WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 12, 1233-1246. Research Article ISSN 2277–7105

FORMULATION AND EVALUATION OF MULTIPLE EMULSION OF CLOTRIMAZOLE

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Article Received on
02 Aug. 2021,

Revised on 23 Aug. 2021,
Accepted on 12 Sept. 2021

DOI: 10.20959/wjpr202112-21661

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ABSTRACT

Multiple emulsions dosage formulation of clotrimazole which has enhanced release and bioavailability properties with less inter and intra- subject variability would be desirable. Thus, it was aimed to formulate and evaluate the multiple emulsion of clotrimazole. By the preformulation studies it is observed that clotrimazole is a white to pale yellow crystals having no odor. λ_{max} was determined at 264 nm by scanning sample from 200-400nm and also calibration curve was obtained by absorbance of aliquots from 5-30 µg/ml. Partition coefficient was 1.797 obtained. Drug: Excipient Compatibility Studies at room temperature, 2^0C -8^0C and 45^0C -50^0C says it is stable. Stability also confirmed by FT-IR studies. All five Clotrimazole multiple emulsion formulations were odorless, washable, homogeneous, stable and free from grittiness and was evaluated under the various parameters, pH of all formulations were observed at 6.8 and viscosity between 40..23 to 76.38 centi poise and percentage of Drug content between 83.31 to 95.23%. *In-vitro* Drug Release of Clotrimazole multiple emulsion formulations were studied and found ME-1(87.61), ME-2(94.18), ME-3 (81.74), ME-4(83.74) and ME-5(78.62). Formulation **ME-2** was found excellent on the basis of cumulative percentage of drug release profile. All five formulations were also tested for stability and found formulation ME-2 was stable. Optimized formulation **(ME-2)** drug release data were fitted into the zero order, first order, Higuchi and Peppas-Korsemeyer model of drug release kinetics.

KEYWORD: Multiple Emulsion, Clotrimazole, Evaluation.

INTRODUCTION

Multiple emulsions are complex liquid description systems in which the droplets of the one dispersed liquid are further dispersed in another liquid.[1] The inner dispersed globule/droplet in the multiple emulsions are separated from the outer liquid phase by a layer of another phase.[2] There are mainly two types of multiple emulsions W/O/W and O/W/O emulsions.[3] Although, w/o/w emulsions have many of the attributes of w/o emulsions, their lower viscosity, derived from water as the external phase, makes them easier to inject.[4] Multiple emulsions have been formulated as cosmetics, such as skin moisturizer. Prolonged release can also be obtained by means of multiple structures.[5] These systems have some advantages, such as the protection of the entrapped substances and the incorporation of several actives in the different compartments. Despite their potential usefulness, applications of multiple emulsions have been limited because of thermodynamic instability and their complex structure.[6,7] Multiple emulsions, which are prepared from oil and water by the emulsification of an existing emulsion so as to provide two dispersed phases, are also of pharmaceutical interest.[8] The basic rational for the use of w/o/w and o/w/o type multiple emulsion as a means of prolonged delivery of drugs is that the drug contained in the innermost phase is forced to partition itself through several phases prior to release at the absorption site.[9,10]

Clotrimazole is a widely used effective antifungal agent.[11] It is a synthetic imidazole derivative shown to be potent and well-tolerated topical agent.[12,13] It is active against dermatophytes (the causative organism of tinea infections) and yeast (*Candida albicans*). Accordingly, multiple emulsions dosage formulation of clotrimazole which has enhanced release and bioavailability properties with less inter and intra- subject variability would be desirable. Thus the aim of the present study is to formulate and evaluate the multiple emulsion of clotrimazole.

MATERIAL AND METHODS

Clotrimazole was obtained from Cipla pvt. Ltd., Indore (M.P.), NaCl, Carbomer (TGC), Capric/caprylic Triglyceride (CT), Cetyl Palmitate (CP) purchased from HiMedia Laboratories Pvt Ltd., Mumbai. Cetyl dimethicone copolyol (CDC), Sorbitan stearate span 60, Cocamidopropyl betaine (CMB), Polysorbate 80 (Tween 80) purchased from Finar Chemical (India) Pvt Ltd., Ahmedabad Deionized water was prepared in college laboratory.

Preformulation Study

Physical appearance: The drug Clotrimazole powder was examined for its organoleptic properties like color taste and odour.

Solubility study: The sample was qualitatively tested for its solubility in various solvents. It was determined by taking certain amount of drug sample in 1 ml of solvent e.g. water, methanol, ethanol, pH buffer 6.8 in small test tubes and well solubilized by shaking upto saturation.

Melting point determination: The Melting point was determined by the capillary method using Digital Melting point apparatus. The capillary tube was fused and filled by pressing the open end gently into pure drug sample and packed by tapping the bottom of the capillary on a hard surface so that the drug packed down into the bottom of the tube. When the drug was packed into the bottom of the tube, the tube was placed into the slot of the apparatus, the apparatus was started and the temperature was noted at which the drug melt.

Determination of partition coefficient

Take 25mg of drug in three separating funnels the separating funnels were shaken for 2 hrs in a wrist action shaker for equilibration. Two phases were separated and the amount of the drug in aqueous phase was analyzed spectrophotometrically the partition coefficient of the drug in phases was calculated by using formula.

$$\text{Partition Coefficient, } K = \frac{\text{Concentration of drug in organic layer}}{\text{Concentration of drug in aqueous layer}}$$

U.V. Spectroscopy of Drug

(a) Determination of Wavelength of Maximum Absorbance (λ_{max})

10mg of drug was weighed accurately and transferred to 10ml of volumetric flask. Then Methanol (suitable solvent) was added to dissolve te drug completely. The volume was made up to 10 ml with solvent. The prepared sample was 1000µg/ml. 01 ml of above solution was then transferred to another 10ml volumetric flask and diluted it upto the mark with solvent. This sample was 100µg/ml. 01 ml of above solution was then transferred to another 10ml volumetric flask and diluted it upto the mark with solvent. This sample was 10µg/ml. Clotrimazole solution (10µg/ml) was scanned in the U.V. range of 200-400 nm using Systronic Double beam UV Visible spectrophotometer.

(b) Preparation of Calibration Curve of Clotrimazole

The calibration curve was plotted between the concentration and absorbance. The different concentrations between 5-30µg/ml were scanned at 264nm and absorbances were recorded.

Fourier-Transform Infra Red spectroscopy (FT-IR)

The IR spectrum of drug substance was authenticated using IR spectroscopy. The presence of characteristic peaks associated with specific structural characteristics of the drug molecule was noted.

Method of Preparation of Multiple Emulsions

Multiple emulsions (W/O/W) were prepared by two step emulsification process:

a) Preparation of primary emulsification

b) Secondary emulsification.

(a) Primary emulsification: Primary W_1/O emulsion was prepared by slow addition of the aqueous phase containing the electrolyte (NaCl) to the oil phase containing Clotrimazole (1%, *w*/*w*) at 80 ± 2°C under continuous stirring at 250 rpm until approximately 25 °C.

The oily phase was prepared dissolving Clotrimazole in a combination of drug solvent and cosolvent (LT and CP) aided with the lipophilic emulsifying agents (CDC and sorbitan stearate) at 80 ± 2 °C.

(b) Secondary emulsification: External aqueous phase (W_2) had been prepared previously by dispersing the cross-linked TGC polymer, in a co-solvent system of deionized water and the hydrophilic emulsifying agents (CMB and/without polysorbate 80) were neutralized with 10% NaOH solution (10%, *w*/*v*) to obtain a pH value of 6.5–7.0.

The obtained primary emulsions were slowly added to the corresponding outer aqueous phases (W_2) 50:50 under 250 rpm at room temperature. After complete addition of the primary W_1/O emulsion over the external gelified aqueous phase, the resulting mix was paddle stirred for a further 10 min until a homogeneous $W_1/O/W_2$ multiple emulsion had been completely formed.

Evaluation

Physical Stability of Emulsion Formulations

Organoleptic characters were monitored to detect any visible signs of instability such as creaming, cracking phase separation or color changes.

Globule size

Microscopic analysis was performed on optical microscope (400X) in order to measure globule size and multiple natures of all formulations. The oil droplet size was measured by dynamic laser light scattering technique using particle size analyzer (Malvern Mastersizer 2000).

Viscosity

The viscosities of the samples were determined at 25°C spindle speeds ranging from 100 to 200 rpm while using a spindle CP 41 in a Brookfield programmable Rheometer. Rheocalc V 2.6 (Microsoft Corporation) software was used as a support program to produce the results of rheological behavior.

Determination of pH

The pH of fresh sample and samples kept in different storage conditions was determined by a digital pH meter. The pH measurements were also taken for the samples at 24 h on days 7, 15, and 30.

Drug content

Percentage of Drug Content was determined by taking freshly prepared W/O/W multiple emulsions and immediately centrifuged at 4000 rpm for 10min. Then 1ml of the aqueous phase (the lower layer) was precisely withdrawn through 2ml hypodermic syringe and diluted properly with phosphate buffer 6.8pH. The solution was filtered with a Millipore filter (0.22mm in pore size) and drug content was analyzed on UV spectrophotometer at 264 nm. The Drug Content was determined by following equation.

$$\%\ \textbf{Drug Content} = \frac{\textbf{Amount of drug present in formulation}}{\textbf{Calculated Amount in formulation}}\ \textbf{X 100}$$

***In-vitro* drug release study**

The *in-vitro* drug release study was carried out on a simple dissolution cell using cellophane membrane (thickness-200mm, breaking strength- 2.7 kg/cm). Prior to release studies, the

cellophane membrane was soaked in distilled water for 6 hours, washed frequently 4 times by changing distilled water, then immersed in 5% v/v glycerol solution for at least 60 min and washed finally with 5 portions of distilled water. 15 ml freshly prepared multiple emulsion was added to donor chamber, made up of a hollow glass tube (2.5 cm in diameter and 10 cm in length) and membrane was tied on bottom end of the tube with a nylon string. This tube was dipped into 250 ml vessel containing 100 ml of PBS pH 6.8 and was stirred at 100 rpm on a magnetic stirrer and maintained at 37 °C which acted as receiving chamber. Aliquots of 1ml were collected from receiving chamber at predetermined time intervals and the drug content was determined on UV spectrophotometer at 264nm after suitable dilution.

Kinetics models of drug release

There are several models to represent the drug dissolution profiles where f (t) is a function of time related to the amount of drug dissolved from the pharmaceutical dosage form.

a) Zero order kinetics
b) First order kinetics
c) Higuchi Model
d) Peppas-Korsmeyer Model

Stability of optimized multiple emulsion formulation (ME-2)

Optimized multiple emulsion formulation was stored in cool and dry place for 30 day. After 30 days stored formulation was observed visually for creaming, creaking, phase separation and color change reaction.

RESULTS AND DISCUSSION

Preformulation Studies revealed Organoleptic Properties of Drug Clotrimazole was white to pale yellow crystals, odorless and characteristic in taste with melting point of 149^0 C and soluble in Phosphate Buffer 6.8pH, 0.1 N HCl and 0.1 N NaOH and Freely Soluble in ethanol and methanol. Wavelength of Maximum Absorbance (λ_{max}) determined in phosphate buffer was 264nm. Calibration curve of Clotrimazole was prepared by concentration range of 5- 30 µg/ml and obtained linearity equation y = 0.024x – 0.01 with regression coefficient (R^2) 0.999. Partition coefficient was 1.797 determined, physically and Chemically Compatible by FT-IR.

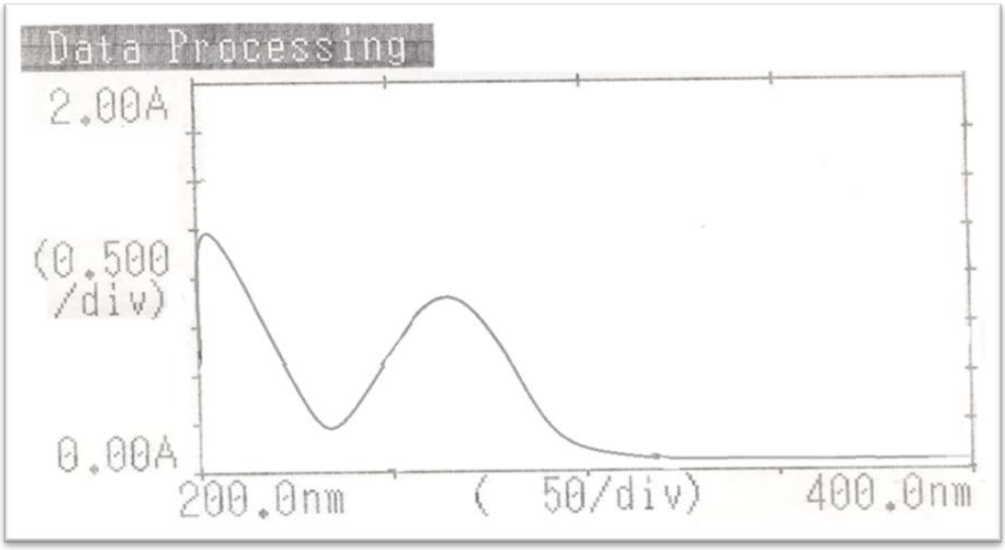

Figure no. 1: scanning of Wavelenth of Clotrimazole.

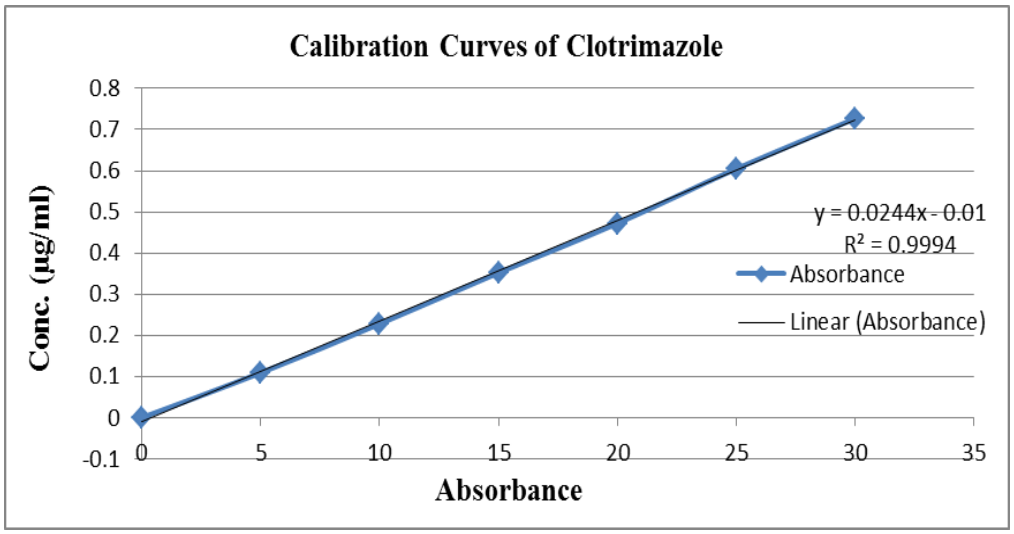

Fig. no. 2: Calibration Curve of Clotrimazole in 6.8 pH buffer.

Formulation Development

Table no. 1: Formulation of Multiple Emulsions

Components	Percentage Composition (w/w)				
	ME-1	ME-2	ME-3	ME-4	ME-5
Oil phase (O)					
Clotrimazole	01.00	1.00	1.00	1.00	1.00
Capric/caprylic Triglyceride (CT)	11.00	11.00	11.00	11.00	11.00
Cetyl Palmitate (CP)	02.00	2.00	2.00	2.00	2.00
Cetyl dimethicone copolyol (CDC)	01.50	1.50	1.50	1.50	1.50
Sorbitan stearate (Span 60)	02.00	2.00	2.00	2.00	2.00
Internal Aqueous Phase (W_1)					
Sodium chloride (NaCl)	0.25	0.25	0.25	0.25	0.25
Purified water at pH 6.6	32.25	32.25	32.25	32.25	32.25

External aqueous phase (W2)					
Carbomer (TGC)	0.10	0.20	0.30	0.40	0.50
Cocamidopropyl betaine (CMB)	0.70	0.60	0.50	0.40	0.30
Polysorbate 80 (Tween 80)	0.00	0.50	1.00	1.50	2.00
Purified water at pH 6.6	49.20	48.70	48.20	47.70	47.20

Evaluation of prepared Multiple Emulsion formulation

Physical evaluation of Multiple Emulsion formulation

Table no. 2: Physical evaluation of all prepared formulation.

S. No.	Code of formulation	Visual observation	Phase Separation	Thermo dynamic Stability
1	ME-1	White, Cloudy	No Phase separation	Stable
2	ME-2	White, Coludy	No Phase separation	Stable
3	ME-3	White, Coludy	No Phase separation	Stable
4	ME-4	White, Coludy	No Phase separation	Stable
5	ME-5	White, Coludy	No Phase separation	Stable

Globule size determination

Table no. 3: Globule size determination of formulations.

Formulation Code	Droplet size (µm)	Zeta potential (mV)	Poly Dispersity Index (PDI)
ME-1	2.42 ± 4.24	14.2 ± 1.42	0.402 ± 0.03
ME-2	1.94 ± 2.53	13.2 ± 1.45	0.329 ± 0.04
ME-3	1.83 ± 1.39	12.4 ± 2.66	0.424 ± 0.01
ME-4	1.99 ± 1.02	14.6 ± 1.37	0.299 ± 0.05
ME-5	2.13 ± 2.33	13.5 ± 1.86	0.443 ± 0.03

Note: All the values are mean of triple reading ± standard deviation

Determination of physical properties pH, Viscosity and Drug content.

Table no. 4: Physical properties, pH, Viscosity and Drug content.

formulation Code	pH	Viscosity (cP)	Drug content (%)
ME-1	6.8	40.23	87.21 ± 1.65
ME-2	6.8	55.43	95.23 ± 1.01
ME-3	6.8	54.55	84.32 ± 1.23
ME-4	6.7	68.32	84.52 ± 1.41
ME-5	6.8	76.38	83.31 ± 2.13

***In-vitro* Release studies of Clotrimazole Multiple emulsion formulations**

Table no. 5: Cumulative Percentage of drug release from multiple Emulsions.

Time (hr.)	ME-1	ME-2	ME-3	ME-4	ME-5
0	0	0	0	0	0
1	06.9	15.00	12.91	11.04	08.75
2	17.9	27.43	27.78	20.55	18.37
3	30.60	40.59	35.20	31.81	26.65
4	46.20	51.45	47.60	43.01	35.91
5	58.76	61.59	59.31	51.37	42.86
6	69.84	74.02	65.93	63.08	56.71
7	80.60	86.34	74.99	74.43	67.25
8	87.61	94.18	81.27	83.74	78.62

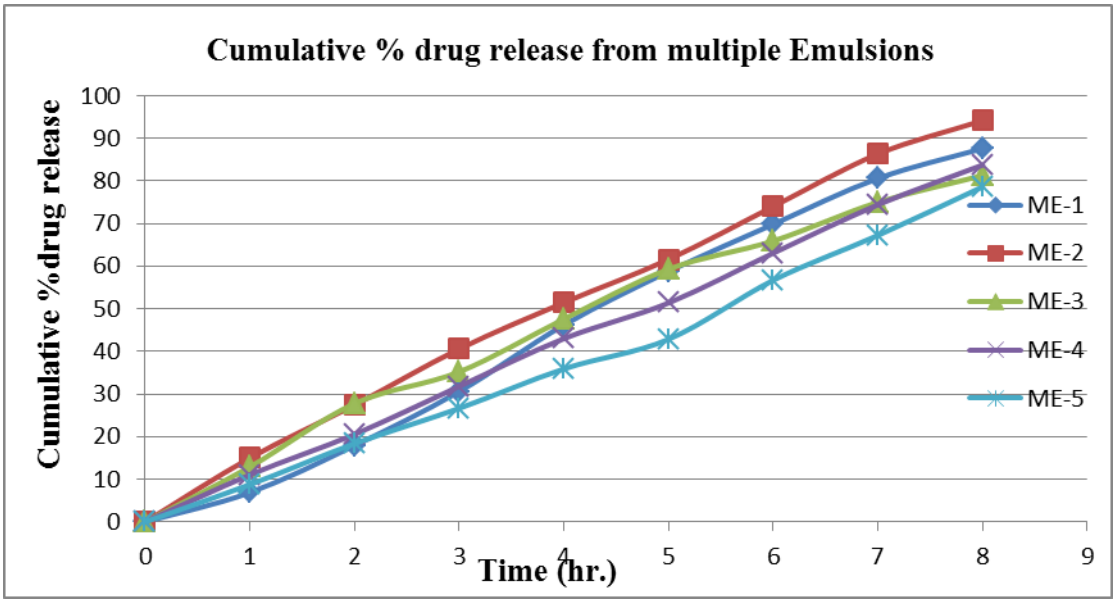

Figure no. 3: *in-vitro* cumulative % drug release from Clotrimazole multiple emulsion.

Kinetic modeling for multiple emulations formulation (ME-2).

Table no. 6: *In-vitro* clotrimazole Release Profile for (ME-2).

Time (hr.)	S.R.T	Log T.	% C.R	Log % C.R	% Drug remaining	Log% drug remaining
0	0	0	0	0	100	2
1	1	0	15.00	1.176	85.00	1.929
2	1.141	0.301	27.43	1.438	72.57	1.861
3	1.732	0.477	40.59	1.608	59.41	1.774
4	2	0.602	51.45	1.711	48.55	1.686
5	2.236	0.699	61.59	1.789	38.41	1.584
6	2.449	0.778	74.02	1.869	25.98	1.414
7	2.646	0.845	86.34	1.936	13.66	1.135
8	2.828	0.903	94.18	1.973	05.82	0.765

Zero Order Kinetics

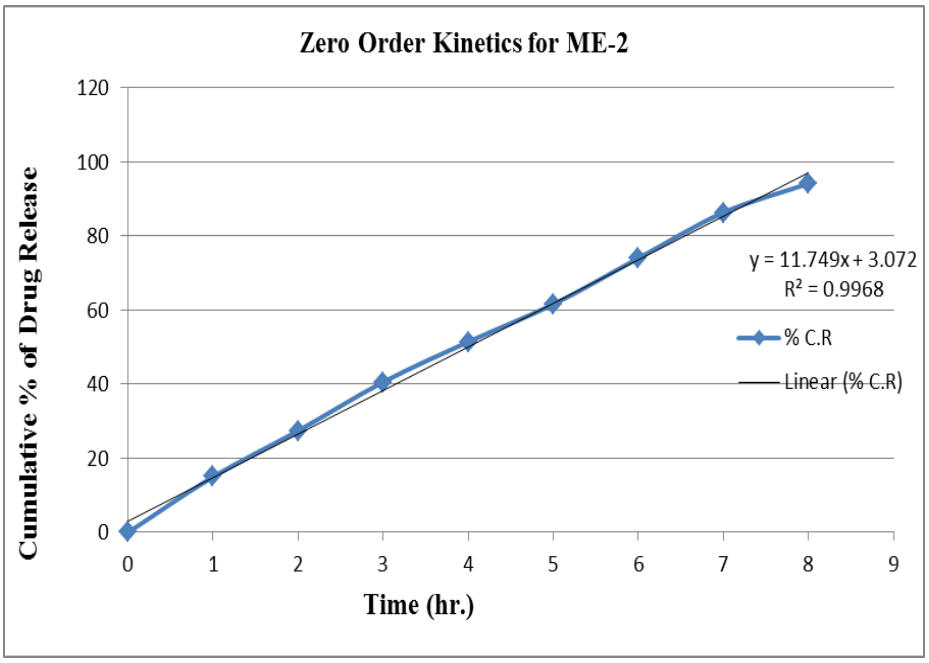

Fig. no. 4: Zero Order plots for ME-2.

First Order Kinetics

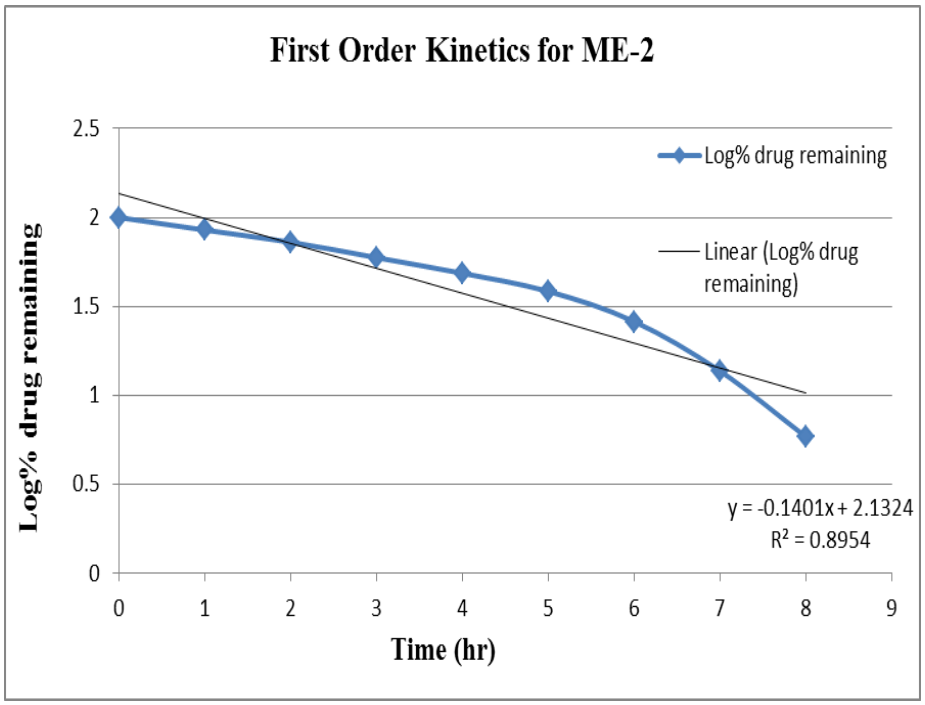

Figure no. 5: Fig. no. 5: First Order plots for ME-2.

Higuchi Model of Drug Release Kinetics

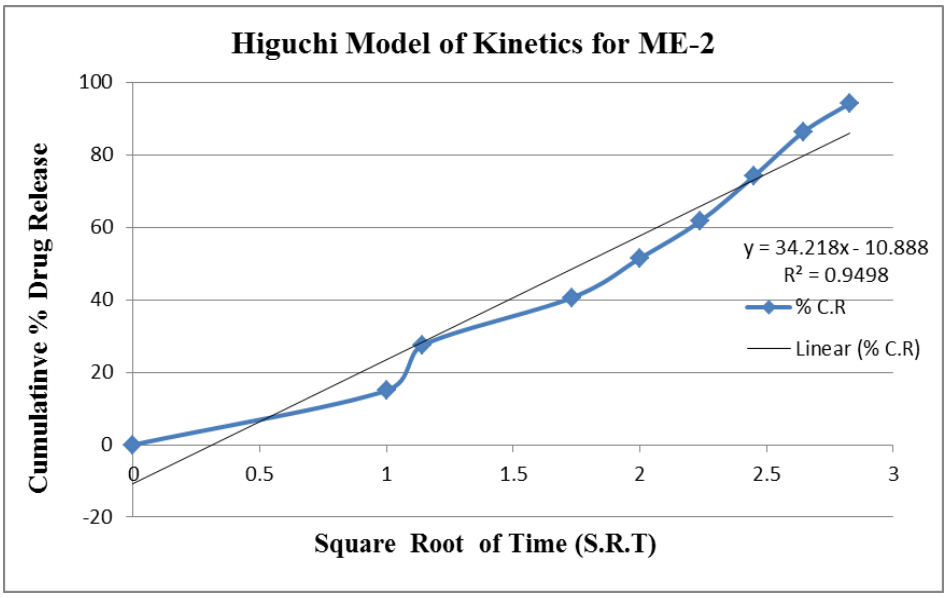

Fig. no. 6: Higuchi model plots for ME-2.

Peppas- Korsemeyer model of drug release kinetics

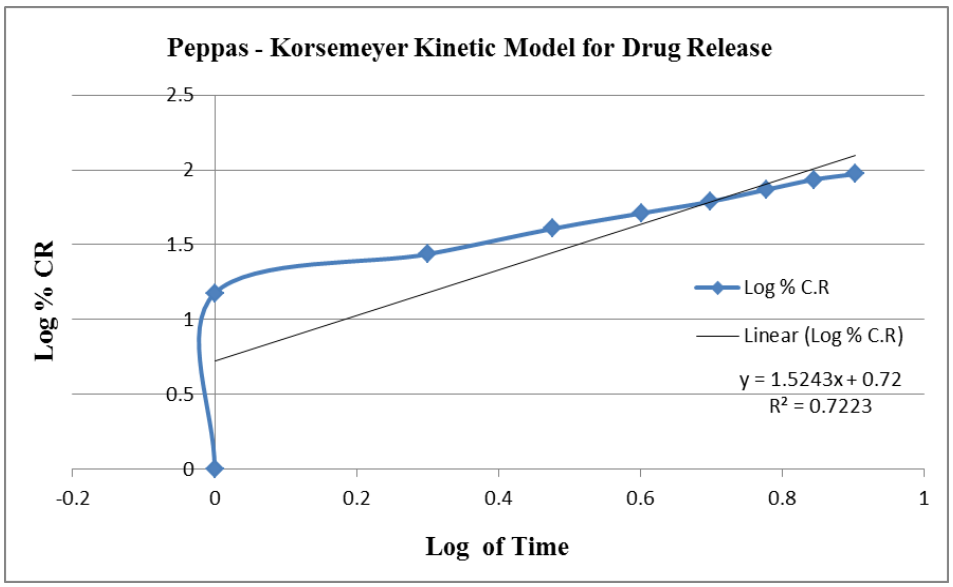

Fig. no. 7: *Peppas-Korsemeyer* model plots for ME-2.

Table no. 7: comparative study of kinetic models for ME-2.

Name of Model	Linearity Equation	R^2 value
Zero Order Kinetics	y = 11.74x + 3.072	R² = 0.996
First Order Kinetics	y = -0.140x + 2.132	R² = 0.895
Higuchi Model	y = 34.21x - 10.88	R² = 0.949
Peppas- Korsemeyer model	y = 1.524x + 0.72	R² = 0.722

Stability Testing

Table no. 8: Stability testing under following parameters.

Formulation Code	Stability testing after 30 days			
	Color Change	**Creaming**	**Creaking**	**Phase Separation**
ME-1	Observed	Observed	Not Observed	Not Observed
ME-2	Not Observed	Not Observed	Not Observed	Not Observed
ME-3	Not Observed	Not Observed	Not Observed	Observed
ME-4	Not Observed	Observed	Not Observed	Not Observed
ME-5	Not Observed	Not Observed	Not Observed	Observed

DISCUSSION

Multiple emulsions dosage formulation of clotrimazole which has enhanced release and bioavailability properties with less inter and intra- subject variability would be desirable. Thus, it was aimed to formulate and evaluate the multiple emulsion of clotrimazole. By the preformulation studies it is observed that clotrimazole is a white to pale yellow crystals having no odor. Solubility was determined in various solvents found that freely soluble in ethanol and methanol, slightly soluble in Distilled Water, soluble in Phosphate Buffer 6.8pH, 0.1N HCl and 0.1N NaOH. Melting point was observed in range of 147-149 ^{0}C. λ_{max} was determined at 264 nm by scanning sample from 200-400nm and also calibration curve was obtained by absorbance of aliquots from 5-30 µg/ml with following linear equation y=0.024x-0.01 R^2 = 0.999. Partition coefficient was 1.797 obtained. Drug: Excipient Compatibility Studies at room temperature, 2^{0}C -8^{0}C and 45^{0}C -50^{0}C says it is stable. Stability also confirmed by FT-IR studies.

Five different type formulations (ME-1 to ME-5) formed using fixed amount of oil phase having Capric/caprylic Triglyceride (CT), Cetyl Palmitate (CP), Cetyl dimethicone copolyol (CDC) and Sorbitan stearate (Span 60) and Internal Aqueous Phase contain Purified water at pH 6.6 and Sodium chloride. Different concentration of External Aqueous Phase contains Carbomer (TGC), Cocamidopropyl betaine (CMB) and Polysorbate 80 (Tween 80) in Purified water at pH 6.6. Then observed visually that all formulations were white and cloudy, there was no phase separation and Thermo dynamically Stable. Characterization of all Clotrimazole multiple emulsion formulations were evaluated for Droplet size, Zeta potential and Poly Dispersity Index (PDI) all results showed in table.

All five Clotrimazole multiple emulsion formulations were odorless, washable, homogeneous, stable and free from grittiness and was evaluated under the various parameters, pH of all formulations were observed at 6.8 and viscosity between 40..23 to

76.38 centi poise and percentage of Drug content between 83.31 to 95.23%. *In-vitro* Drug Release of Clotrimazole multiple emulsion formulations were studied and found ME-1(87.61), ME-2(94.18), ME-3 (81.74), ME-4(83.74) and ME-5(78.62). Formulation **ME-2** was found excellent on the basis of cumulative percentage of drug release profile.

All five formulations were also tested for stability and found formulation ME-2 was stable after 30 days against color change, creaming, creaking and phase separation. Optimized formulation **(ME-2)** drug release data were fitted into the zero order, first order, Higuchi and Peppas-Korsemeyer model of drug release kinetics. Formulation ME-2 was followed Zero Order Kinetics explained as continuous and steady release of Clotrimazole from the formulation.

CONCLUSION

The prepared multiple emulsions of Clotrimazole had shown excellent promising results for all the evaluated parameters. On the basis of *in-vitro* drug release and drug content results, **ME-2** formulation was better drug release as compare to ME-1, ME-3, ME-4 and ME-5 which shows higher percentage of drug release.

In- vitro release profile was applied on various kinetic models like Zero order, First order, Higuchi equation and Peppas-Korsemeyer model. The best fit with highest regression coefficient was found with Zero order. The rate constants are calculated from the slop of the respective plots the release mechanism of Multiple Emulsion. **ME-2** formulation can be further study for preclinical and clinical evaluations.

CONFLICTS OF INTEREST

There are no conflicts of interests.

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