How to Cite:
Dhone, P., Gujaram, M., Sabitri, B., Neeta, R., & Neha, M. (2022). Development & characterization of clotrimazole multiple emulsion. *International Journal of Health Sciences*, *6*(S4), 1689–1701. https://doi.org/10.53730/ijhs.v6nS4.6403

Development & characterization of clotrimazole multiple emulsion

Dhone PG
Professor & Head, Department of pharmacology, GMC Ambikapur

Marandi Gujaram
Assistant professor, Bhima Bhoi Medical College and Hospital, Balangir

Beshra Sabitri
Assistant professor, Bhima Bhoi Medical College and Hospital, Balangir

Rai Neeta
Department of pharmaceutics, Vishwakarma University, Pune, Maharashtra, India

Munot Neha
Department of pharmaceutics, Vishwakarma University, Pune, Maharashtra, India

***Abstract*---**The present study is the development and characterization of clotrimazole multiple emulsion. The purpose of this research is to enhance the release of drug as well as the bioavailability of particular drug. After formulation of multiple emulsion of drug clotrimazole some characterization like pre-formulations and evaluation study was performed. It shows excellent results for all parameters. On the basis of in vitro drug release the zero-order release study shows that the ME-2 formulation is more stable and effective for antifungal activity.

***Keywords*---**clotrimazole, poly-dispersed, surfactant.

Introduction

Multiple emulsions are complex system in which one liquid is dispersed in to another liquid. In multiple emulsions inner dispersed droplets are separated by outer liquids by layer of another liquids. (Akhtar . N et .al 2010)
They are basically two types: (Bhatia N.et al 2013)

Water in oil in water (W/O/W)
Oil in water in oil (O/W/O)

***Corresponding author**: Rai Neeta; Email: neetarai.2012@rediffmail.com*
Manuscript submitted: 18 Feb 2022, Manuscript revised: 9 March 2022, Accepted for publication: 1 April 2022

In this research the drug clotrimazole is used. It is generally used as antifungal treatment. It is an imidazole derivative. It is more effective against fungus and dermatitis through multiple emulsion. (Collings, A,1997, Deshmukh et.al ,2014)

Materials & Methods

In this research the Drug Clotrimazole is used. The other analytical reagents caprylic Triglyceride, Cetyl Palmitate, Cetyl dimethicone copolyol, Span 60, Sodium chloride are also used in the research. (Ghosh S.,et al ,2011)

Methods

Pre-formulation study: (Hanpramukkun N,et.al ,2009)
Physical appearance: (Kumar R.,et al,2012)

Powdered Clotrimazole examined by its organoleptic properties i e color, taste and its odour.

Solubility study: To determine the solubility of given sample in certain solvents like polar or non-polar solvents. (Kumar R.,et al,2012)
Melting point determination: It is determined by Digital melting point apparatus.
Partition coefficient determination: (Kumar R.,et al,2012)

Take 25mg of drug in three separating funnels then it is shaken for 2 hrs in a wrist action shaker for equilibration ,then two phases were separated and the amount of the drug in aqueous phase was analyzed u.v spectro-photometrically .the partition coefficient of the drug in phases was calculated by using formula:

$$\textbf{Partition Coefficient} = \frac{\textit{Concentration of drug in organic solvent}}{\textit{Concentration of drug in aqueous solvent}}$$

U.V. Spectroscopy of Drug: (Madaan V., et al 2014)
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 the 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.

Preparation of Calibration Curve of Clotrimazole (Madaan V., et al 2014)
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) (Madaan V., et al 2014)
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

Formulation of Multiple Emulsions (Nimbekar T.P et al,2012, Prajapati S.B et al,2013)
Multiple emulsions (W/O/W) were prepare by two steps emulsification process: -

Primary emulsification:
Multiple emulsion was prepared by mixing of aq. phase that contains electrolyte to the oil phase which contain drug (1%w/w) at 80 ± 2°C.the primary emulsion was prepared by mixing of aq Phase to oil phase at continuous stirring at 250 RPM. Then oil phase was prepared by dissolving the drug solvent and co-solvent aided with lipophilic emulsifying agents

Secondary emulsification

It is external process W2 which has been prepared previously by dispersing the cross linked TCG polymer ,in co-solvent system By deionized water and hydrophilic emulsifying agents which as neutralize with NaOH i.e 10 %w/v at optimum pH 6.5-7.0.then in obtain emulsions add slowly the aq phase W2 at 250 RPM at RT . After completion it formed in gel form by continuous stirring for 10 min until homogenous emulsion was formed.

Components	Percentage Composition (w/w)				
	ME-1	ME-2	ME-3	ME-4	ME-5
Oil phase					
Clotrimazole	1.00	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	2	2	2.00	2.00
Cetyl dimethicone copolyol (CDC)	1.5	1.5	1.5	1.50	1.50
Span 60	2	2	2	2.00	2.00
Aq.Phase W1					
Sodium chloride	0.25	0.25	0.25	0.25	0.25

Purified water	32.25	32.25	32.25	32.25	32.25
Aq 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

Table no 1: Composition of clotrimazole emulsion

Results
Pre-formulation Studies
Organoleptic properties of Clotrimazole

Table no. : 2 Organoleptic Properties of drug Clotrimazole

Test	Specification	Observations
Color	White to pale Yellow Crystals	Complies
Taste	Characteristic	Complies
Odor	Odor less	Complies

Melting point

Melting point of drug Clotrimazole is 149^{0}C

Solubility study

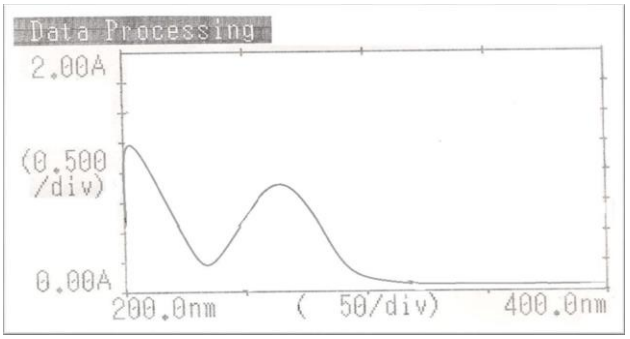
Table no. 3 Solubility profile of Clotrimazole in different solvent

S. no.	Solvents	Solubility
1.	Distilled water	Slightly Soluble
2.	Ethanol	Freely Soluble
3.	Methanol	Freely Soluble
4.	Phosphate Buffer 6.8Ph	Soluble
5.	0.1 N HCl	Soluble
6.	0.1 N NaOH	Soluble

Determination of Wavelength of Maximum Absorbance (λ_{max}):-

The maximum absorbance at scanning range 200-400 m at concentration range 10(µg/mL) is 264nm

Fig no 2 Scanning of Wavelength of Clotrimazole



Preparation of the Calibration Curves of Clotrimazole

Table no. 4 Linearity of Clotrimazole 6.8 pH buffer

Conc. (µg/ml)	0	5	10	15	20	25	30
Absorbance	0	0.109	0.228	0.352	0.472	0.605	0.727

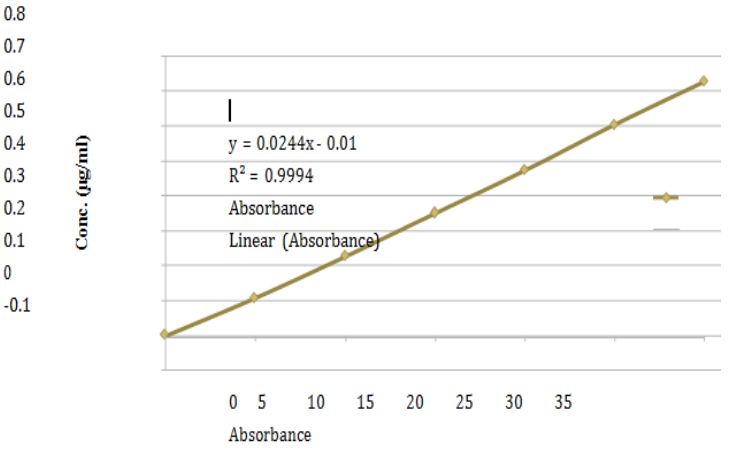


Fig. no. 3: Calibration Curve of Clotrimazole in 6.8 pH buffer

Linearity Equation : y = mx + c, y = 0.024x – 0.01
y = abs. of unknown sample,
m= slope = 0.024
x = Conc.(micro-gm/ml),
c = Intercept = 0.01, r^2=0.999

Partition Co-efficient:

Partition coefficient = concentration of n- Octanol / concentration in water is 57.208/31.83 = 1. 797.

Table No. 5: Partition Co-efficient

Sr. No.	Solvents	Absorbance
1.	Water	0.754
2.	n- Octanol	1.363

Characterization of clotrimazole multiple emulsion:
Physical evaluation of all prepared Multiple Emulsion formulation

Physical evaluation of all prepared formulation is non-separation, white cloudy and all thermo dynamic stable.

Globule size determination

Table no. 6: Globule size determination of formulations

Code	Droplet size	Zeta potential(mV)	PDI
ME-1	2.42 ± 0.24	14.2 ± 1.42	0.402 ± 0.03
ME-2	1.94 ± 0.53	13.2 ± 1.45	0.329 ± 0.04
ME-3	1.83 ± 0.39	12.4 ± 2.66	0.424 ± 0.01
ME-4	1.99 ± 0.02	14.6 ± 1.37	0.299 ± 0.05
ME-5	2.13 ± 0.33	13.5 ± 1.86	0.443 ± 0.03

Determination of physical properties pH, Viscosity and Drug content

Table no.7 Physical properties, pH , Viscosity and Drug content

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**

Table No.8 Invitro Drug Release Rate

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

Cumulative drug release studies of Clotrimazole Multiple emulsion formulations

Table no.9 Cumulative drug release studies of Clotrimazole Multiple emulsion formulations

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

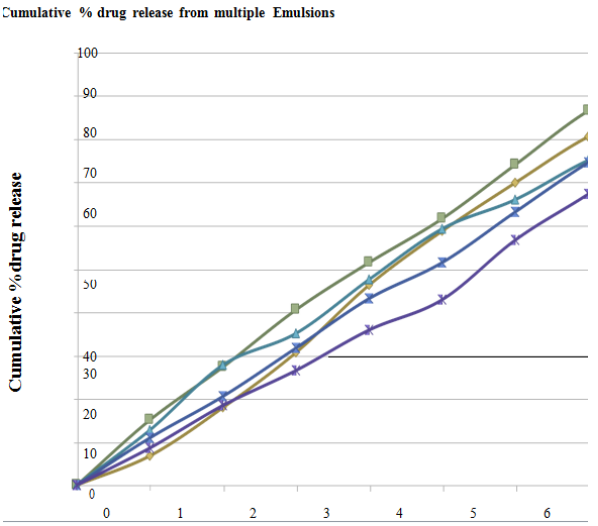


Fig No 4: Cumulative Drug Release Studies Of Clotrimazole Multiple Emulsion Formulations

Kinetic modelling for multiple emulations formulation (ME-2)

Table no. 10: In-vitro clotrimazole Release Profile for (ME-2)

Time hr	S.R.T	Log T.	% C.R	Log %C.R	%Drug remaining	Log% D.R
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

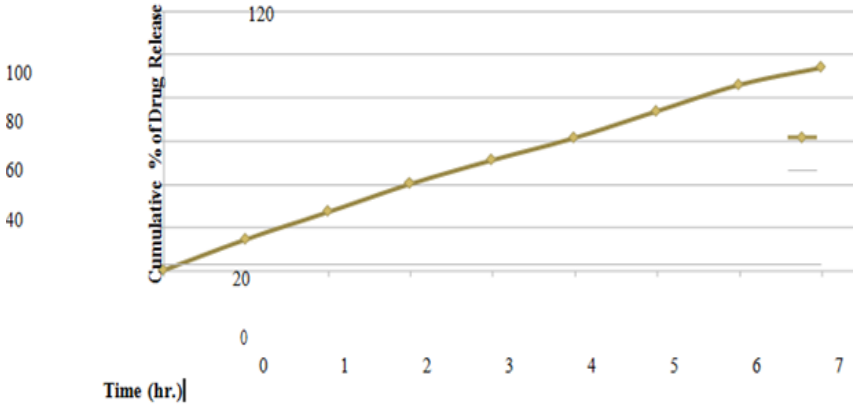


FIG NO 5 Zero Order Kinetics for ME-2

Log of Time

Table no.11: Comparative study of kinetic models for ME-2

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

Stability Testing

Table no. 12: Stability testing under following parameters

Code	Stability study 30 days			
	Color Change	Creaming	Creaking	Phase Separation
ME-1	Observed	Observed	No	No
ME-2	No	No	No	No
ME-3	No	No	No	Observed
ME-4	No	Observed	No	No
ME-5	No	No	No	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. □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 formulations are more stable and free from grittiness which was evaluated by certain parameters, pH, Viscosity and percentage of drug content of formulation are observed was 6.8, 76.38 cp and 83.31 to 95.23%. *In-vitro* Drug release was found to be 8 7 . 6 1 - 78.62 and it follow the zero-order release rate.

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 ug 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 drug release profile was seen by various models like zero, first , higuchi and peppas- koresmeyer models. The best fit model was found to be zero order and rate constant are calculated by slop of respective plots by different mechanism of multiple emulsions. After several clinical and preclinical studies, we found that ME2 is best formulations.

References

Akhtar N, Mahmud A; et al., "Formulation and characterization of a multiple emulsion containing 1% l-Ascorbic acid", Bull. Chem. Soc. Ethiop. 2010, 24(1), 1-10.

Bhatia N., Pandit S., Agrawal S. and Gupta D. A review on multiple emulsions. International Journal of Pharmaceutical Erudition. 2013; 3(2): 22-30.

Collings, A, J, British Patent no.1235667, 19971.

Deshmukh R.L. Multiple emulsion: Strategic and technology. Asian Journal of Pharmaceutical Education and Technology, 2014; 2(2): 1-19.

Ghosh S., "Formulation and Characterizations of Multiple Emulsions with Various Additives", international journal of research in pharmaceutical and biomedical sciences. Apr – Jun 2011, Vol. 2 (2).

Gohla, S, H and Nielsen, J.SOFW J, 1995, 121, 707.

Hanpramukkun N, Kongmuang S, et al., "The stability of Clindamycin phosphate in w/o/wmultiple emulsions," Int J Pharm Sci Tech, 2009, 3(2): 0975-0525.

Kumar R., Kumar M.S. and Mahadevan N. Multiple emulsions: A review. International Journal of Recent Advances in Pharmaceutical Research. 2012; 2(1): 9-19.

Madaan V., Chanana A., Kataria M.K. and Bilandi A. Emulsion technology and recent trends in emulsion applications. International Research Journal of Pharmacy. 2014; 5(7): 533-542.

Matsumoto, S, Kita Y. and Yonezawa, D, J Colloid Interface sci, 1976, 57 353.

Nimbekar T.P., Wanjari B.E., Sanghi D.K. and Gaikwad N.J. 2012. Formulation and evaluation of sustained release multiple emulsion of hydroxyprogestrone. International Journal of Pharmacy and Pharmaceutical Sciences. 4(1): 76- 80.

Prajapati S.B., Bhatt H., Koli A., Dharamsi A. and Shah S.A. An overview of preparation, evaluation and applications of multiple emulsions. International Journal for Pharmaceutical Research Scholars. 2013; 2(1): 142-150.

Vyas S.P. and Khar R.K. Multiple emulsions: Novel carrier systems. CBS Publishers and Distributors. First Edition, 2004; 303-328.

Yoshioka, T, Ikeuchi,K, K, Hashida, M, Muranishi, s, and sezaki, H,chem. Pharma, Bull,1982,20.1408.