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EVALUATION OF COMMERCIAL BENZOYL METRONIDAZOLE SUSPENSION

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ABSTRACT

Benzoyl metronidazole is available in the Egyptian market as suspension. It was reported that its suspension is physically unstable. Hence, it is aimed in this study to evaluate marketed benzoyl metronidazole suspension. Four batches of benzoyl metronidazole suspension were evaluated for sedimentation volume, drug content, particle length distribution, dissolution rate and bioavailability of the drug. The results revealed significant differences in these parameters between and among the batches of the same brand. The commercial suspensions showed physical and chemical stability along 10 months of aging concerning the preceding parameters, except that the number of the large particles increase by aging. The in-vivo evaluation of two benzoyl metronidazole batches was performed in healthy human volunteers. A direct correlation could exist between the in-vitro dissolution characteristics and the in-vivo parameters.

INTRODUCTION

The ester benzoyl metronidazole is a prodrug of metronidazole and because of its poor solubility in water it is tasteless and is used clinically in the form of oral aqueous suspension[1]. The suspension proved to be successful for treatment of amoebiasis[2]. Formulation of oral benzoyl metronidazole suspension implies physical stability problems which may lead to an increase of the particle size compromising the intended effect of the substance in the treatment of aerobic bacterial infections[3]. The crystal growth may occur specially when the preparation was stored in the cold[3]. This increase in particle size has recently been shown to be due to a phase transition of the anhydrous form to the monohydrate form of metronidazole ester[3].

The designation "quality" applied to a drug product according to Academy of Pharmaceutical Sciences (APS), recognizes that drug product may undergo changes with time which result in a loss in biological and therapeutic activity, even though the product originally completely complies with the potency and purity standards and no significant decomposition has occurred[4].

In order to guarantee the quality of a product Quality Assurance Systems embrace the entire manufacture and product life cycle from evaluation of starting material suppliers, through manufacture, monitoring testing distribution and finally to consumer. The ever increasing demands for higher margins of safty result in more and more regulatory control[5]. Systematic surveillance of the products by the authorities of all pharmaceutical companies of a country according to the same standard is desirable[5].

The aim of the present study is : 1st: to evaluate the marketed metronidazole suspension, 2nd to correlate in-vitro dissolution testing with in-vivo results.

EXPERIMENTAL

Materials :

Flagyl suspensions (Four batches of one brand) were purchased from the local market. Benzoyl metronidazole powder (Alexandria Co., Egypt). All chenicals and reagents were either of analytical or Pharmaceutical grade and were used without further purification.

Evaluation of Flagyl Suspensions

a-sedimentation Volume (Vu/Vo) : The bottles containing flagyl suspension were shaken and each bottle was transferred to 100-ml graduated cylinder. The sedimentation volumes (Vu) of the sediment were measured throughout a period of 24 hrs. and were divided by the total volumes (Vo) of the suspensions.

b-Redispersibility: The stoppered graduated measures containing the suspensions were turned upside down through 360^0 at a constant speed of 20 inversions per minute. The smaller the number of inversions necessary to restore homogeneity of suspensions the easier is the redispersibility of the sediment.

c-Drug Content : Five ml of the suspension were transferred to 100-ml volumetric flask, 50 ml

methyl alcohol were added and the flask was shaken until all the drug was dissolved. The volume was completed with distilled water. Samples were withdrawn and after appropriate dilutions these samples were measured spectrophotometrically at 320 nm (Shimadzu UV-150-02 Japan). No interference of the additives with the assay procedure was detected.

d-Particle Length : The linear measurments of particles were made by utilizing a calibrated scale incorporated within the projection microscope. (MP_3 Nr 1307 Poland).

e-Dissolution Studies : The same procedure adopted for the tablets[6] was used except that 5 ml of the thoroughly pre-shaken suspension equivalent to 250 mg metronidazole were added to the dissolution medium after the removal of an equal Volume. Then, the same steps mentioned before[6] were followed.

f-Thin-Layer Chromatography : The thin layer chromatographic method was carried out for the determination of the stability of benzoyl metronidazole suspension after aging for 10-months. The system consisted of chloroform, acetone and glacial acetic acid at ratios 85:10:7.5 respectively. The system was proved to be capable of separating the degraded product. Ultraviolet lamp or iodine vapour was used for the detection of metronidazole spots.

g-Bioavailability Studies : The same procedure adopted for tablets was used[6]. Ten ml of flagyl suspension equivalent to 500 mg of metronidazole were administered orally by each of six volunteers (age ranged from 26-36 years and body weight from 56-68 kg) with a glass of water in the morning after collecting a urine sample from each volunteer to act as his own blank. Then, the procedure was completed exactly as mentioned before[6].

RESULTS AND DISCUSSION

Evalution of Marketed Benzoyl Metronidazole Suspension:

Four batches of benzoyl metronidazole from locally manufactured brand of suspension in the Egyptian market were collected and four bottles from each batch were tested for the following requirements :

1- The sedimentation Volume : It was observed that the suspended particles separated into two layers. One settled down and the other floated upward. The volume of the sediment equals the summation of the two layers.

Statistical analysis of the results showed that there were significant differences between bottles from each batch at $P \leq 0.1$ level of certainity. Batch No X 4, gave the highest sedimentation volume up to 6 hours (Fig. 1). While batch No X 3 gave the lowest sedimentation volume, and the other batches were in between. Statistical analysis of the data revealed that the difference between batches was significant at $P \leq 0.05$.

2- Redispersibility : The sediment was redispersed after four or five times of inversion until the bottom of the stoppered measures became clear.

There was no difference in redispersibility between bottles.

3- Drug Content : The percent recovered from the labeled amount was found in the range of 87.4-97.1 (Table 1).

Table 1 : Drug Content of Marketed Benzoyl Metronidazole Suspension (Flagyl).

Batch No.	Bottle No.	% drug content from the labeled amount
X 1	1	93.0
	2	94.3
	3	94.3
Mean	4	94.0
	-	93.9
X 2	1	97.1
	2	93.4
	3	94.6
	4	96.0
Mean	-	95.3
X 3	1	94.7
	2	94.0
	3	93.4
Mean	4	94.6
	-	94.2
X 4	1	87.4
	2	92.9
	3	94.3
	4	96.9
Mean	-	92.8

Statistical analysis of these data revealed that there were significant differences in drug content between bottles of batch No X 1 and X 4 at $P < 0.1$. However the difference was not significant ($P < 0.1$) in case of the bottles drawn from batches No X 2 and X 3. Moreover, it was found by statistical analysis that there were significant differences between batches at $P < 0.05$.

4- Particle Length : Figure 2 showed the variation of the particle length between bottles of batch No X 2. The histogram represented the different lengths of the drug particles present in each bottle. Figure 3 illustrated wide variation between batches. Statistical analysis of these data showed that there were significant differences between bottles and also between batches. From the microscopic examination of the upper and lower layers of the sediment Figure 4, it was observed that there were large number of the small particles in the upper layer than that of the lower one. For this reason the small particles floated at the top forming the upper layer. However, the melting points of the separated crystals from both layers were found to be the same. Thus, it can be deduced that the crystals separated were of the same nature and there was no possibility of the presence of polymorphs and the only difference was in particle length.

5- Dissolution Behaviour of Marketed Benzoyl Metronidazole Suspension (Flagyl) : It was observed that there was intrabatch (Table 2) and interbatch (Table 3) variations in dissolution rate of the drug.

Table 2 : Intrabatch Dissolution Rate Constant (K) of Flagyl Suspension Before Storage.

Batch No.	Bottle No.	r	K x 10^2 (min^{-1})
X 3	1	0.9964	1.824
	2	0.9984	1.829
	3	0.9989	1.952
	4	0.9953	0.9557
X 2	1	0.9958	1.328
	2	0.9976	1.253
	3	0.9975	1.994
	4	0.9956	1.094
X 4	1	0.9669	1.519
	2	0.9842	1.78
	3	0.9686	1.359
	4	0.9717	1.331
X 1	1	0.9987	1.298
	2	0.9948	1.186
	3	0.9985	1.013
	4	0.9907	0.9557

Table 3 : Interbatch Dissolution Rate Constant (K) of Flagyl Suspension before Storage.

Batch No.	Corr.	K X 10^2(min^{-1})
X 3	0.9992	1.543
X 2	0.9981	1.352
X 4	0.9720	1.474
X 1	0.9959	1.169

r : Correlation Coefficient.

It was found that bottle No 3 and 4 from batch No X 2 gave the highest and lowest values of K respectively. The percent dissoluted after 30 minutes was 66.5% and 92.5% after 105 minutes from bottles No 3 while the corresponding value for bottle No 4 were equal to 62% and 79.5% respectively. Batch No X 3 showed the highest dissolution rate constant (K) followed in order by batches No X 2, X 4 and X 1 (Table 3). The highest percent dissoluted of benzoyl metronidazole from batch No. 3 was found to be 72.5% after 30 minutes and 90% after 105 minutes, while the batch No. 1 gave 47.5% after 30 minutes and 77.5% after 105 minutes (Fig. 5). Figure 5 showed the dissolution behaviour of benzoyl metronidazole powder compared with the dissolution of the suspension. It was clearly obvious that the dissolution rate of drug powder was much higher than suspension. Percent drug dissoluted after 60 minutes from powder was 90% while the highest amount dissoluted in case of suspension was 83% after the same time. The results revealed that, there were significant differences at $P < 0.2$ between bottles and significant differences between batches at $P \leq 0.1$ for the percent of benzoyl metronidazole dissoluted and K value after 10, 30 and 105 minutes.

It could be concluded that the dissolution characteristics were highly dependent on the particle size and the particle size distribution. It was apparent that batch No X 3 of benzoyl metronidazole suspension dissolved much faster than the other batches. This can be attributed to the difference in particle size. Hence, batch No X 3 was characterized by a higher population of the smallest particles than other batches. This agreed with the reported findings that

particle size reduction is generally a mean of dissolution rate enhancement[7,8].

Bioavailability of Benzoyl Metronidazole Suspension :

The two batches which gave the highest and the lowest dissolution rate were used in the bioavailability study. Figure 6 showed the mean cumulative urinary excreted amounts of benzoyl metronidazole. It was clearly observed that batch No X 3 showed higher excretion rate than batch No X 1. Using student t-test for comparison between the mean amount excreted, the data showed significant differences after 2 & 24 hr. at $P \leq 0.05$ and significant differences after 6 hr, at $P \leq 0.2$ significance level. The elimination rate constant (K) of benzoyl metronidazole may then be obtained from plots of log excretion rate versus time (Fig. 7). The mean excretion rate constant obtained equals to 0.05826 hr^{-1} which corresponded to half-life of 11.89577 hr.

A comparison of the *in-vivo* and *in-vitro* results demonstrated a direct correlation between the dissolution characteristics of the two suspensions and the *in-vivo* parameters.

The suspensions were reevaluated after storing for 10 months for the preceding parameters. The results obtained indicated that the sediment also separated into two layers. The sedimentation volume, drug content and the dissolution characteristics of benzoyl metronidazole suspensions were not changed by aging for ten months. The dissolution rate constants (K), were calculated from the slope of the lines obtained by plotting log amount of drug remained versus time. The results were tabulated in Tables 4 and 5.

Table 4 : Intrabatch Dissolution Rate Constant (K)of Flagyl Suspension after Storage.

Batch No.	Bottle No.	r	K X 10^2(min^{-1})
X 3	1	0.9972	1.796
	2	0.9969	1.612
	3	0.9979	1.888
	4	0.9920	1.188
X 2	1	0.9963	1.054
	2	0.9942	1.245
	3	0.9873	1.156
	4	0.9903	1.158
X 4	1	0.9653	1.393
	2	0.9788	1.531
	3	0.9618	1.276
	4	0.9821	1.404
X 1	1	0.9950	1.515
	2	0.9989	1.204
	3	0.9948	0.982
	4	0.9930	0.981

Table 5 : Interbatch Dissolution Rate Constant (K) of Flagyl Suspension after Storage.

Batch No.	r	K X 10^2(min^{-1})
X 3	0.9987	1.612
X 2	0.9943	1.146
X 4	0.9738	1.404
X 1	0.9965	1.156

r : Correlation Coefficient.

It was observed that generally there was no significant change in these values after aging (compare Tables 2 and 3 with 4 and 5). Reexamination of particle length after aging revealed that the number of the large particles increased, after ten months of shelf-storage (Fig. 3). This was in agreement with the reported results about physical instability of benzoyl metronidazole oral suspension. As the commercial benzoyl metronidazole is available in an anhydrous form. This exhibits phase transition to the hydrate in water followed by drastic increase in the particle size[3].

The thin layer chromatographic method was used to test the stability of the drug substance in pharmaceutical suspension after aging. From the data obtained it was observed that the drug was stable during storage.

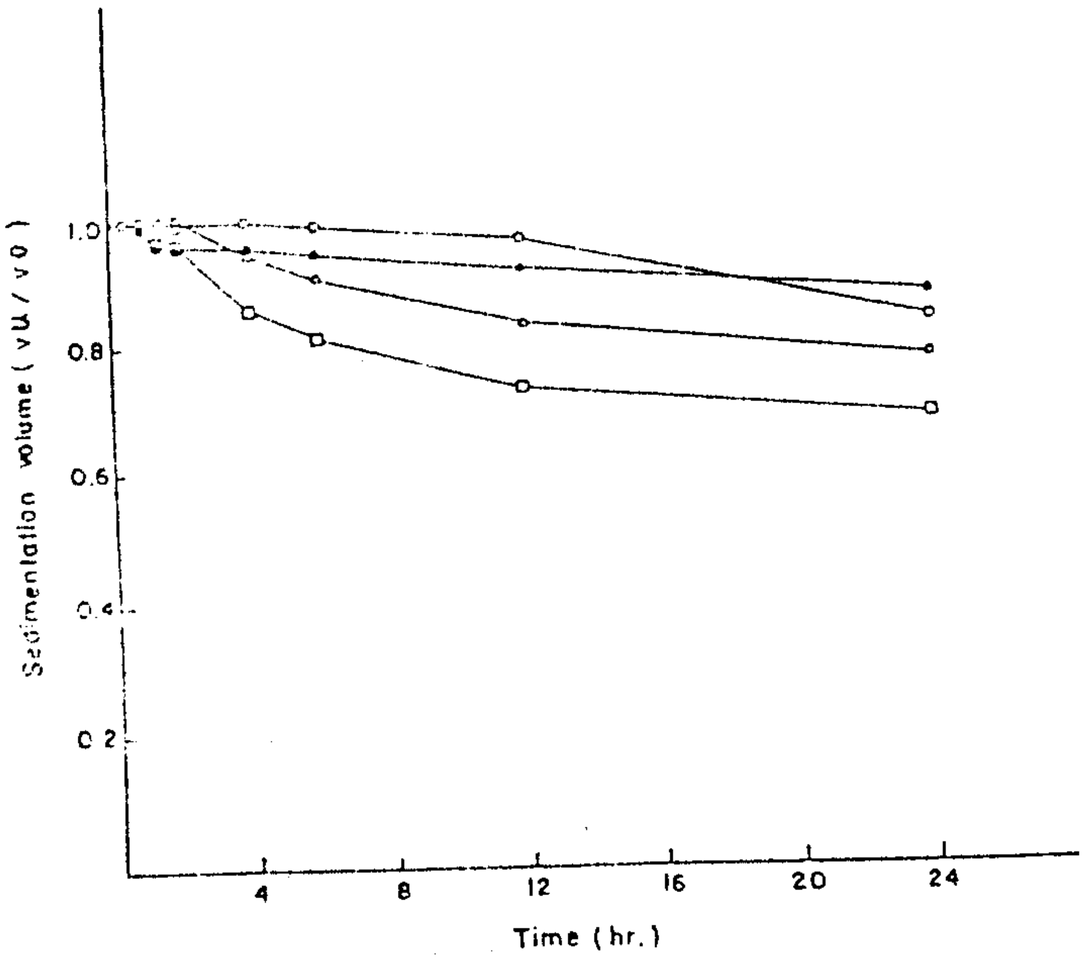

Fig. (1): Interbatch sedimentation volume of flagyl suspension.
- ○ batch No x 4
- ▲ batch No x 2
- △ batch No x 1
- □ batch No x 3

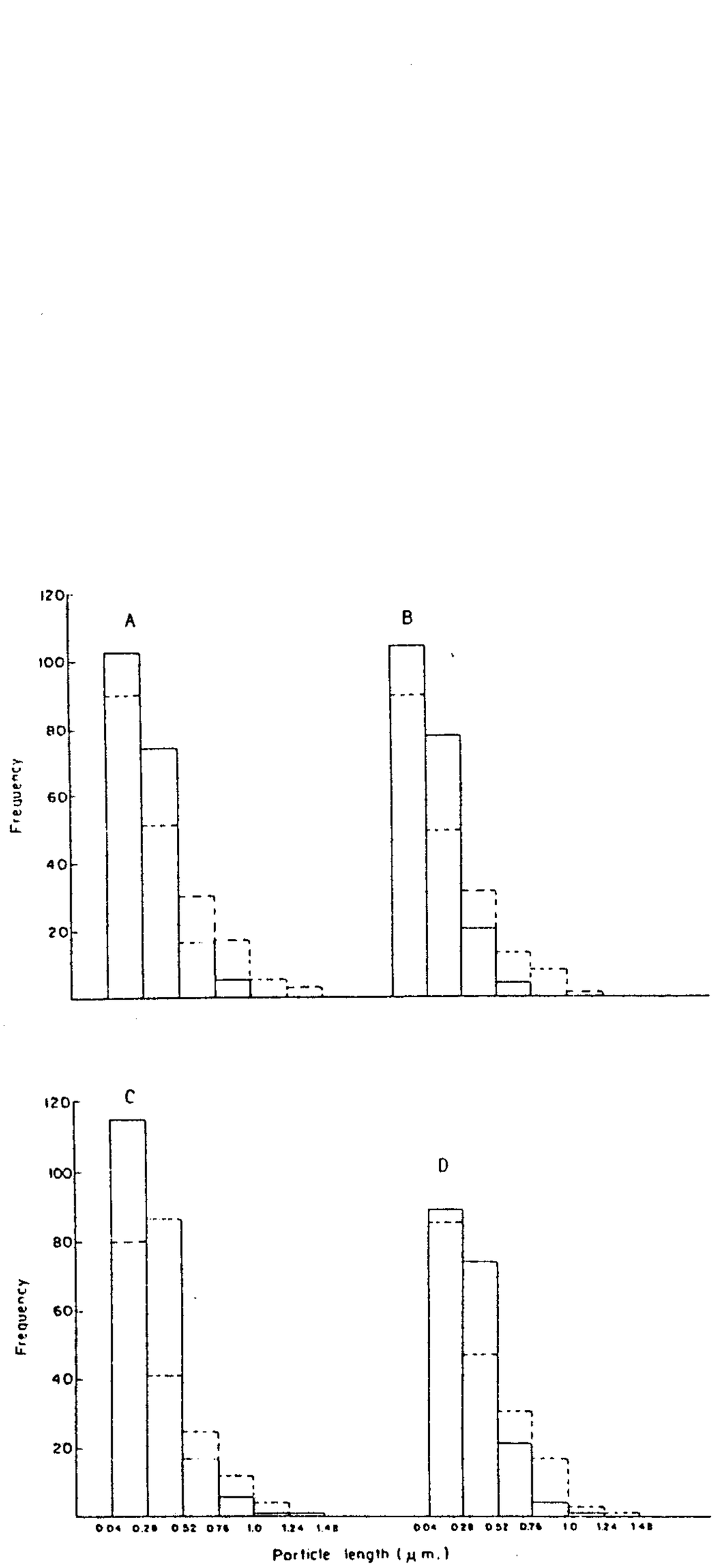

Fig (2): Intrabatch particle length distribution before (——) and after (- - -) 10 monthes aging.
For bottles from batch No. x 2
- A bottle No. 1
- B bottle No. 2
- C bottle No. 3
- D bottle No. 4

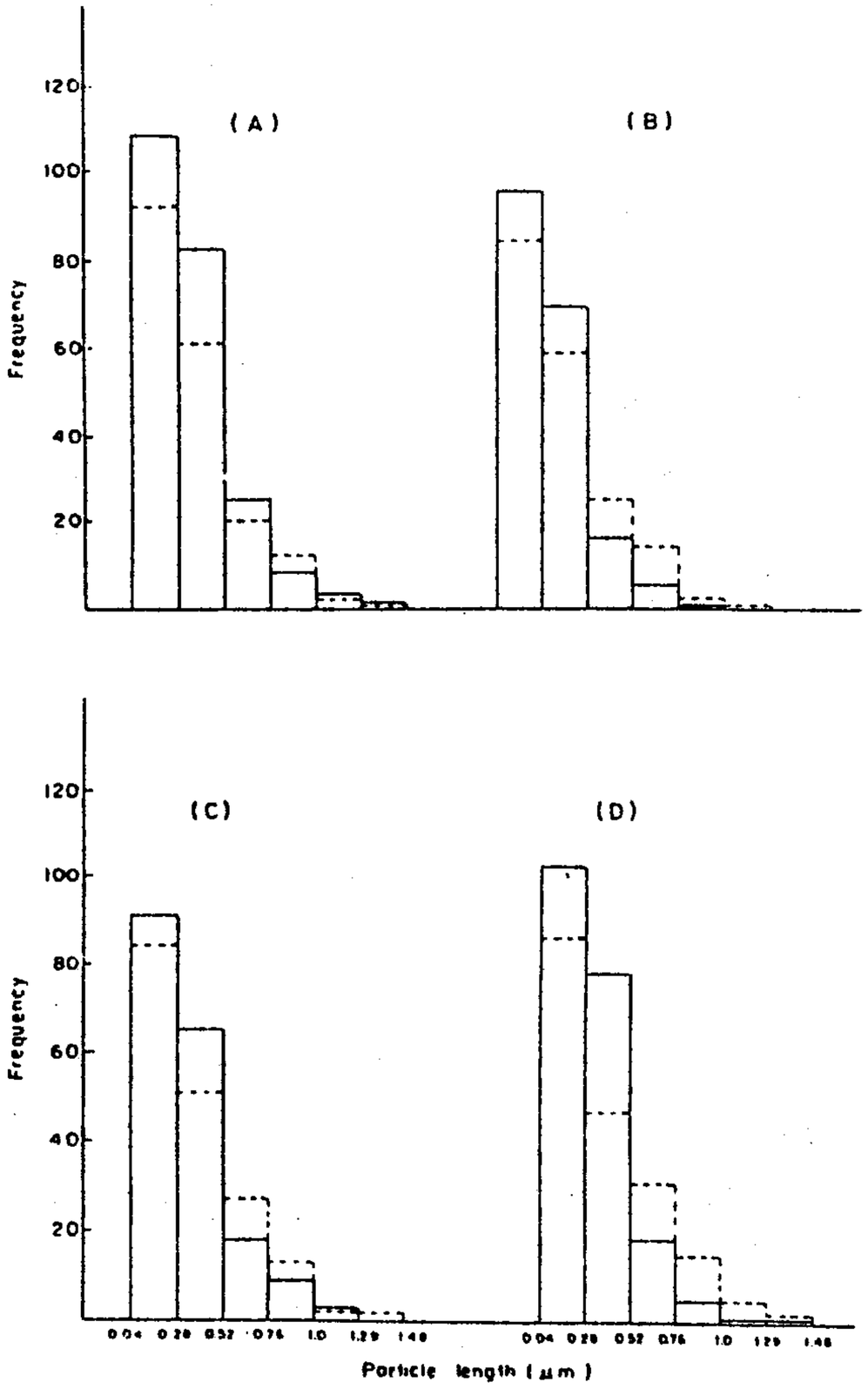

Fig.(3) : Interbatch particle length distribution before (——) and after (- - -) 10 months aging.

A batch No X 3 B batch No X 4

C batch No X 1 D batch No X 2

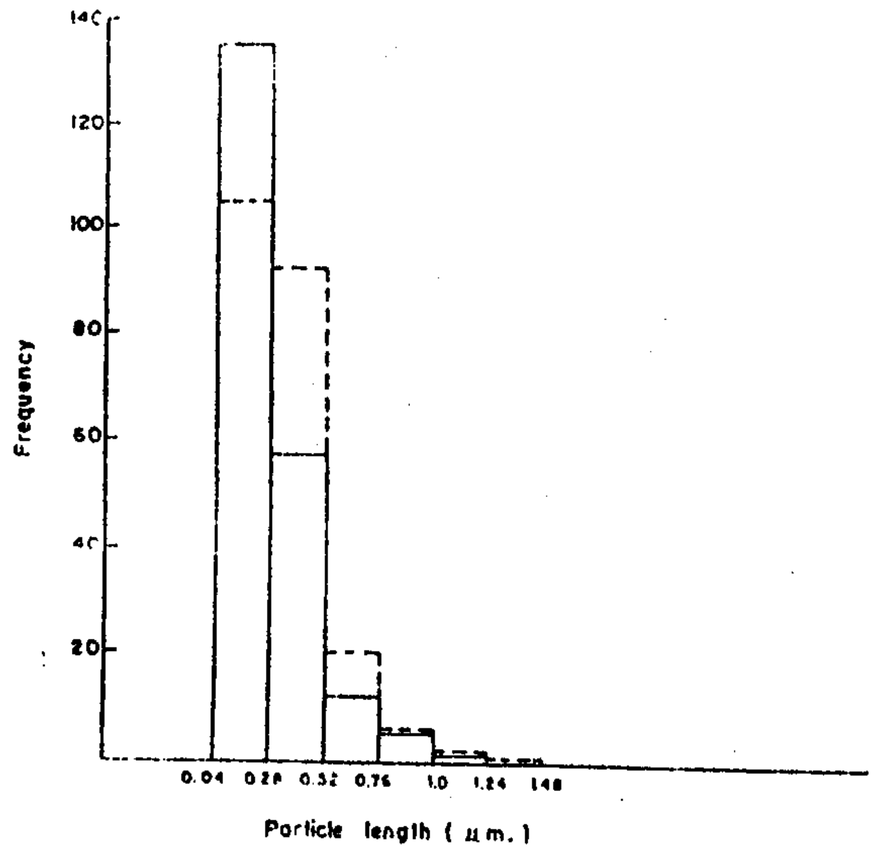

Fig.(4) : Particle length distribution of flagyl suspension of upper and lower layers of the sediment.
(——) upper layer (- - -) lower layer.

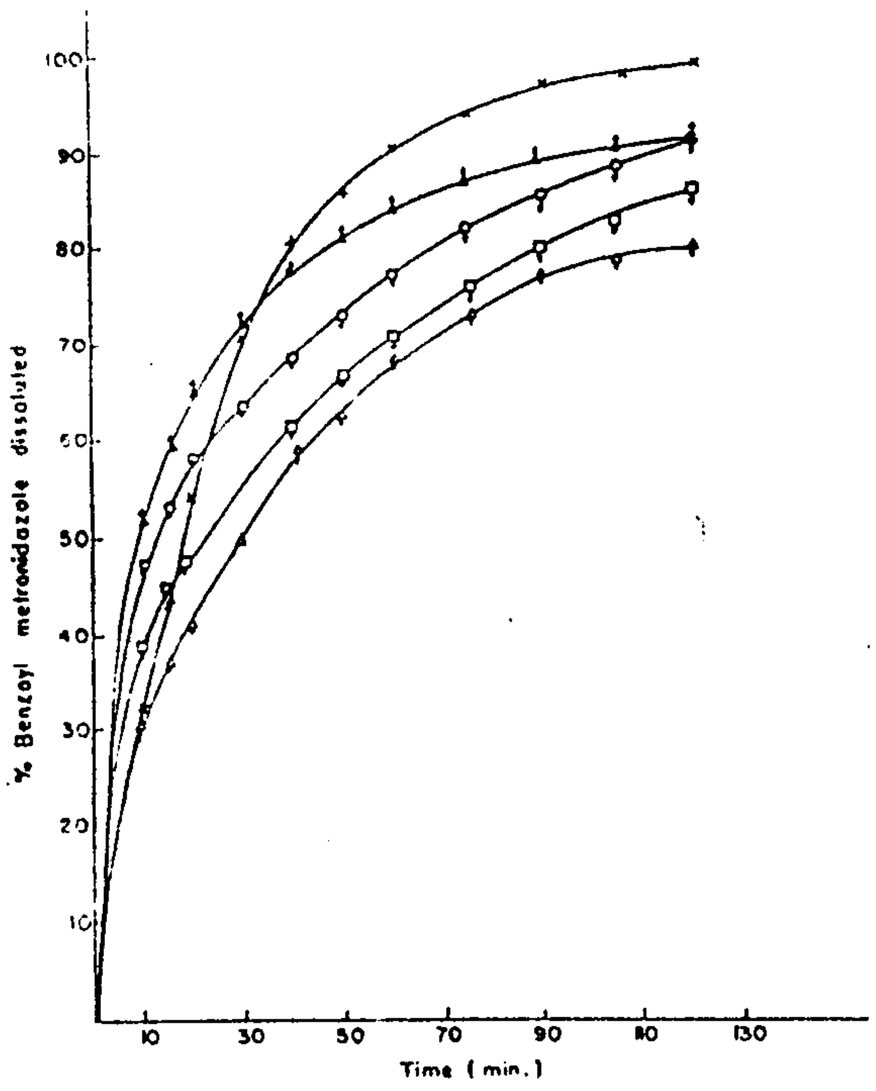

Fig.(5): Interbatch dissolution profile for flagyl suspension .
x powder ▲ batch No X 3
o batch No X 2 □ batch No X 4
▲ batch No X 1

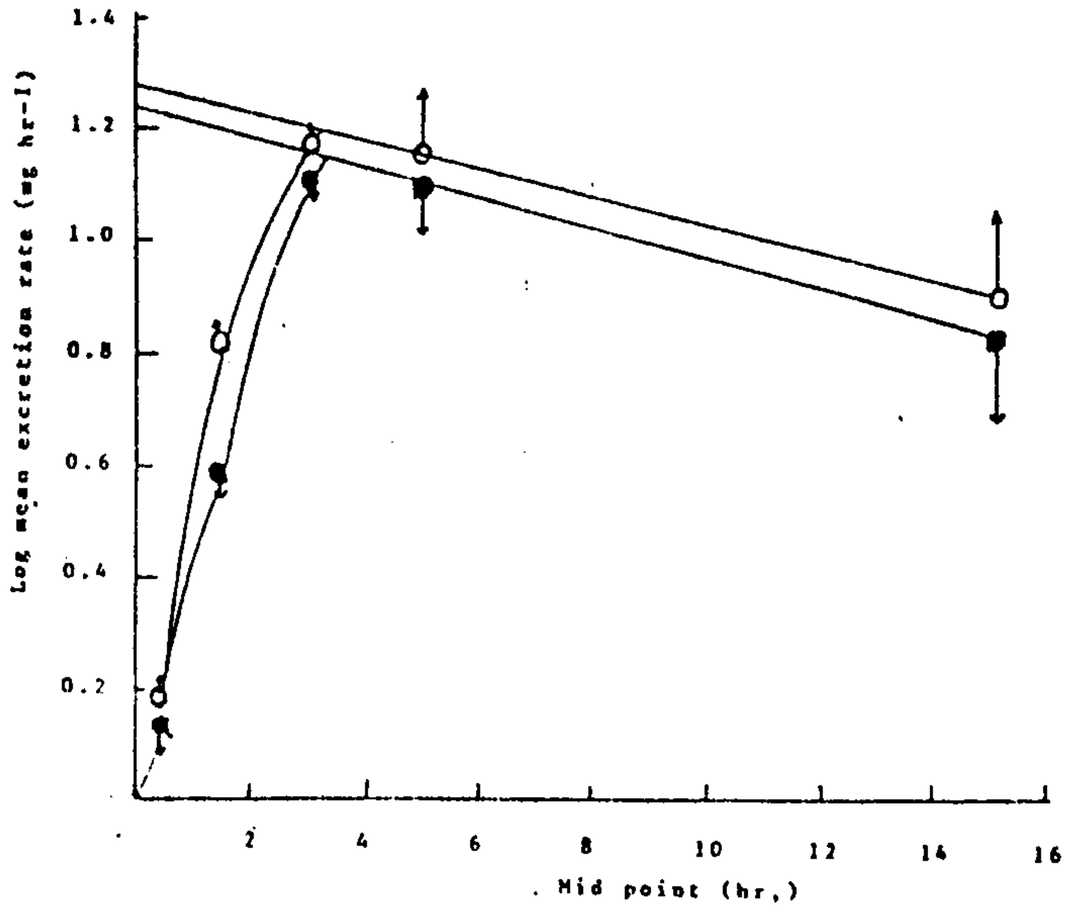

Fig. (7) : Logarithmic plot of excretion rate of flagyl suspension versus time.
o batch No X 3 • batch No X 1

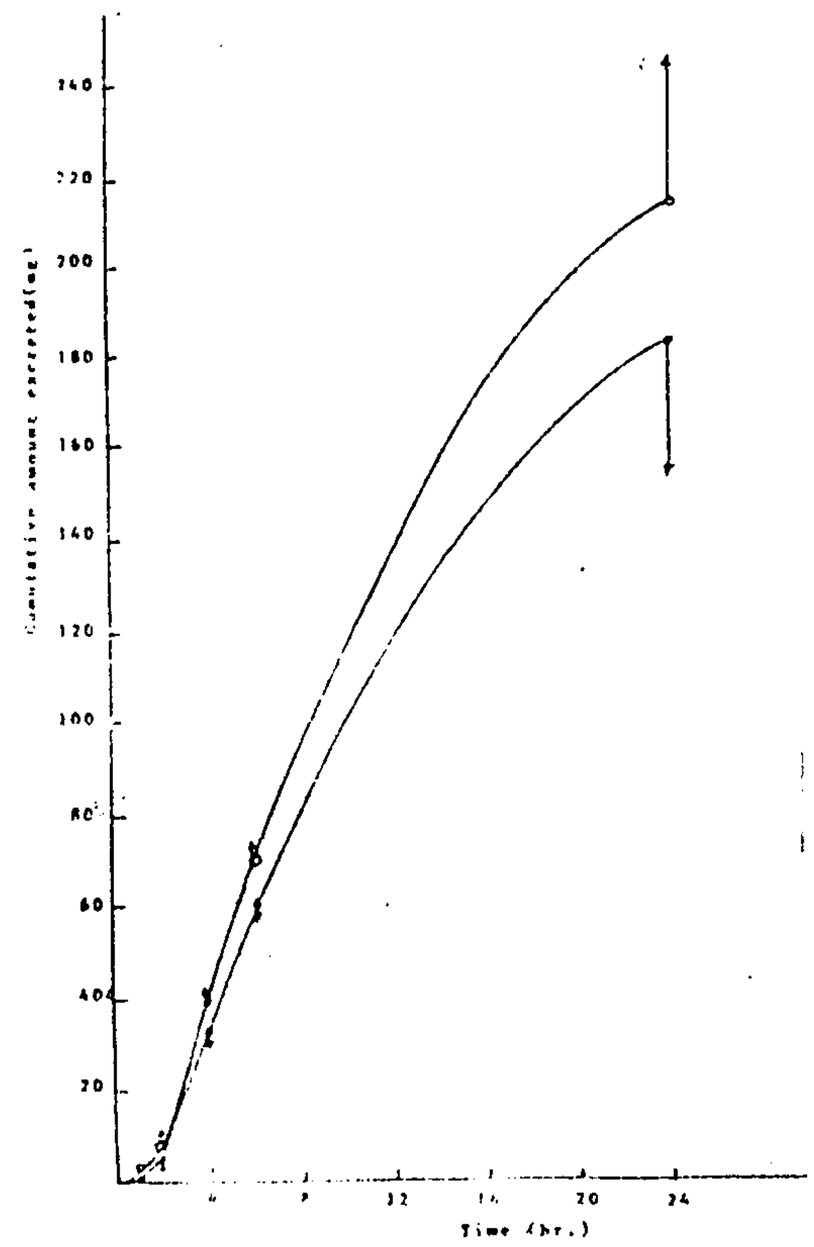

Fig.(6): Mean cumulative urinary excretion of flagyl suspension after oral administeration .

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تقـــــــــــييم معلق بنزوات المترونيدازول المتوفر فى السوق المحلى

السيد على ابراهيم ـ سوزان شوقى طوس ـ تهانى حسن الفحام ـ مها عبدالعظيم حسن

قسم الصيدلانيات ـ كلية الصيدلة ـ جامعة اسيوط ـ اسيوط

توجد بنزوات المترونيدازول فى السوق المصرى على هيئة معلق وقد دلت الابحاث على عدم ثبات هذا المعلق من الناحية الفيزيائية . ولذلك فقد استهدفت هذه الدراسة تقييم المعلق المتوفر فى السوق المحلى .

وعلى ذلك فقد تم تقييم اربع تشغيلات من المعلق من ناحية حجم الراسب وكمية العقار وطول الجسيمات وتوزيعها ومعدل الذوبان وكذلك التوافر الحيوى للمعلق .

وقد اسفرت النتائج على وجود تباين معنوى فى هذه المقاييس بين وخلال هذه التشغيلات . وكذلك فان هذه المقاييس لاتتغير حتى بعد مضى عشرة شهور من تخزينها عند درجة حرارة الغرفة (٢٥ م) ما عدا عدد الجسيمات الكبيرة قد زاد بواسطة التخزين على حساب عدد الجسيمات الصغيرة .

ومن دراسة التوافر الحيوى لكل من المعلق الذى اعطى اعلى واقل معدل ذوبان فقد وجد ان هناك علاقة مباشرة بين الدراسة المعملية لمعدل الذوبان وبين دراسة التوافر الحيوى داخل جسم الانسان وقد وجد ان ثابت معدل افراز العقار فى البول يساوى ٠٥٨٢٦ر ساعة$^{-1}$ وان زمن نصف العمر للعقار هو ١١ر٨٩ ساعة .

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