

FORMULATION AND EVALUATION OF TRIMETHOPRIM-
SULFAMETHOXAZOLE SUPPOSITORIES.

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ABSTRACT

Suppositories containing a mixture of trimethoprim (80 mg) and sulfamethoxazole (400 mg) per each were prepared using the fusion method. Witepsol H15 with and without 10% Tween 60 as the mixture of PEG 6000, 4000, 1540 (47:33:20) were used as suppository bases. The physico-chemical properties, in-vitro and in vivo availability were investigated. The amount of drug released showed that Witepsol H15 with 10% Tween 60 gave the highest amount of SMZ and TMP released. Meanwhile, no much difference has been found in case of suppositories made with Witepsol H15 and the mixture of PEG bases. This indicated that the incorporation of Tween 60 in 10% concentration has a great role in enhancing the release properties of both drugs. The bioavailability study has proved the same findings of the in-vitro investigations, as it was found that Witepsol H15 incorporated with surfactant was the most suitable base for the preparation of TMP-SMZ suppositories.

INTRODUCTION

Trimethoprim (TMP) and sulfamethoxazole (SMZ) antibacterial spectrum is quite similar, but the former drug is usually 20 to 100 times more potent than the latter¹. The combination of those two drugs is bactericidal, while each one alone is bacteriostatic agent². Extensive clinical studies have shown

that this combination has therapeutic efficacy in urinary tract infections^{3,4}, respiratory tract infections, primary acute and chronic bronchitis^{5,6}; gonorrhoea⁷; and salmonella infections⁸. Pharmacokinetic studies of TMP and SMZ alone or in combination have been reported⁹⁻¹¹. TMP-SMZ combinations (co-trimoxazole) are currently supplied in different official preparations as suspensions, tablets and injections¹². Meanwhile, no work has been done concerning the formulation of the combination of TMP-SMZ in form of suppositories. This initiated our interest to present another route of administration which might be suitable for children, and when the oral or injectable administration is not recommended. In the present study, an attempt has been made to formulate suppositories containing the combination of TMP-SMZ using a fat soluble base (Witepsol H15) and water soluble base (mixture of PEG). Incorporation of 10% Tween 60 in Witepsol H15 and its effect on the release characteristics has been studied. Furthermore, the effect of formulation factor on in-vitro and in-vivo availability has been also investigated.

EXPERIMENTAL

Materials:

Sulfamethoxazole (SMZ) and Trimethoprim (TMP) (Kahira Pharm. Co., Egypt); Witepsol H15 (Dynamit Nobile, W. Germany); PEG 6000, 4000, 1540 (Prolabo, France); Cellophane membrane, Spectrapor M.W. Cutoff: 12,000-14,000 (Fisher Sci. Co., U.S.A.); N-(1-Naphthyl)-ethylene diamine hydrochloride (E-Merck, Dermstadt); Hydrochloric acid and chloroform (Prolabo, France) and other chemicals used were analytical grade.

Formulation and Evaluation of Trimethoprim-Sulfamethoxazole Suppositories.

Equipments:

Erweka hardness tester, model SBT (W. Germany); Erweka deformation tester model SSP (W. Germany); Two-gram suppository mould (Erbo, GMBH, Albstad, W. Germany); Spectrophotometer (Perkin Elmer 505); Colorimeter corning, model 252- (Medical corning Ltd., Kalstead, Essex CO 92 DX England) and MSE minor centrifuge model II (MSE, Scientific Inst. Manor Royal, Crawley RH 10 2 QQ, Sussex, England).

Methods:

1- Preparation of suppositories:

Fusion method was used to prepare 2 gm suppositories, each containing 400 mg SMZ and 80 mg TMP. Witepsol H15 with and without 10% Tween 60 was used as an example of fatty base; also a mixture of PEG 6000, 4000 and 1540 (47:33:20) was selected as a water soluble base. The prepared suppositories were stored at 3-5°C for two days, then for another two days at room temperature before testing.

2- Evaluation of the prepared suppositories:

A- Physical Properties:

Suppositories were evaluated for hardness and deformation according to USP, BP and BPC requirements, as well as the content uniformity.

B- Spectrophotometric determination:

The U.V. spectra of pure drugs as well as the drugs in presence of PEG were recorded along 230-300 nm. The I.R. spectra were also recorded using KBr disc method.

C- Thin layer chromatography (TLC):

This was carried out to detect any interaction or complex formation if present between any of the two drugs with the mixture of PEG used for the preparation of suppositories of SMZ-TMP.

A certain weight of each drug separately as well as an equivalent weight of suppository mass were dissolved in certain volume of alcohol. The solutions were then spotted on the prepared fluorescent silica gel plates. They were developed by a mixture of chloroform and methanol at a ratio of 100 : 10¹³ and by a mixture of n-butanol, chloroform and diethylamine in a ratio of 45:45:5¹⁴, in case of SMZ and TMP respectively. After development, the plates were dried and the spots were located by means of U.V. lamp at 254 nm, where the spots appeared violet on a green black ground in case of SMZ; however in case of TMP the spots were located by iodine vapour.

D- Drug release characteristics and determination of SMZ and TMP:

One suppository was placed onto a glass tube (1.5x 3.5 cm) covered with a cellophane membrane firmly tied¹⁵. The tube was vertically suspended in a beaker containing 30 ml of distilled water to be used as dissolution medium and the temperature of which was maintained at 37°C ± 0.5°C using a thermostatically controlled water-bath. One-ml aliquot was withdrawn at certain time intervals and replaced by the same volume of distilled water. The sample taken was diluted with distilled water and adjusted to pH 7.2 by addition of 1N NaOH and then measured spectrophotometrically at 240.5 and 255 nm. The amount of SMZ and TMP was determined using the equation of Pernarowski et al¹⁶ which was applied in case of SMZ and TMP by Ghaneim et al¹⁷ as follows

$$C_{\text{TMP}} = \frac{Q_0 - Q_{\text{SMZ}}}{Q_{\text{TMP}} - Q_{\text{SMZ}}} \cdot \frac{A}{a_i}$$

$$C_{\text{SMZ}} = \frac{Q_0 - Q_{\text{TMP}}}{Q_{\text{SMZ}} - Q_{\text{TMP}}} \cdot \frac{A}{a_i}$$

Formulation and Evaluation of Trimethoprim-Sulfamethoxazole Suppositories.

Where:

C_{TMP} and C_{SMZ} are the concentration in gm/litre of TMP and SMZ respectively; Q_o is the absorbance ratio value of the binary mixture; Q_{TMP} and Q_{SMZ} are the absorbance ratio values of TMP and SMZ respectively; $Q_{255:240.5}$ of TMP and SMZ were found to be 0.212 and 1.378 respectively¹⁷; A and a_i are the absorbance of the mixture and the absorbitivity of the components respectively, both at the isoabsorptive wave length.

E- Bioavailability Study:

Six adult healthy male human volunteers weighing 65-90 Kg were selected for this investigation. All subjects were refrained from any medications one week before proceeding the experiments. Each subject received the three formulae one week apart and served as a control. Along 24 hours the urine samples were collected quantitatively. For the first three hours, the urine was collected each hour, then after 3,6,12 and 24 hours.

TMP was determined spectrophotometrically by using the method of Bushby and Hitchings¹⁸. The urine samples were centrifuged at 9000 r.p.m. for five minutes, then extracted twice by chloroform after its alkalization with sodium carbonate. The combined chloroform extracts were washed with water and then extracted with 0.1N hydrochloric acid and measured spectrophotometrically at 270.5 nm. SMZ was determined using Bratton-Marshall method¹⁹.

RESULT AND DISCUSSION

1- Physical Properties:

Suppositories containing a combination of SMZ (400 mg) and TMP (80 mg) were prepared as a new formulation containing those two drugs. The prepared suppositories were exhibiting a good mechanical properties and were found to be in a good agreement with USP, B.P and EPC requirements. The drugs content uniformity was within the limits prescribed by B.P(1980). The deformation time for the prepared suppositories was found to be dependent on the type of the base and it was found to be 11, 19 and 29 minutes for Witepsol H15 containing 10% Tween 60, Witepsol H 15 and mixture of PEG respectively. The hardness was found to be 3.0, 3.4 and 3.5 kg for the same sequence of bases. Incorporation of Tween 60 into the Witepsol H15 was found to decrease both deformation time and hardness of suppositories. This is in agreement with the findings of Mezey and Regdon²⁰ and Swinyard¹², where the disintegration time of suppositories decreased by adding Tween or spans.

The absence of any interaction between the drugs and PEG polymers was confirmed by the spectrophotometric and TLC investigations. The λ max of U.V spectra of both drugs either free or in the presence of P G polymers used was the same. I.R spectra were unaffected due to the presence of PEG. TLC showed only two spots corresponding to the parent drugs.

2- Release Characteristics:

Figure 1. represents the release characteristics of SMZ and TMP as a function of suppository bases. For both drugs the release pattern could be arranged as follows; Witepsol H15 with 10% Tween 60 > PEG mixture > Witepsol H15. Suppositories of Witepsol H 15 containing surfactants shows the highest amount

Formulation and Evaluation of Trimethoprim-Sulfamethoxazole Suppositories.

of SMZ and TMP released, while the release pattern was almost the same in case of using PEG and Witepsol H15 bases. The effect of surfactant on increasing the release rate from Witepsol H15 might be due to the self emulsifiable character of Tween 60 which might aid dispersion of medicaments throughout the surrounding medium. The surfactants may increase the rate of diffusion through the cellophane membrane. Ravin et al²² reported that, the effect of surfactants might be attributable to their surface character and rapid wettability of the drug with the diffusion medium. The results obtained were in agreement with Mezey and Regdon²⁰, Mihaly²³ and Ayres et al²⁴.

3- Bioavailability Study:

The urinary excretion method in human applied in this work for the assessment of the bioavailability of TMP and SMZ has been reported to correlate well with the corresponding blood concentration data in the assessment of the bioavailability of sulfa drugs²⁵ and TMP²⁶. The mean cumulative amount of TMP and SMZ excreted unchanged in urine at each sampling time for the formulae of suppositories is given in Table 1; and graphically illustrated in Figure 2. Table 1. shows that, the mean of the amounts for 24-hours urine recovery is ranging from 8.6 - 20.2 mg and from 9.8 - 16.0 mg for TMP and free SMZ respectively. Suppositories of Witepsol H15 incorporated with Tween 60 (10%) showed the highest extent of excretion for both drugs at various time intervals. For suppositories made from Witepsol H15, Witepsol H15 with 10% Tween 60 and PEG, the cumulative amount of TMP excreted after 24 hrs. were 31.3, 35.9 and 31.4 and for SMZ were 32.8, 62.1 and 42.8 respectively. Several investigations were reported for rectal absorption of sparingly soluble drugs from suppository bases incorporated with surfactants²⁷⁻³⁰. Analysis of variance³¹ for the mean cumulative excreted amount from the three formulae showed a significant differences among the three formulations ($P < 0.05$).

The elimination rate constant K_E for TMP and SMZ were determined from the slope of the linear semilogarithmic plots of the amount remaining to be excreted in the urine at various time intervals²⁶ as represented in Figure 3. Values obtained in Table 2 for K_E in case of SMZ and TMP were in agreement with those reported values^{12,32,33}. The $t_{\frac{1}{2}}$ was calculated to range from 23 to 24 hrs. for TMP, and from 17.3 to 28.8 hrs. for SMZ and these values were found to comply with literature^{26,34}.

In conclusion, the combination of SMZ and TMP could be formulated in form of suppositories as an alternative route of administration which would be more preferable in case of patients suffering from difficulties in oral or injectable administration. Rectal route of administration could be more convenient in case of children. In this formulation and from the data obtained in the present investigation, Witepsol H 15 with surfactants is highly recommended as suppository base for the mixture of SMZ and TMP.

Formulation and Evaluation of Trimethoprim-Sulfamethoxazole Suppositories.

Table 1: Amount of Unmetabolized SMZ and TMP Excreted in urine after rectal administration of one suppository containing 400 mg and 80 mg of SMZ and TMP respectively.

Time in hours	Suppository bases					
	Witepsol H 15		Witepsol H 15 + 10% Tween 60		PEG mixture	
	SMZ mg.	TMP mg.	SMZ mg.	TMP mg.	SMZ mg.	TMP mg.
1	1.050	0.467	1.479	2.900	0.438	2.680
2	1.726	0.646	5.230	3.950	1.083	2.308
3	1.650	0.782	5.150	3.900	1.931	3.127
6	7.105	2.226	14.856	8.028	14.245	4.949
12	8.053	7.062	20.441	8.571	12.212	8.563
24	13.257	20.197	16.000	8.579	12.931	9.838
(*)	32.841	31.380	62.156	35.928	42.840	31.470

(*) Cumulative amount excreted in urine for SMZ and TMP.

Table 2. Elimination Rate Constant and Half-Lives of Elimination of Unmetabolized SMZ and TMP from Different Suppository Formulations.

Formulae	SMZ		TMP	
	K_E	$t_{1/2}$ (h)	K_E	$t_{1/2}$ (h)
Witepsol H 15	0.030	23.10	0.020	34.65
Witepsol H 15+ 10% Tween 60	0.040	17.33	0.025	27.72
PEG Mixture	0.024	28.88	0.030	23.10

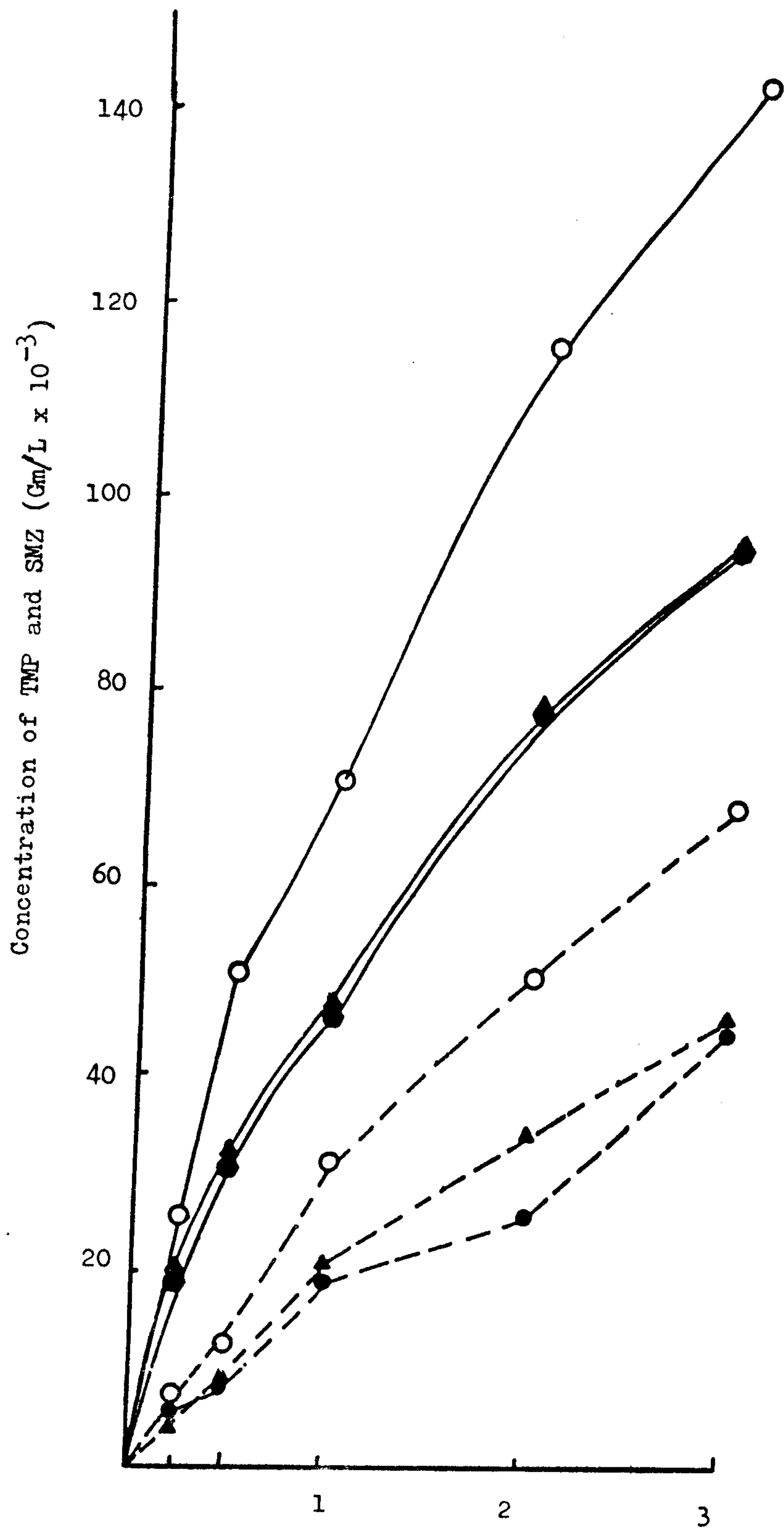


Figure 1: Amount released of SMZ (---) and TMP (—) from suppositories made with ● Witepsol H15; ○ Witepsol H15+10% Tween 60 ▲ PEG mixture.

Formulation and Evaluation of Trimethoprim-Sulfamethoxazole Suppositories.

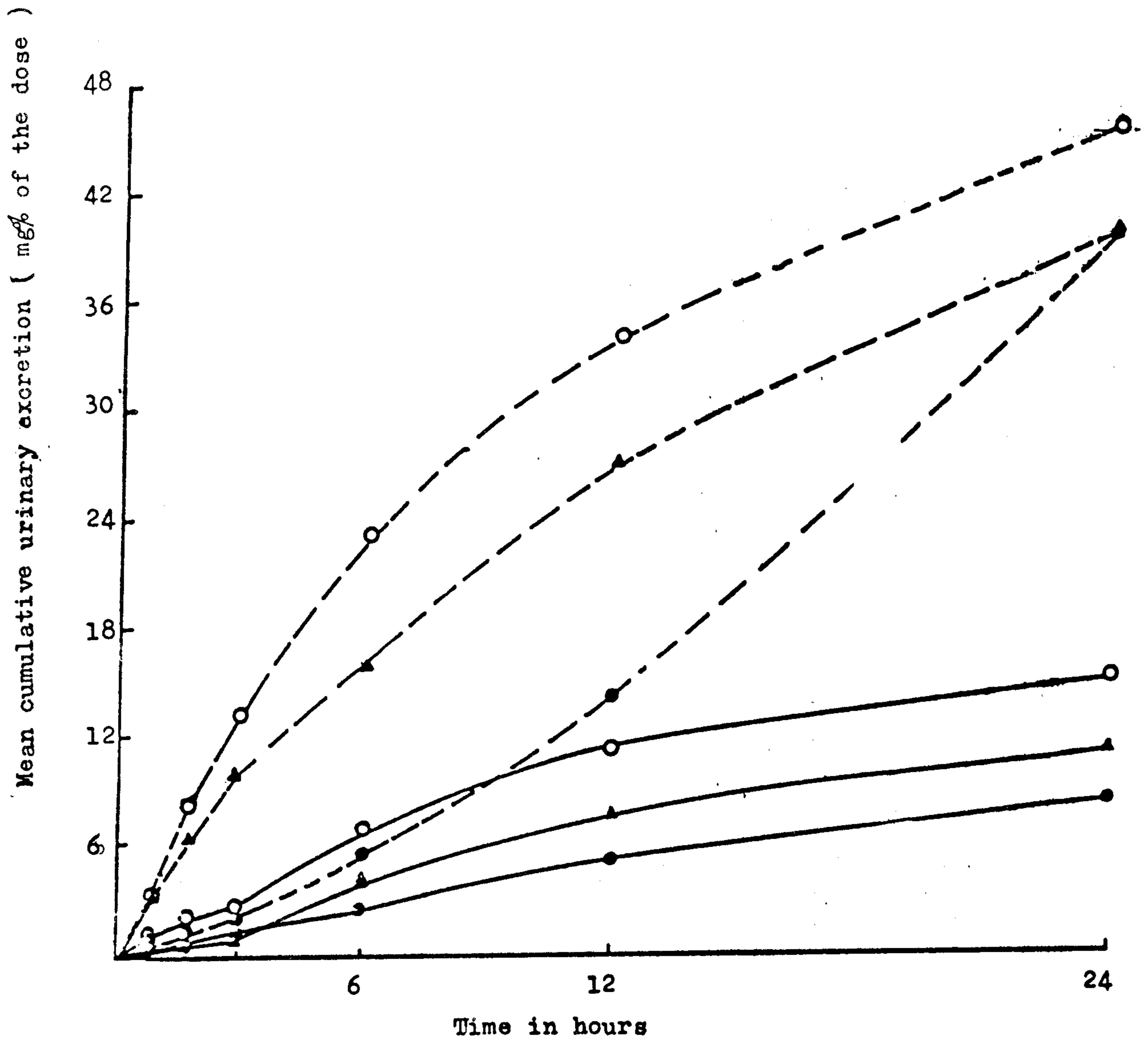


Figure 2: Mean cumulative percent of unmetabolized TMP (----) and unmetabolized SMZ (—) excreted in urine following rectal administration.

Key: As in Figure 1.

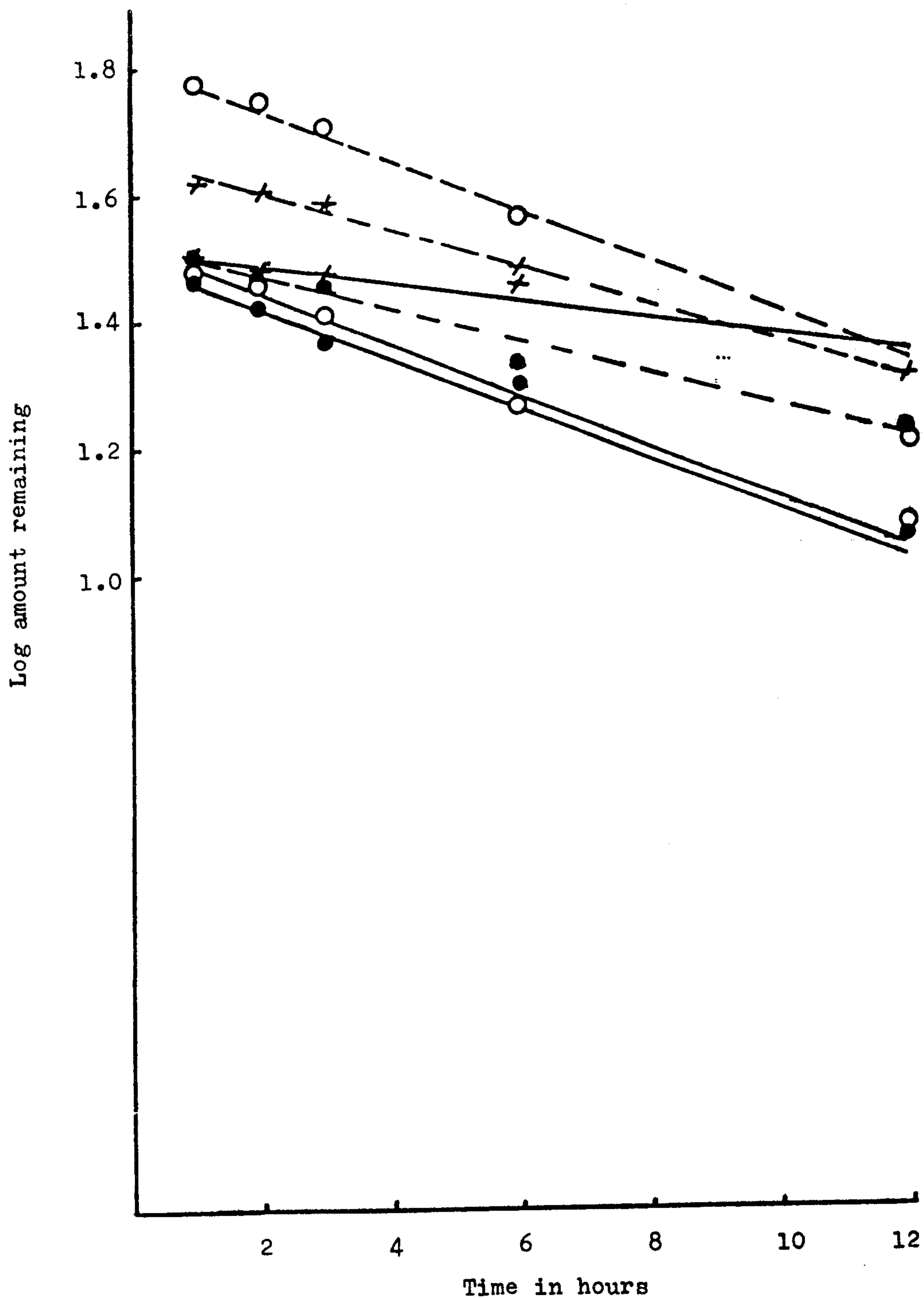


Figure 3. Calculation of the elimination rate constant by semilogarithmic plotting of the amount of SMZ (---) and TMP (—) remaining to be excreted in the urine at various time intervals after rectal administration.
 ● Witepsol H 15; ○ Witepsol H15 + 10% Tween 60 and X PEG mixture.

Formulation and Evaluation of Trimethoprim-Sulfamethoxazole Suppositories.

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صياغة وتقييم اقماع الترايميثوبريم والسلفا ميثوكسازول

عبد الجواد حلمى عبد الجواد - اسماعيل رمضان - احمد طلعت نـوـح
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يستخدم خليط الترايميثوبريم والسلفاميثوكسازول فى علاج بعض الامراض الناتجة عن الاصابات الجرثومية . ويتواجد هذا الخليط بنسبة ١ : ٥ فى العديد من الصور الصيدلية مثل الاقراص وحقن فى العضل وفى معلقات بينما لا يتواجد فى صورة اقماع شرجية . لذلك يهدف هذا البحث الى صياغة هذا المخلوط فى اقماع شرجية وقد حضرت هذه الاقماع باستخدام قاعدة ه ١٥ وخليط من عديد الايثلين جليكول كما استخدم توين ٦٠ بنسبة ١٠ % مع ويتبسول ه ١٥ لزيادة اتاحة كلا الدوائين وقد اظهرت النتائج الى اتاحة الدواء قد زادت باستخدام توين ٦٠ مع ه ١٥ الى جانب ان الاتاحة البولوجية قد زادت ايضا باستخدام توين ٦٠ .

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