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GELLIFIED EMULSION FOR SUSTAIN DELIVERY OF ITRACONAZOLE FOR TOPICAL FUNGAL DISEASES

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ABSTRACT

Gellified emulsion (Emulsion in gel) have emerged as one of the most interesting topical drug delivery system as it has dual release control system i.e. emulsion and gel. Also the stability of emulsion is increased when it is incorporated in gel. Itraconazole is an orally or topically active antifungal agent with a broad spectrum of activity. The objective of this project was to develop a Gellified emulsion for control delivery for Itraconazole. In present work we prepare emulsion and then incorporated in carbapol gel. The prepared formulation was evaluated on basis of pH, spreadability, Viscosity, drug content, in vitro release and Stability Studies. The microbial assay and Skin irritation studies on rabbit was also performed. The result of studied reveled that the optimized batch shows 95.08% release in 48 hours and stable for around three. The result of microbial assay compared with marketed product, the result shows 46.6% inhibition of optimized batch where as marketed preparation shows only 32.3% inhibition. While result of skin irritation test shows no edema and erythema. Hence it can be concluded that emulsion based system is more effective and safe system for sustain delivery of antifungal agent(s).

Keywords: Emulsion, Gel, Gellified emulsion, Itraconazole, Topical drug delivery

INTRODUCTION

Itraconazole is an orally or topically active antifungal agent with a broad spectrum of activity. In addition, the drug has an interesting tissue distribution, which has made possible effective and rapid treatments of candidiasis, when the drug is administered topically. A topical itraconazole-containing formulation may be of use for several reasons including the opportunity to generate high local tissue levels and lower systemic exposure. Most pharmaceutical drug substances are lipophilic compounds, which are practically insoluble in water. For skin care and the topical treatment of dermatological disease, a wide choice of vehicles ranging from solids to semisolids. Itraconazole is effective against several fungal strains such as *Candida albicans* and *Candida topicalis*, which are responsible for topical candidiasis in more than 25% of patients suffering from this condition. Candida-related fungal infection is a common skin disease affecting two thirds of all persons at least once during their lifetime

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Topical formulations apply a wide spectrum of preparations, both cosmetic and dermatological, to their healthy or diseased skin[1]. These formulations range in physicochemical nature from solid through semisolid to liquid. Drug substances are seldom administered alone, but rather as part of a formulation, in combination with one or more nonmedical agents that serve varied and specialized pharmaceutical functions. Drugs are administered topically for their action at the site of application, or for systemic effects[2]. Drug absorption through the skin is enhanced if the drug substance is in solution, if it has a favorable lipid/water partition coefficient, and if it is a nonelectrolyte. For the most part, pharmaceutical preparations applied to the skin are intended to serve some local action and, as such, are formulated to provide prolonged local contact, with

minimal systemic drug absorption. Drugs applied to the skin for their local action include antiseptics, antifungal agents, skin emollients, and protectants. The main advantage of topical delivery system is to bypass first pass metabolism. Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes, gastric emptying time are other advantage of topical preparations[3-4]. The topical drug delivery system is generally used where the others system of drug administration fails or it is mainly used in fungal infection. Human skin is a uniquely engineered organ that permits terrestrial life by regulating heat and water loss from the body whilst preventing the ingress of noxious chemicals or microorganisms. It is also the largest organ of the human body, providing around 10% of the body mass of an average person, and it covers an average area of 1.7 m^2. Whilst such a large and easily accessible organ apparently offers ideal and multiple sites to administer therapeutic agents for both local and systemic actions, human skin is a highly efficient self-repairing barrier designed to keep 'the insides in and the outside out'[5].

Emulgel is emulsions, either of the oil-in-water or water in oil type, which are gelled by mixing with a gelling agent[6]. Several antifungal agents are available on the market in different topical preparations (e.g. creams, ointments, and powders for the purpose of local dermatological therapy). One of these antifungal agents is Itraconazole, which has both antifungal and antibacterial properties[7-9]. It is applied locally in mild uncomplicated dermatophyte and other cutaneous infections. Gellified Emulsion is stable one and better vehicle for hydrophobic or water insoluble drugs[10-16]. It is an emulsion either of the oil-in-water or water in oil type, which are gelled by mixing with a gelling agent [11]. Oil-in-water emulsions are most useful as water washable drug bases and for general cosmetic purposes, while water-in-oil emulsions are employed more widely for the treatment of dry skin and emollient applications[17-20].

EXPERIMENTAL

Materials and methods

Itraconazole was received as a gift sample from Ranbaxy laboratories Ltd., dewas (India).Carbopol 934; Carbopol 940; Light liquid paraffin; Tween 20; Span 20; Propylene glycol; Methyl paraben; Propyl paraben were purchased from loba chemie , Mumbai. Ethanol; were purchased from c.y.company china. Double distilled water was used for all experiments. All chemicals were pharmaceutical grade and used without further modification.

Preparation of gellified emulsion

Itraconazole Gellified Emulsion was prepared. The Gel in formulations were prepared by dispersing Carbopol 934 in purified water with constant stirring at a moderate speed and Carbopol 940 in purified water with constant stirring at a moderate speed then the pH are adjusted to 6 to 6.5 using Tri Ethanol Amine (TEA).

The oil phase of the emulsion were prepared by dissolving Span 20 in light liquid paraffin while the aqueous phase was prepared by dissolving Tween 20 in purified water. Methyl and Propyl paraben was dissolved in propylene glycol whereas drug (Itraconazole) was dissolved in ethanol and both solutions was mixed with the aqueous phase. Both the oily and aqueous phases were separately heated to 70° to 80°C; then the oily phase were added to the aqueous phase with continuous stirring until cooled to room temperature. And add Glutaraldehyde in during of mixing of gel and emulsion in 1:1 and to obtain the Gellified Emulsion[5].

Optimization of emusified gel

Experimental design: Eight Itraconazole Gellified Emulsion formulations (Table 1) were prepared according to a 2^3 factorial design employing the qualitative factors and levels show in table 1 and table 2.

Table 1: Factor level for the 2^3 factorial designs

Factors	Levels
(A) Gelling agent type	+10 % - 5 %
(B) Liquid paraffin concentration	+7.5% -5%
(C) Emulsifying agent concentration	+2.5% - 1.5%

Table 2: Composition of Itraconazole gellified emulsion formulation

Composition	Formulation	Composition		
		A	B	C
(1)	F1	+	-	-
A	F2	+	+	-
B	F3	+	-	+
AB	F4	+	+	+
C	F5	-	-	-
AC	F6	-	+	-
BC	F7	-	-	+
ABC	F8	-	+	+

A, Gelling Agent type, B, liquid paraffin concentration, C, emulsifying agent concentration,Factor at low level, +, factor at high level.

Characterization of gellified emulsion

Physical appearance: The prepared Itraconazole Gellified Emulsion formulations were inspected visually for their color, homogeneity, consistency and pH. The pH values of 1% aqueous solutions of the prepared Gellified Emulsion were measured by a pH meter (Digital ph meter DPH 115 pm).

Spreadability: one of the criteria for a Gellified Emulsion to meet the ideal quantities is that it should possess good spreadability. It is term expressed to denote the extent of area to which gel readily spread on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from Gellified Emulsion and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability. It is calculated by using the formula (Fig. 1).

$S = M. L / T$

Where M = wt. tied to upper slide

L = length of glass slides

T = time taken to separate the slides

Extrudability study: In conducting the test, a closed collapsible tube containing above 20 grams of gel was pressed firmly at the crimped end and a clamp was applied to prevent any rollback. The cap was removed and the microencapsulated gel was extrudes until the pressure was dissipated (Fig. 2).

Rheological Study: The viscosity of different Gellified Emulsion formulation was determined at 37^0C using a brook field viscometer (Brookfield DV-E viscometer) with spindle 61. The recorded viscosities are collected in figure 3.

Drug Content Determination: drug concentration in Gellified Emulsion was measured by spectrophotometer. Itraconazole content in Gellified Emulsion was measured by dissolving Known quantity of Gellified Emulsion in solvent (methanol) by Sonication. Absorbance was measured after suitable dilution at 282 nm in UV/VIS spectrophotometer (UV-1700 CE, Shimadzu Corporation, Japan).

In Vitro Release Study: Franz diffusion cell (with effective diffusion area 3.14 cm^2 and 15.5 ml cell volume) was used for the drug release studies. Gellified Emulsion (200 mg) was applied onto the surface of egg membrane evenly. The egg membrane was clamped between the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with freshly prepared PBS (pH 5.5) solution to solubilize the drug. The receptor chamber was stirred by magnetic stirrer. The samples (1.0 ml aliquots) were collected at suitable time interval. Samples were analyzed for drug content by UV visible spectrophotometer at 282 nm after appropriate dilutions. Cumulative corrections were made to obtain the total amount of drug release at each time interval .The cumulative amount of drug released across the egg membrane was determined as a function of time.

Microbiological assay: Ditch plate technique was used. It is a technique used for evaluation of bacteriostatic or fungistatic activity of a compound. It is mainly applied for semisolid formulations. Previously prepared Sabouraud's agar dried plates were used. Three grams of the Gellified Emulsion are placed in a ditch cut in the plate. Freshly prepared culture loops are streaked across the agar at a right angle from the ditch to the edge of the plate. After incubation for 18 to 24 hours at 25°C, the fungal growth was observed and the percentage inhibition was measured as follows (1, 32):

% inhibition = L2 / L1 × 100

Where L1 = total length of the streaked culture, and

L2 =length of inhibition.

Skin irritation test: A 0.5 gm sample of the test article was then applied to each site (two sites per rabbit) by introduction under a double gauze layer to an area of skin approximately 1" x 1" (2,54 x 2,54 cm) square. The Gellified Emulsion re applied on the skin of rabbit. Animals were returned to their cages.

After a 24 hour exposure, the Gellified Emulsion are removed. The test sites were wiped with tap water to remove any remaining test article residue.

Accelerated stability studies of Gellified Emulsion: Stability studies were performed according to ICH guidelines. The formulations were stored in hot air oven at 37 ± 2°, 45 ± 2° and 60 ± 2° for a period of 3 months. The samples were analyzed for drug content every two weeks by UV-Visible spectrophotometer at 282 nm. Stability study was also carried out by measuring the change in pH of gel at regular interval of time.

RESULTS AND DISCUSSION

Physical appearance: The prepared Itraconazole Gellified Emulsion formulations were white viscous creamy preparation with a smooth and homogeneous appearance. The pH values of all prepared formulation

ranged from 5.4 to 5.8, which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

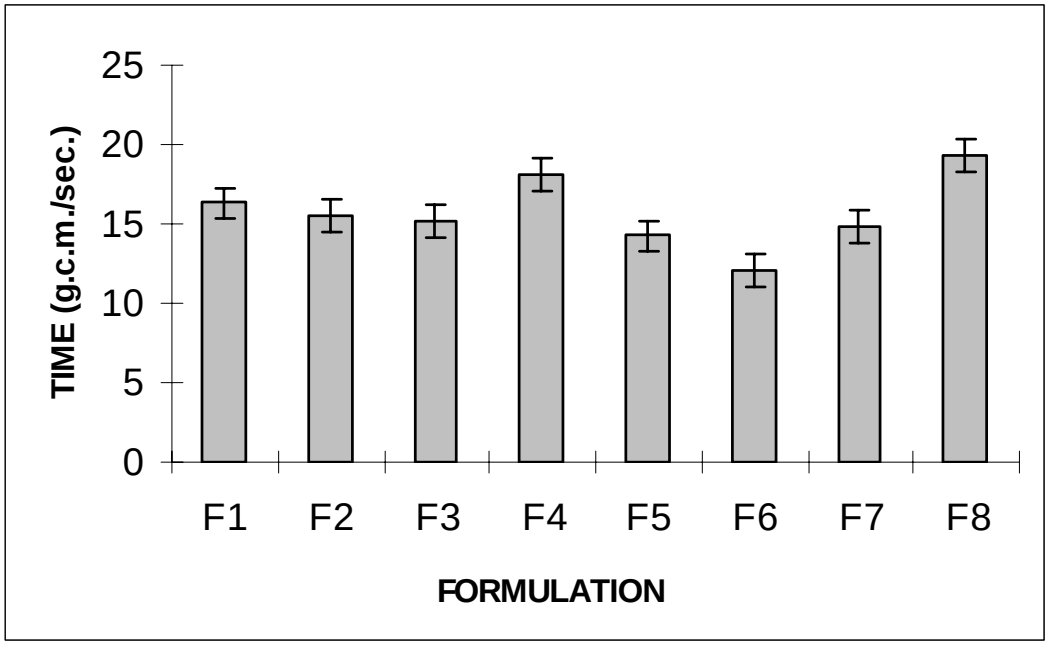

Fig. 1: Spreadability of the various gellified emulsion formulations (Mean ± S.D.)

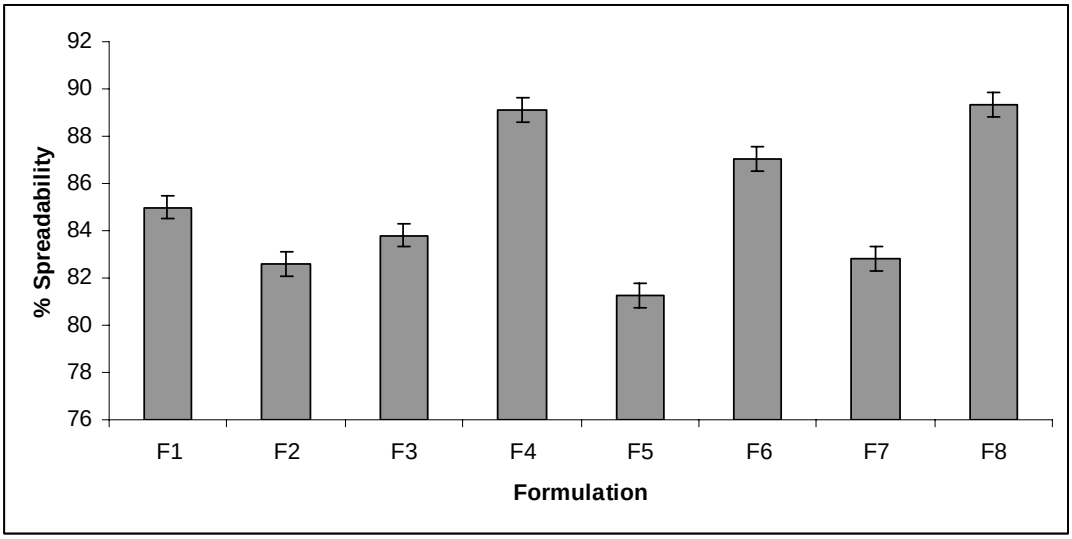

Fig. 2: Extrudability of the various gellified emulsion formulations (Mean ± S.D.)

Rheological studies: The measurement of viscosity of the prepared Gellified Emulsion was done with Brookfield viscometer (Brookfield DV-E viscometer). The Gellified Emulsion were rotated at 10 (min.) and 100 (max.) rotation per minute with spindle 61. At each speed, the corresponding dial reading was noted. The viscosity of the Gellified Emulsion was obtained (Fig.3).

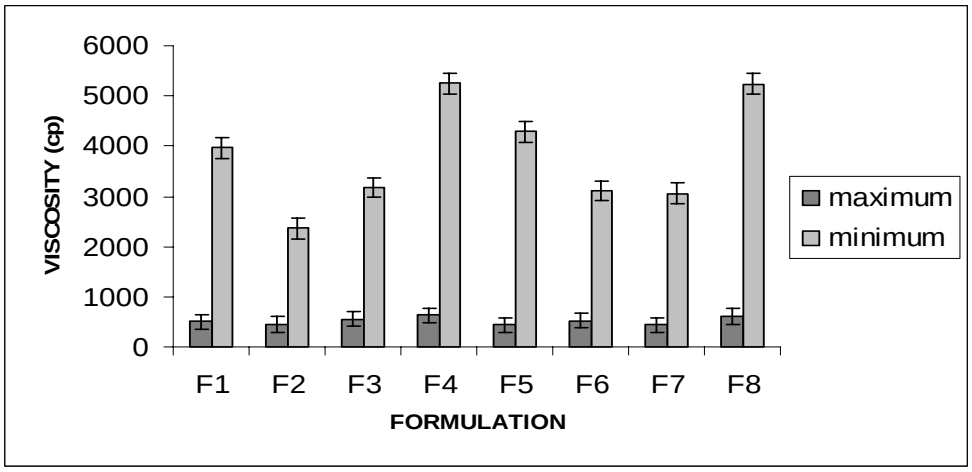

Fig. 3: viscosity of itraconazole gellified emulsion (Mean ± S.D.)

Drug content determination: 1 g of the prepared Gellified Emulsion was mixed with 100 ml of suitable solvent (methanol). Aliquots of different concentration were prepared by suitable dilution after Sonication and filtering the stock solution and absorbance was measured. Drug content was calculated using the equation, which was obtained by linear regression analysis of calibration curve. The drug content of all Gellified Emulsion formulation is given below (Fig.4).

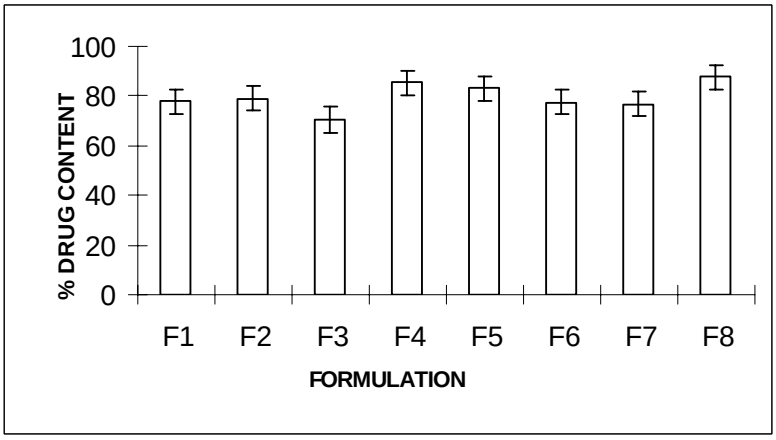

Fig. 4: comparing of drug content of various formulation of itraconazole gellified emulsion (Mean ± S.D.)

In vitro Drug Release: The in vitro release profiles of Itraconazole from its various Gellified Emulsion formulations are represented in Figure 5. It was observed that all the formulation had become liquefied and diluted at the end of the experiments, indicating water diffusion through the membrane. In general, it can be observed from figures that the better release of the drug from all Gellified Emulsion formulation. The release of the drugs from its Gellified Emulsion formulation can be ranked in the following descending order: F4 > F8> F5 > F6 > F3 > F1 > F7 > F2, Where the amounts of the drug release of the drug released after 24 hours were 95.8%, 84.2%, 67.94%, 66.14%, 60.7%, 58.06%, 45.06% and 34.19%, respectively. Thus the higher drug

release was observed with formulations F4 and F8. This finding may be due to presence of liquid paraffin in its High level and the emulsifying agent in its high level respectively, which lead to an increase in the hydrophilicity of the Gellified Emulsion, which, in turn, facilitates penetration of the release medium into the Gellified Emulsion and diffusion of the drug from the Gellified Emulsion. Add 0.1% of gluteraldehide to give the retard the release of drug from Gellified Emulsion formulation. Its proved that the presence of liquid paraffin led to retardation of Itraconazole release from its Gellified Emulsion formulation. The lower drug release from F5, which is Carbopol 940-based, than the drug release from F4 and F8, which is Carbopol 934-based, may be due to the higher viscosity of Carbopol 934 Gellified Emulsion formulations as observed in table 2 ,it may also be due to the drug content in the network structure of Carbopol 934. Opposing to F4 and F8 formulation, F7 and F2 showed the lowest drug release. In formulation F7 and F2, liquid paraffin in its low level / high level, while the emulsifying agent in its high level / low level respectively. Thus the 3 studied factors can be arranged according to their effect on the drug release from the Gellified Emulsion formulation as follows: the emulsifying agent concentration > liquid paraffin concentration > the gelling agent type.

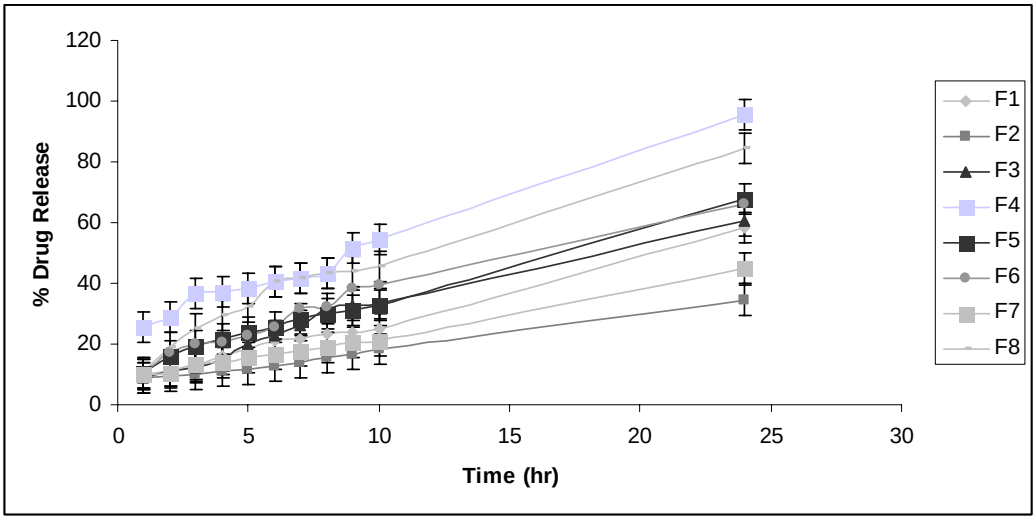

Fig. 5: Release profiles of Itraconazole from its emulgel formulations at 24 hours (Mean ± S.D.)

Microbiological assay: The use of control plates showed that the plain Gellified Emulsion bases were microbiologically inert toward the tested candida albicans strains. The antifungal activity of Itraconazole in its different Gellified Emulsion formulation as well as in its commercially available cream form. Percentage inhibition was taken as a measure of the drug antifungal activity. The Gellified Emulsion formulations were found to have the same rank order in their antifungal activities as in the in vitro release studies. Thus, the greatest activity was observed with formula F4 and F8, where the percentage inhibition reached up to 46.6% and 42.9% respectively, while the lowest activity was found with F2 and F3, where the percentage inhibition was 30.19% and 31.6% respectively.

Table 3: Percentage inhibition as a criterion for the antifungal activity of Itraconazole in its different gellified emulsion formulations

Formulation	% Inhibition
F1	41.6%
F2	30.9%
F3	31.6%
F4	46.6%
F5	39.7%
F6	38.5%
F7	32.6%
F8	42.9%
Marketed product	32.3%

Skin Irritation Test: The Primary Irritation Index of the test article was calculated to be 0.00; No irritation was observed on the skin of the rabbits

.Table 4: Skin irritation test of Itraconazole gellified emulsion on rabbit skin

No. of Rabbit	Erythema			Edema		
	4 hr	24hr	72hr	4hr.	24hr.	72hr
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0

Accelerated stability studies of emulsion based gel

The accelerated stability studies were performed according to ICH guidelines for 3 months and the results were found to be stable in varying temperature as shown in Table 5 and 6.

Table 5: Accelerated stability study of optimized emulsion based gel formulation F4

Storage Temp. ^{0}C	Period of studies in week		
	1 months	2 months	3 months
37 ± 2	93.6%	92.8%	93.9%
45 ± 2	94.8%	94.9%	95.8%
60 ± 2	92.8%	94.8%	92.9%
pH	5.6	5.7	5.6

Table 6: Accelerated stability study of optimized emulsion based gel formulation F8

Storage Temp. ^{0}C	Period of studies in week		
	1 months	2 months	3 months
37 ± 2	83.5%	85.2%	86.7%
45 ± 2	84.2%	82.6%	83.3%
60 ± 2	83.1%	85.2%	85.8%
pH	5.6	5.7	5.6

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