in the case of brand D, where five individual capsules weretested to illustrate the intra-batch variation.

FIG. iB. Variation in Uniformity of Content of Ampicil in per Capsule A, J, I, G, C.



The effect of temperature was determined by repeating the test on the slowest dissolving brand over a temperature range of 31 ^{o}C to 44 ^{o}C.

RESULTS AND DISCUSSION

The U.S.P. specification on weight variation requires that in the initial tests, none of the twenty capsules should deviate in weight from the mean weight by more than 10%. This requirement was fulfilled by all the brands except B and D as shown in Table 2(a). In order to ascertain whether the observed deviation for these two brands was due to variation in capsule

shell weight or not, a further test was performed. The results, Table 2(b) show that the variation in both cases was not due to variation in shell weight, since the shell weights had standard deviations of 1.93mg. and 1.92mg. and coefficients of variation of 2.43% and 2.45% respectively. The U.S.P. specification which states that the weights of the contents should not be more than 10% in any two capsules and in no capsule by more than 25%, was met by brand B but, not by brand D. On opening the brand D capsules. it was observed that there was considerable variation in depth in fill. This seems to imply that the U.S.P. test, may in fact have failed the B.P. test if it had been applied to them.

Applying the standards reported by Pietra and Setnikar (16) for tablets in which a coefficient of variation not exceeding 2% indicates excellent uniformity of weight, it can be seen from Table 2(a) that brands F, G and J which showed very little variation are excellent while, brands A, C and H are only just good. On the contrary, brands I, E, B and D exhibited marked variation (the variation increasing in that order) and this is reflected in the fact that brands B and D failed the initial weight variation tests.

The results on the content unformity, Figs. 1(a) and (b), show marked variations similar to that of the weight variation test.

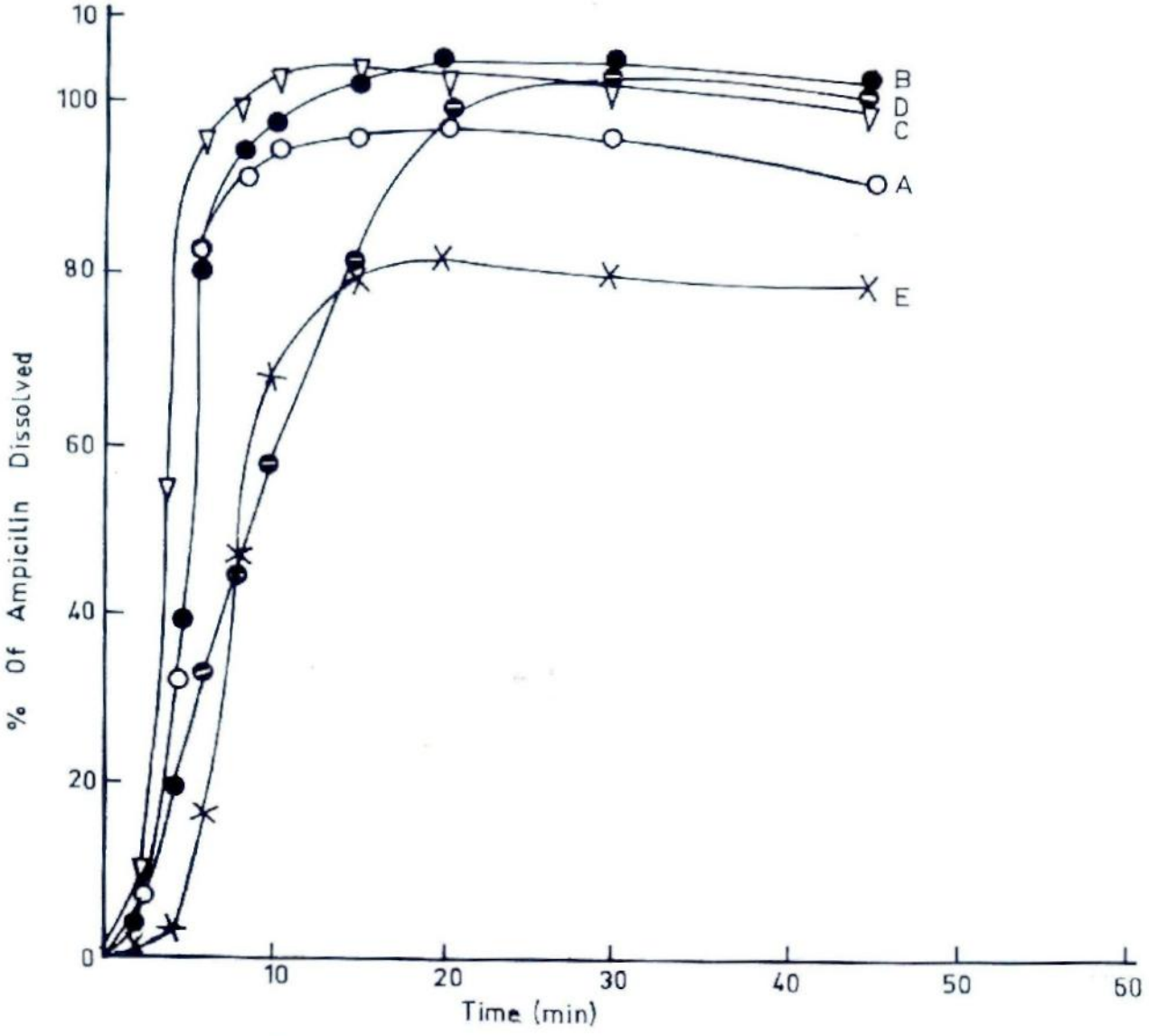


FIG. 2A. Dissolution Profiles of Brands A, B, C, D, and E at 30%

TABLE 3

Results of disintegration time tests

Brand Code	D.T. for 5 capsules in one basket (min)	Longest time for one capsule (min)	Mean D.T. for 10 capsules (one per basket (min)	Range of D*.T. for the 10 capsule (min)	D*.T. in simulated Gastric Fluid (min)	Remarks
A	9.5	6.0	5.5	4.4-7.1	5.4	Passed
B	21.1	8.2	8.2	7.0-10-08	6.4	Passed
C	18.4	13.5	8.6	6.2-14.4	4.6	Passed
D	31.5	16.2	15.5	13.0-21.4	11.5	Failed+
E	17.2	11.3	10.1	8.4-11.5	6.2	Passed
F	19.2	17.6	15.4	7.6-20.3	13.5	Failed+
G	8.2	6.1	5.4	5.5-6.5	5.3	Passed
H	16.5	11.4	10.4	8.5-13.1	6.5	Passed
I	27.5	27.2	14.2	6.6-27.3	7.1	Failed+
J	11.1	6.5	6.1	5.0-7.3	5.2	Passed

*: D.T. = Disintegration Time
+: Failed B.P. specifications only.

TABLE 4

Summary of all results

Brand Code	Weight	Content Uniformity		Disintegration Time (Min)		Dissolution RAte		Overall Ranking
	Coeffi-cient of variation (%)	Mean Content (%)	Coeffi-cient of variation (%)	B.P. Indi-vidual Capsule Test	U.S.P. (Mean)	T50% (Min)	T90% (Min)	
A	2.62	93.93	4.79	6.0	5.5	4.3	6.0	3
B	6.49	95.20	6.24	8.2	8.2	4.5	6.6	6
C	2.36	102.69	3.76	13.5	8.6	3.6	4.4	4
D	9.57	95.44	23.10	16.2	15.5	8.8	16.5	10
E	4.72	73.55	6.23	11.3	10.1	8.2	+	7
F	1.62	94.49	3.63	17.6	15.4	25.8	60	8
G	1.25	98.49	1.62	6.1	5.4	3.8	6.4	1
H	2.91	88.49	2.58	11.4	10.4	5.0	12.6	5
I	4.64	92.32	7.69	27.3	14.2	6.8	10.0	9
J	1.48	96.17	2.21	6.4	6.1	4.3	6.0	2

+ Brand E released only 83% of ampicillin.

It is evident from the results that brands A, B, C, F, G and J all passed the test with no capsule outside the 85%-115% limits as specified in the U.S.P. Whereas brand H only just passed as one of the capsule had ampicillin content below the 85% limit but above the 75% limit. The result also shows that two capsules of brand I were below the 85% limit and therefore this brand failed the test. Similarly, brand D and E failed because the ampicillin content of a capsule of brand D was 31.95% while no capsule of brand E had ampicillin content of up to 85%. In fact, the mean content for brand E was 73.55%, and since all the capsules were found to be fully filled on opening (as reflected by the low variation of the capsule weight—it had a coefficient of variation of 4.72%), the low ampicillin content could be attributed either to underweighing of the ampicillin during production of the batch, or to the use of an old stock of ampicillin in the manufacture. The fact that the expiry date for this brand was only one month away from the time of the assay may be responsible for this low ampcillin content, because if an old stock of ampicillin had actually been used, its potency would have reduced considerably before the labelled expiry date. The general practice is to give an allowance of about three years for expiration (from the time of manufacture) within which period the potency of the drug substance should not fall below 90% of its original, labelled value (17). Another reason may be due to poor storage conditions of this brand since the time of its manufacture which may have led to some degradation of the ampicillin.

Calculations of the standard deviation (6) and coefficients of variation (C.V) show that brand G had the lowest variation (6-4mg.) while, brand D had the highest (6-55.13mg).

Although no official limits have been set for these parameters, they give a clear indication of the real objective of the content uniformity test (i.e. variation from capsule to capsule). Statistical analysis of the coefficients of variation obtained in this test and those in the weight variation test employing the student's 't' test, shows that there was significant correlation at the 95% confidence level (P 0.05) and also, it seems to justify the fact that the U.S.P. specifies limits for content uniformity only as an alternative to weight variation, since the order of variation in both tests were approximately the same. However, the results of brand E which had an "excellent" weight variation but a "poor" ampicillin content, illustrates the necessity of both tests in order to fully characterise a product.

Data on the disintegration tests of the different brands of ampicillin capsules are summarised in Table 3. Determination of the end point of the disintegration time proved difficult when testing five capsules in one basket since the capsules softened and formed an adhesive gelatin mass which stuck to the mesh and entrapped within it, some of the solid content. Thus, as can be seen from the date, only brands A, G and J passed the five-capsule-per-basket test. Brands C, E and H failed due to aggregation of the ampicillin capsules which was clearly evident during the test. However, these three brands passed the repeat test using only one capsule per basket. Similarly, brand B failed the five capsule/basket test due to the presence of some large granules but, eventually passed the repeat test of one capsule/basket. Brands I and F however failed because of the presence of large and poorly water permeable aggregates which remained in the disintegration medium after the gelatin shell had completely dissolved. They also failed the repeat tests due to the formation of these water impermeable aggregates which was observed to occur mainly around the cap-body junction, indicating a low porosity of these brands. It can be inferred that their poor porosity may have resulted from the application of excessive "pressures" during capping of the capsule body, which led to the compression of some of the powders around the junction. In addition, the high proportion of fines in the solid content of both brands may also have contributed to the observed found to exhibit poor porosity after compression(4).

Brand D exhibited a peculiar disintegration in that one half of the capsule shell was hardly water permeable with the result that it never dissolved or disintegrated in the course of any of the tests. Because some solid content were stuck to this part of the capsule shell for a long time, they could not be released within the specified limit, and as a result, the brand failed.

It is evident, Table 3, that in general, the disintegration times for the repeat test (i.e. one capsule/basket) were shorter than those for the five capsule/basket test due to the aggregation which occurred with the five capsules tested together.

A similar observation made by Jones and Cole (3), led them to suggest an alternative in the B.P. specifications such that only one capsule/basket should be used instead of five and the disintegration time being calculated as the mean of five or more determinations as is the case in the U.S.P.

U.S.P. specifies that capsules shoud disintegrate between 15 and 30 min. This relatively liberal limit made it possible for all the brands to pass the U.S.P. test as shown in Table 3. Nevertheless, brands C, D, F and I exhibited wide ranges in disintegration times the difference between the fastest and slowest being not less than 8 min. in all the four brands.

The results, Table 3, also shows that all the brands showed faster disintegration in simulated gastric fluid than in water, largely because the gelatin shell was more soluble in the simulated gastric fluid (3). However, brand D still exhibited its characteristic of one half of the capsule failing to disintegrate, although this insoluble part finally broke up into shreds within 12 min.

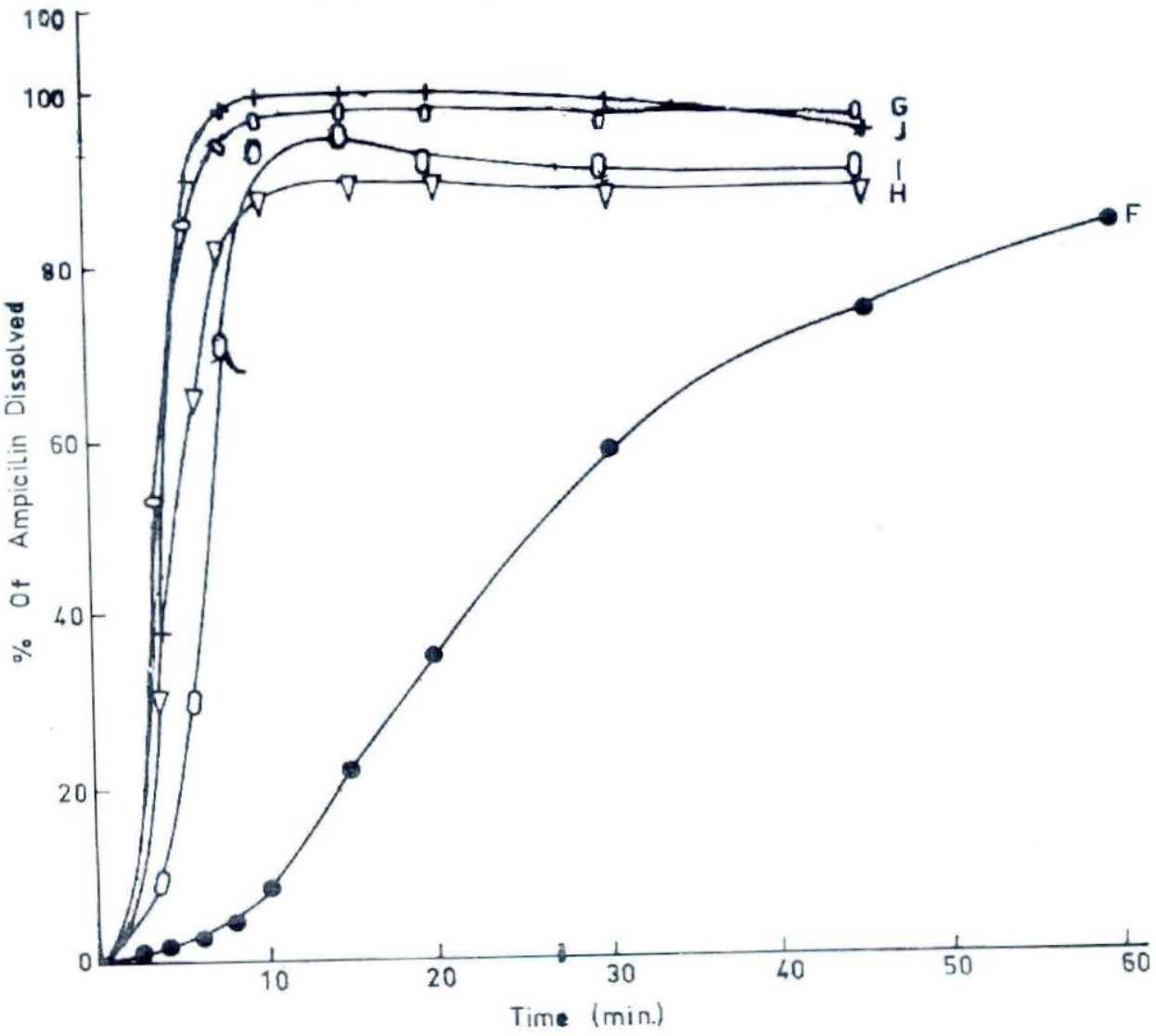


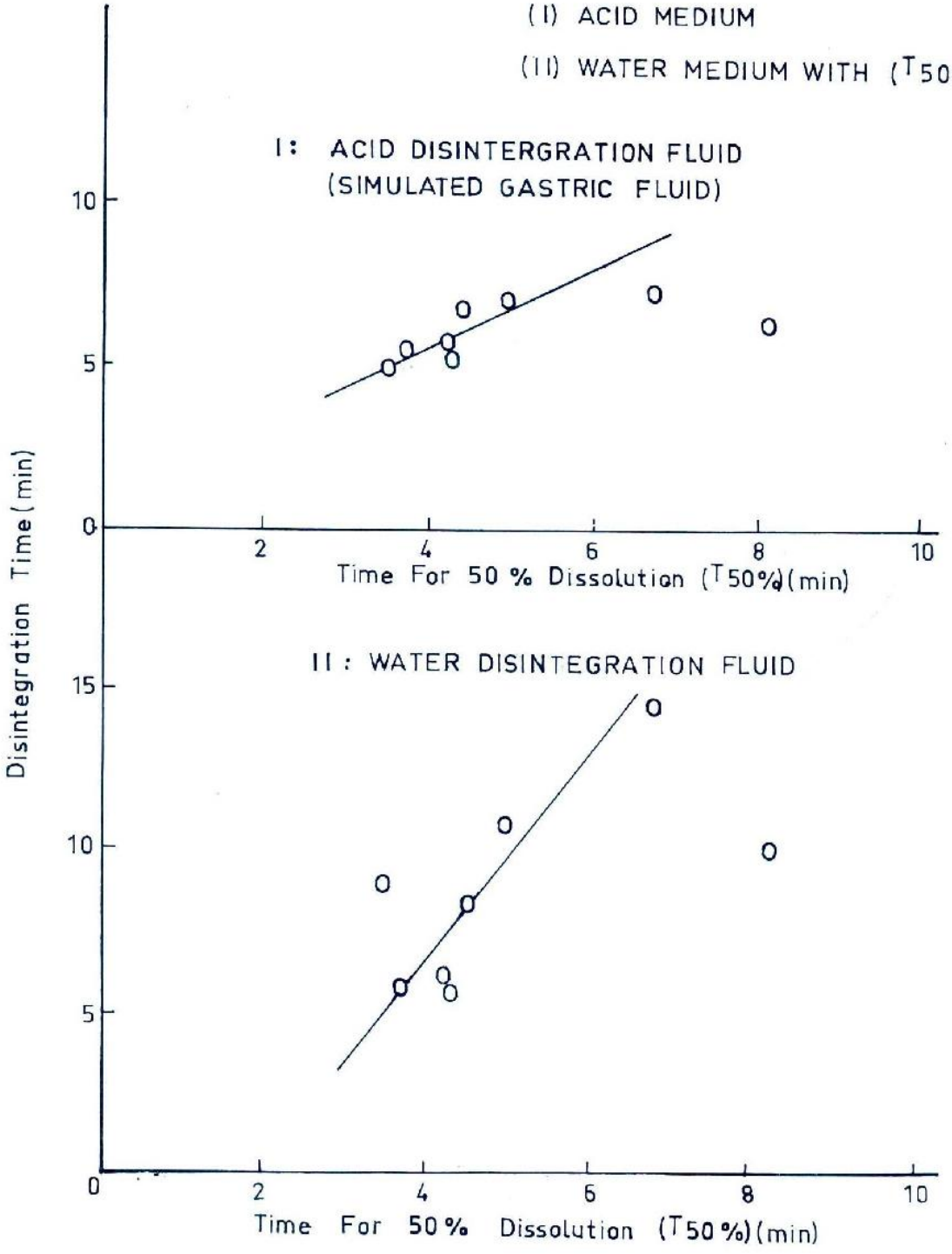
FIG. 2B Dissolution Profile of Brands F, G, H, I and J at 37°C

The results of the dissolution rate tests are plotted in Figs 2(a) and (b). Due to the absence of specifications in both the B.P. and the U.S.P. on dissolution rate of ampicillin capsules the test is at present essentially a comparative one. In fact, a recent B.P. policy statement on dissolution testing of ampicillin capsules (10) merely regards the test as a measure of proportion of drug capable of going into solution under standardised in-vitro testing conditions within a reasonable time. What this "reasonable time" should be, is currently under investigation. Nevertheless, from Figs. 2(a) and (b) it can be seen that brands C. G. J. A, H and I exhibited good dissolution rate profits since their T50% ranged between 3.3 and 6.5 min., while brands D and E have slower dissolution rates with T50% of 8.5 and 8.1 min. respectively. For brand D, the gelatin cap did not dissolve as it did in the disintegration test, hence adherent ampicillin particles were only slowly released from it and a slow dissolution rate was observed. Although brand E exhibited a good disintegration time, the maximum percentage of ampicillin dissolved was only 81.6%. This is in good agreement with the low mean content of ampicillin recorded for this brand (see Fig. 1(a). Consequently, the slow dissolution rate is more likely due to this low mean content of ampicillin since dissolu-

tion rate follows roughly a first order rate law. This is supported by the fact that brand C which had the highest mean content of ampicillin (see Fig. 1(b) also had the shortest T50% of 3.6 min.

Brand F had the poorest dissolution rate profile of all the brands studied and it was observed that once the gelatin shell dissolved, the solid content formed a lumpy mass or aggregate which settled to the bottom of the basket. The release rate from this aggregate was so slow that even at 45 min. only 75.7% of ampicillin was in solution. The inability to deaggregate, coupled with the equally poor disintegra-

FIG. 3. CORRELATION OF DISINTEGRATION TIMES IN:
(I) ACID MEDIUM
(II) WATER MEDIUM WITH ($T_{50\%}$)



tion of this brand, shows that the presence of the labelled amount of active ingredient in capsule formulations does not necessarily imply that the active ingredient would be made available to the body on ingestion of the capsule.

Fig 3 shows the correlation between the disintegration times and the time required for 50% dissolution of ampicillin from the capusles. Although the correlation was not very significant, the results showed a good correlation when ranked (coefficient of rank correlation was found to be 0.842) and this implied that the differences in the disintegration times and T50% were of the same order in all the brands. It also seems to justify the earlier reliance of most official compendia on disintegration time tests as an indicator of release rate.

Comparison of the dissolution behaviour of brands containing the trihydrate and those containing the anhydrous forms of ampicillin, by means of the student's 't' test (18) showed the difference in mean value of T50% for the two forms to be statistically insignificant ($p > 0.1$). This suggests that the dissolution of both forms of ampicillin is essentially similar and thatthe variations in dissolution rates observed in the present study on the different brands must be due to differences in their formulations and manufacturing processes. This is in agreement with the results obtained by Baner (19), Whyatt at al (20) and Hill et al.

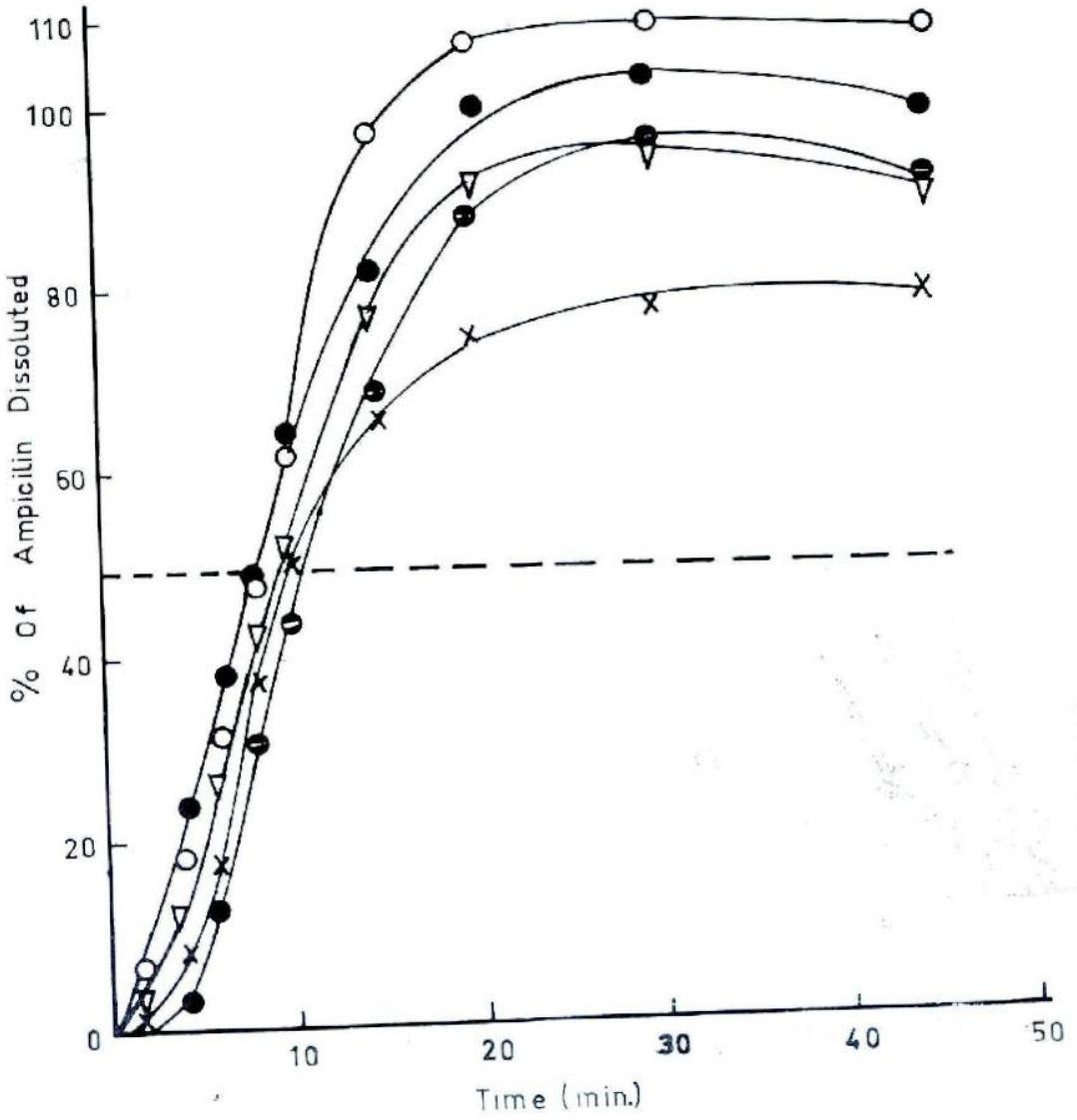


FIG.4. Intra-batch Variation in dissolution rate profile within Brand D.

(21) who showed that formulation factors exert a greater effect on the dissolution rates of ampicillin capsule formulations than the absolute solubility of the ampicillin powder..

Brand D was chosen to illustrate the variation in dissolution rate profiles within a batch because it exhibited the greatest variation in content uniformity. As shown in Fig. 4, the dissolution behaviour reflects this variation in ampicillin content. The values of T50% ranged from 7 to 11 min. whereas, the T90% ranged from 14 to 23 min. for four of the capsules; The fifth capsule, released a maximum of only 80% of ampicillin even after an hour. This variation would result in fluctuating blood levels when this brand is administered orally.

Fig. 5 illustrates the effect of temperature on the dissolution rate of brand F and it is clearly evident from the plots that a change of temperature from 31.50C to 44oC increased the dissolution rate by as much as 600%.

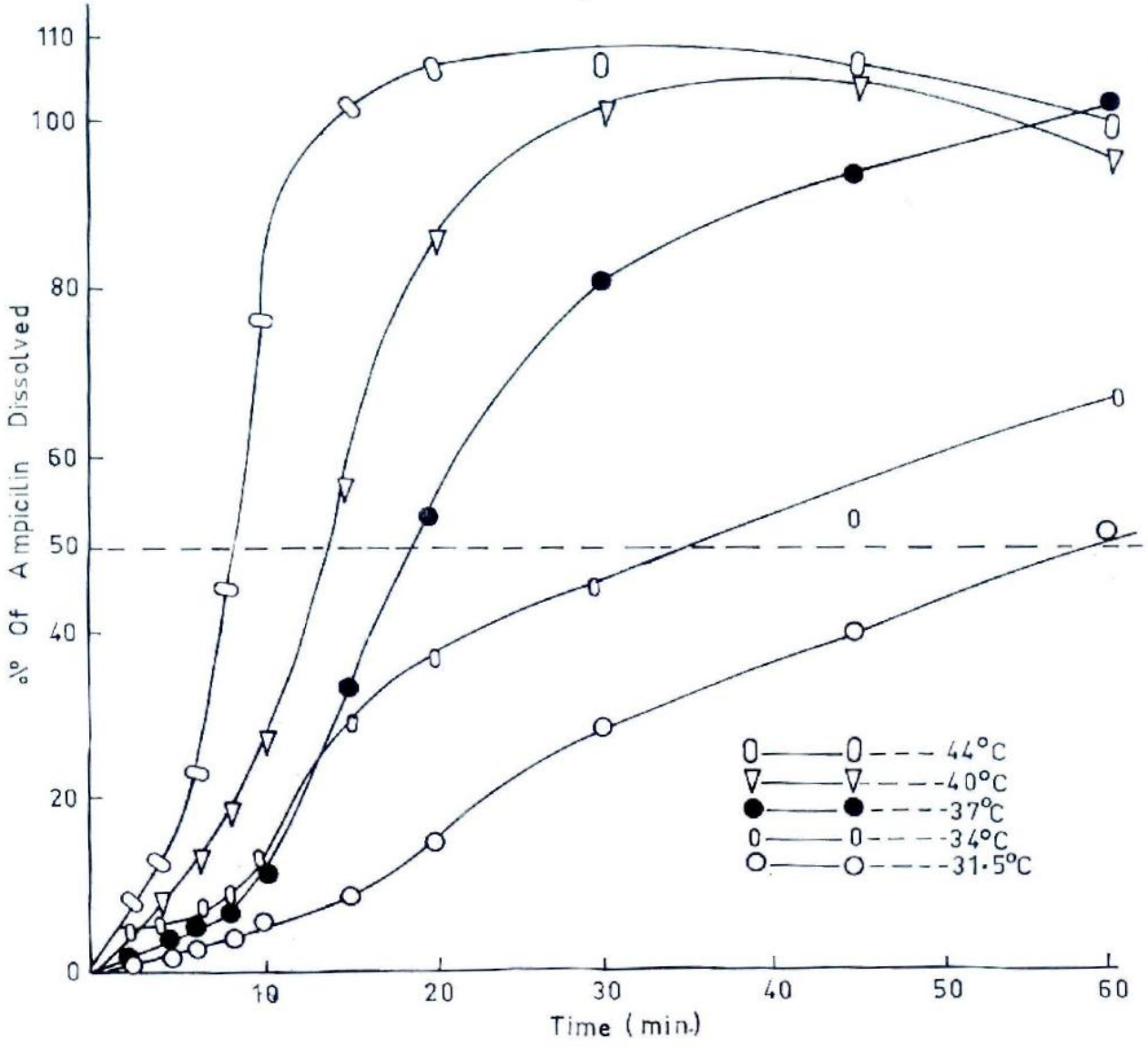


FIG. 5: Effect of Temperature on Dissolution Rate profile of Brand. F

Finally, Table 4 shows a summary of the results of all the tests performed. Assuming all the tests to be of equal importance, an attempt was made to rank the ten brands on the basis of overall performance in all the tests and the following rank order was arrived at G J A C H B E F I D (i.e. Brand G is the best of the

brands, while brand D had the poorest performance).

CONCLUSIONS:

The results of weight variation tests can reasonably indicate the uniformity of content of a hard gelatin capsule, as shown by the correlation between the two. This, however, is not always so, since, in the case of brand B, a good weight variation did not correspond with the content of ampicillin.

Disintegration tests carried out on five or more capsules singly, and, calculating the mean of the results, are better at portraying the disintegration behaviour of a capsule formulation, since agglomeration occurs when multiple capsules are used in one basket. In addition, the test on individual capsules correlates more with dissolution rate tests than those from multiple capsule tests.

It has been found that the disintegration of ampicillin capsules in acid medium is shorter than in distilled water medium and its dissolution rate is faster at higher temperatures.

A good content uniformity result is not enough to characterise fully the in-vitro release patterns of a capsule formulation since, if it fails to disintegrate and dissolve, to release the active ingredient, its desired therapeutic effects will not only attained. This was demonstrated by brand F.

The poor results obtained for content uniformity and dissolution rates for some of the brands (particularly bands B, D, E, F and I) emphasises the importance of adequate quality control of all pharmaceutical products imported into the country. This will eliminate bad products and prevent the undermedication of a patient when a generic brands of ampicillin formulation is administered.

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