In-vitro Availability of Erythromycin From Some Commercial Tablets

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Abstract ❑ The *in-vitro* characteristics of nine batches of film-coated erythromycin stearate tablet brands marketed in Egypt and one batch of enteric-coated erythromycin base tablets were examined. The disintegration times and dissolution rates of erythromycin stearate tablets indicated wide inter-brand and inter-batch variability as well as inter-tablet variability in some batches of brand B tablets. Enteric-coated tablets showed excellent *in-vitro* properties. The results strongly pointed to formulation and coating problems in addition to variations in the intrinsic dissolution properties of erythromycin stearate raw material, as potential causes of the relatively poor *in-vitro* performance of erythromycin stearate tablets. The correlation obtained between dissolution data of the tablet products under study and the bioavailability parameters of the same products reported earlier, indicated the usefulness of simple *in-vitro* tests for the continued monitoring of the production and bioavailability of erythromycin tablets.

Keyphrases ❑ Erythromycin stearate film-coated tablets, enteric-coated erythromycin tablets, dissolution rate, *in-vitro* / *in-vivo* correlations.

Erythromycin base is rapidly inactivated by gastric acid (1,2) and is protected by enteric coating in oral preparations. The sparingly soluble stearate salt, reported also to be significantly acid labile [2], is produced commercially in film-coated tablets. Erythromycin stearate dissociates in the intestine yielding free erythromycin base (3). It is obvious that erythromycin bioavailability from such coated preparation would depend intimately on pharmaceutical formulation and quality of the coat.

Variation in the technical characteristics of coated erythromycin preparations from different manufacturers are likely to result continually in biononequivalent commercial products. This has been emphasized in comparative bioavailability studies of oral erythromycin products over the past two decades (3-6). Attempts were made to use simple ***in-vitro*** characterization data to predict the ***in-vivo*** performance of film-coated and enteric-coated erythromycin tablets. Disintegration and dissolution parameters of film-coated erythromycin stearate tablets proved useful in describing bioavailability parameters (7, 8).

In the present study, the pharmaceutical ***in-vitro*** properties of some commercial brands of film-coated erythromycin stearate tablets marketed in Egypt and one brand of enteric-coated erythromycin tablets were assessed. Further, an attempt was made to correlate the results obtained with bioavailability data for these products reported in an earlier ***in-vivo*** study (9).

Experimental:

Nine batches of different brands of erythromycin stearate film-coated tablets manufactured locally and one batch of an imported brand of enteric-coated tablets of erythromycin base were examined . These products are listed in ***Table I*** . They include the batches used in the bioequivalence study (9). The following tests have been carried out:

1- Uniformity of weight: Determined using 20 tablets.

2- Drug content: The erythromycin content of tablets was determined spectrophotometrically at 236 nm following controlled alkaline hydrolysis. The method is outlined under dissolution rate.

3. Disintegration time: was determined according to the USP XXI disintegration tests for plain coated and enteric-coated tablets, respectively.

4. Dissolution rate: Dissolution rates were measured using the USP rotating basket dissolution apparatus (6-station dissolution tester with an automatic sampling device, Dissoette, Model QC 72R24-6M, Hanson Research, CA, U.S.A.) at 30± 0.2°C and 100 rpm. For film-coated tablets, the dissolution medium was 900 ml of 0.2M phosphate buffer (pH 7.5). The dissolution rate of enteric-coated tablets was measured in a NaCl/HCl solution (pH 1.2) which was changed after 1h to pH 7.5 by adding 50 ml of dibasic potassium phosphate solution (13.6%) and 7ml of 10N NaOH solution. Dissolution samples were removed periodically for up to 120 min. and were assayed immediately.

Assay method: The amount of erythromycin dissolved

Table I. **Brands of Erythromycin Tablets Under Study**

Brand	Drug form	Strength (mg as base)	Coat	Batch No.
A	Stearate salt	250	film	A_1: 109028
				A_2: 172037
				A_3: 122114
B	Stearate salt	250	film	B_1: 873693
				B_2: 863271
				B_3: 862892
$\bar{B}$	Stearate salt	500	film	$\bar{B}_1$: 873933
				$\bar{B}_2$: 870725
				$\bar{B}_3$: 863019
C	Base	250	enteric	C_1: 672 RD

***Table II: In-vitro* Characterization Data of Erythromycin Products Under Study**

Brand	Product age, month[a]	Av. tablet wgt, g ± SD	Drug content[b]	Disint. time,min.	% dissol. 30'	120'
A_1	-[c]	1.481±0.009	104.4	39	14	44
A_2	1	0.487±0.011	105.9	14	32	69
A_3	>24	0.496±0.021	102.7	32	18	45
B_1	4	0.789±0.005	103.0	54	23	63
B_2	16	0.771±0.010	107.3	21	12	37
B_3	17	0.790±0.013	106.8	43	15	50
$\bar{B}_1$	4	1.138±0.008	103.8	10	42	67
$\bar{B}_2$	11	1.124±0.012	104.6	13	65	89
$\bar{B}_3$	17	1.146±0.030	102.7	5	44	83
C_1	-[c]	0.489±0.006	95.9	-[d]	70	100

a : The difference between manufacturing and experimentation dates. b: Percent of labeled amount as determined by a chemical assay method. c: No manufacture date was stated on the package. d: Tablets did not disintegrate in simulated gastric fluid but disintegrated in 3 min. in simulated intestinal fluid.

was determined spectrophotometrically (10). The method was found to produce results comparable to those obtained from the USP microbiological assay (11). The method involves controlled alkaline hydrolysis of erythromycin, producing a compound having a λ max at 236 nm. For each analyzed sample, an acid-inactivated blank is prepared by treating the erythromycin test solution with 0.05N H_2SO_4 at R.T. for 1h with susbsequent alkaline hydrolysis. This blank corrects for the UV absorbing impurities and degradation products of erythromycin. For maximum absorbance at 236 nm, the samples containing erythromycin should be heated with 0.2% NaOH (pH≈11) in a boiling water bath for 7 min. Dissolution samples were diluted with H_2O before proceeding with the assay. The volumes of 0.2%NaOH and 0.05N H_2SO_4 added to the dissolution samples were varied according to the pH of the dissolution medium in order to adjust the pH of alkaline-treated samples to ≈ 11 and acid-inactivated samples to ≈ 2-3. A calibration curve was constructed using erythromycin powder (Abbott). Beer's law was obeyed in the concentration range tested (1-8 mg/100 ml).

RESULTS and DISCUSSION

All batches met pharmacopoeial requirements of uniformity of weight ***(Table II)***. Erythromycin content of tablets from different batches was within ± 10% of the labeled amount ***(Table II)***.

All batches studied passed the USP XXI disintegration test, though inter-brand and inter-batch variations were observed among film-coated tablet products ***(Table II)***. Further, brand $\bar{B}$ tablets disintegrated more rapidly than brand **B** tablets produced by the same manufacturer. Tablets of some batches of erythromycin stearate products showed complete coat and core disintegration in simulated gastric fluid. This has raised a question concerning possible inactivation of the drug by gastric acid, as erythromycin stearate dissolves in and is decomposed by acids (2). It has been shown that the bioavailability of erythromycin stearate can be increased by protecting the core tablets with a film-coating that withstands low pH and susceptible to disintegrating rapidly at pH values higher than 4.5 (5).

Dissolution rates were determined under conditions precluding erythromycin degradation (pH 7.5 and 30°C). These conditions have been selected carefully based on reported stability data which

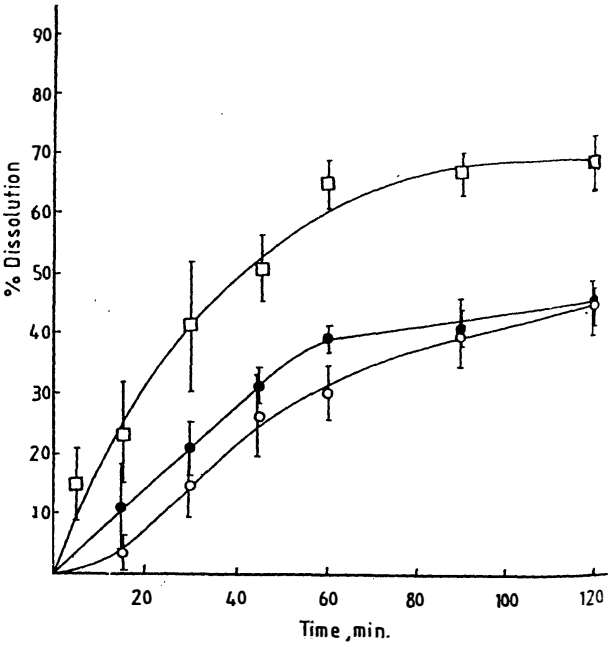

Fig.1: **Dissolution rates of batches A_1 (●), A_2 (□) and A_3 (○) of brand A tablets (250 mg) at pH 7.5 and 30°C.**

have been indicative of the rapid inactivation of erythromycin at pH values higher and lower than the pH-range 7-8 and at 37°C (12,13). Phosphate buffer was used as a dissolution medium since it is the buffer species with the least detrimental effect on erythromycin stability (13). Moreover, this buffer has been selected for the dissolution testing of erythromycin tablets in previous studies (11,14). It should be noted that there was no pharmacopoeial dissolution test for erythromycin stearate tablets at the time the study was conducted. A dissolution test has been introduced later on in USP XXII (15).

The dissolution profiles of the tablet products under study are shown in ***Figs. 1-3***. Film-coated erythromycin stearate tablets exhibited generally poor dissolution characteristics with considerable inter-brand and inter-batch variability in both rates and extents of dissolution. The mean % dissolution of tablets of three batches of brand A ranged from 43 to 70% in 120 minutes.

Dissolution rates of brand **B** tablets were markedly low with wide inter-tablet variability, particularly in the case of batch B_2 ***(Fig.2).*** This is illustrated in ***Fig.4*** which shows the % dissolution at one hour of 12 tablets of this batch. In most cases, dissolution failure was a consequence of the lack of disintegration of tablets during the dissolution experiment, though this batch of tablets passed the USP XXI disintegration test for plain coated tablets using a guided disc ***(Table II)*** . Enhanced break-up of the tablets during the disintegration test results from the up and down movement and impact of the disc. These results obviously indicate formulation problems and/or nonuniform manufacturing conditions, leading to differences in coat thickness and hardness. Differences in the color intensity of the coat were also noted in tablets of this batch.

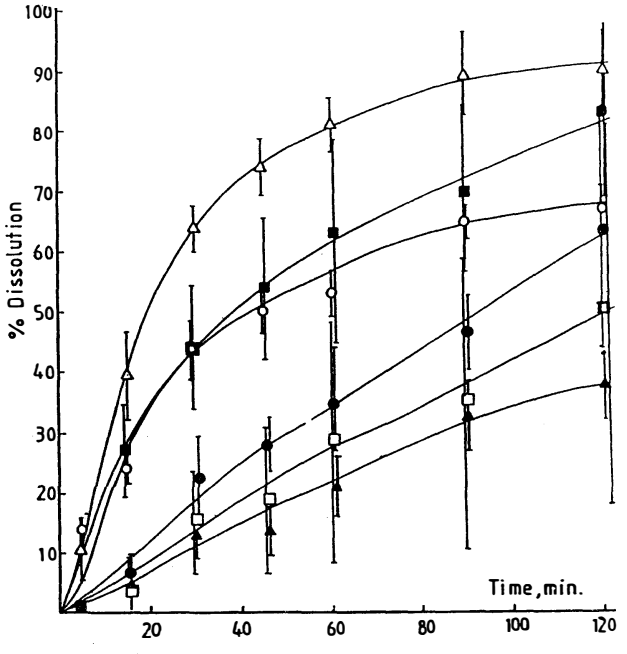

Fig. 2: **Dissolution rates of batches B_1 (●), B_2 (□) and B_3 (▲) of brand B tablets (250 mg) and batches $\bar{B}_1$ (○), $\bar{B}_2$ (△) and $\bar{B}_3$ (■) of brand $\bar{B}$ tablets (500 mg) at pH 7.5 and 30°C.**

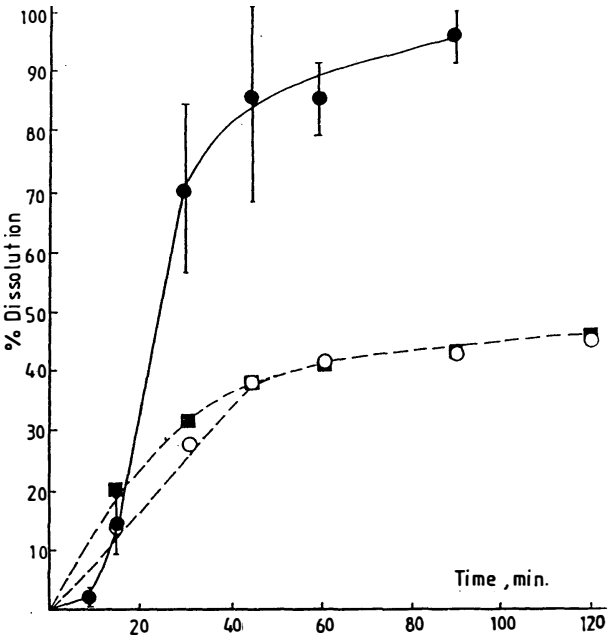

Fig. 3: **Dissolution rate of brand C tablets (●) and erythromycin stearate powder (---) using the rotating basket (○) and the paddle (■) methods at pH 7.5 and 30°C.**

Brand $\bar{B}$ tablets (500 mg) showed a superior dissolution behaviour compared to brand B tablets (250 mg) from the same manufacturer ***(Fig. 2)***. Significant dissolution differences between erythromycin stearate tablet formulations declaring 250 and 500 mg were reported by Philip and Daly (8). As the concentration of the antibiotic and excipients were indentical, the authors attributed such differences to variations in the intrinsic dissolution rate of the two different lots of erythromycin stearate raw material used for the formulations. They also found that the % dissolution at one hour at pH 6.6 and 22°C of ten lots of erythromycin from different sources to vary from 27 to 100%.

Variations in the intrinsic dissolution properties of erythromycin stearate can be accounted for by the varying content of stearic acid and sodium stearate. According to BP specifications, erythromycin may contain up to 18.5% of stearic acid and 6% of sodium stearate. In the present study, a sample of erythromycin stearate powder showed poor dissol-

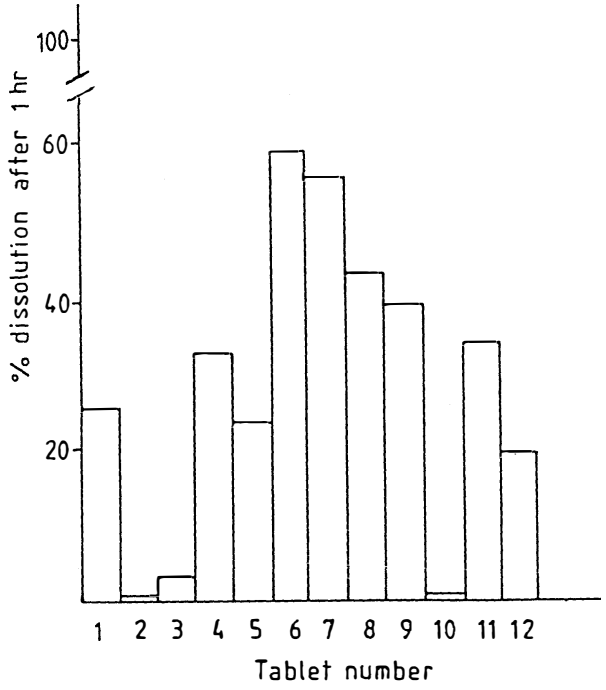

Fig.4: **Inter-tablet variability in % dissolution after 1h of batch B_2 tablets (film-coated erythromycin stearate tablets, 250 mg) at pH 7.5 and 30°C.**

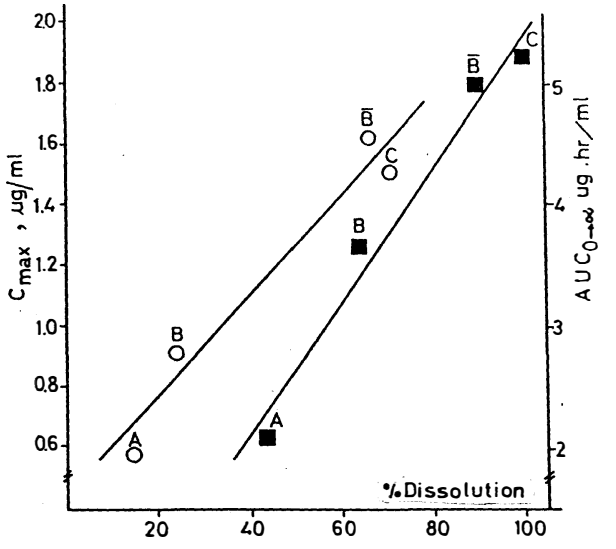

Fig. 5: **Correlation between dissolution data and bioavailability parameters of erythromycin products under study. Relationship between % dissolution at 30 min. and Cmax, µg/ml (○) and relationship between % dissolution at 120 min. and AUC, µg. h/ml (■).**

ution using either the rotating basket or the paddle method ***(Fig. 3).*** The amount of drug dissolved in 120 minutes was less than 50%.

On the other hand, enteric-coated tablets containing erythromycin base (brand **C**) exhibited excellent dissolution properties at pH 7 .5 ***(Fig.3),*** 100% dissolution was attained in 120 minutes. This brand proved to be of superior technical and pharmaceutical quality.

Disintegration times and dissolution rates of commercial brands of film-coated and enteric-coated erythomycin products were reported to decrease upon storage at relatively high humidity and temperatue(11). Product age-dependent changes in the ***in-vitro*** properties of tablets were not noted in the present study. Disintegration times and dissolution data (mean % dissolved at 120 min.) of film-coated erythromycin stearate tablets did not show a rank correlation of these parameters with the product age (difference between manufacturing and experimentation dates, (***Table II***).

In order to assess the usefulness of ***in-vitro*** characterization data of erythromycin tablets in predicting ***in-vivo*** performance, these data were correlated with bioavailability parameters obtained for these tablets in a single-dose fasting bioavailability study in man (9) . The rank order of ***in-vivo*** performance of the products as indicated by $AUC_{0-\infty}$, µg. h/ml values was: brand **C** > brand **B̄**> brand **B** > brand **A**. Disintegration times could not be correlated with these data. It must be noted that the disintegration test has been omitted from the official monograph of erythromycin and erythromycin stearate tablets in USP XXII . A quantitative correlation, however, could be established between dissolution data and ***in-vivo*** parameters. ***Figure 5*** shows correlations between the % dissolution at 30 minutes and C max, µg/ml (r=0.9721) and the % dissolution at 120 minutes and AUC (r=0.9821). This indicates utility of dissolution data in discriminating between fast and slowly absorbed products and predicting erythromycin absorption.

It can therfore be concluded that film-coated erythromycin stearate tablets exhibit considerable inter-brand and inter-batch variability regarding ***in-vitro*** properties. Formulation and coating problems as well as the intrinsic physicochemical properties of the drug substance appear to be greatly implicated in such a variability. Enteric-coated erythromycin base tablets showed a much better performance which can be connected with good bioavailability. ***In-vitro*** studies have to be undertaken continually to ascertain the quality of commercial erythromycin coated tablets, including different strength tablets from the same manufacturer and to reveal potential defects in pharmaceutical formulation and manufacturing conditions. Based on the ***in-vitro/in-vivo*** correlations obtained, dissolution data can be used for the continued monitoring of the production and bioavailability of erythromycin tablet products.

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Evidence for an α_2-Presynaptic Blocking Activity of Levamisole in the Isolated Rat Vas Deferens

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Abstract ❑ The effects of levamisole on the sympathetic transmission and the mechanism underlying these effects were investigated in the field-stimulated rat vas deferens. Levamisole (5-80 μM) potentiated the contraction of the vas deferens induced by field stimulation at low and high frequencies (2, 20 Hz), in a concentration-dependent manner. The extent of potentiation at low frequency was greater than that at high frequency. However, noradrenaline-induced contractions were only potentiated at high concentrations of levamisole (40, 80 μM). The potentiating effect of levamisole on electrically evoked muscle twitches was completely abolished in the presence of yohimbine and only a slight potentiation was obtained with high concentrations of levamisole. Moreover, in the presence of levamisole (20 μM), the concentration-response curve of clonidine was shifted to the right and the ED50 of clonidine was increased by three-fold. Levamisole was capable of recovering the electrically-evoked muscle contraction after being abolished by cocaine. These data provide a strong evidence for an α_2-presynaptic blocking activity of levamisole in the isolated rat vas deferens and confirmed its reported uptake blocking activity, only at high concentration.

Keyphrases ❑ Levamisole, clonidine, noradrenaline, rat vas deferens, α_2-presynaptic receptors.

Levamisole, is a well known broad spectrum anthelmintic (1) with a potent antidepressant activity (2). Most antidepressant agents interfere with peripheral adrenergic nerve endings, mainly through a cocaine-like blockade of neuronal uptake of noradrenaline (NA) (3,4). This cocaine-like action was reported for levamisole on the saphenous vein strips and circulatory system of the dog (5,6), and on the guinea pig vas deferens (7). This action was further supported by the observation of Pires and Co-workers (8) that levamisole inhibited the tyramine-induced inotropic effects on the guinea pig heart. However, Vanhoutte ***et. al.*** (5) found that levamisole augmented the responses of tyramine in the dog saphenous vein strips, a finding that is not in agreement with the proposed uptake blocking activity reported for levamisole. Moreover, levamisole has been reported to increase the tritium overflow of NA elicited by electrical stimulation in the saphenous vein strips pretreated with cocaine, indicating that the neuronal uptake mechanism of levamisole is not solely the mechanism responsible