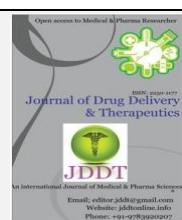


Available online on 15.08.2019 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

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Research Article

Formulation and Evaluation of Nanoemulsion for Topical Application

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ABSTRACT

The aim of present research is to design and develop nanoemulsion of *Econazole nitrate* as effective treatment for tinea versicolor fungal disease. *Econazole nitrate* is an imidazole antifungal agent with broad spectrum activity. It belongs to BCS class II i.e. low soluble and highly permeable drug. Due to its poor solubility, it is incompletely absorbed after oral dosing and bioavailability varies among individuals. The drug efficacy of topical formulation can be limited by instability due to its poor solubility in the vehicle and low permeability. Therefore, to overcome these problems nanoemulsions have been designed. Topical nanoemulsion containing 1 % *Econazole nitrate* with different oils (oleic acid), surfactant (Tween 20), co-surfactant (PEG 200, PEG 400) and distilled water. Various oil-in-water nanoemulsions are prepared by the spontaneous emulsification method. The nanoemulsion formulations that passed thermodynamic stability tests were characterized for appearance, pH, FTIR, viscosity, drug content, % drug entrapment efficiency and *in-vitro* drug release study of *Econazole nitrate* determined by Franz diffusion cell and stability study.

Keywords: Nanoemulsion, Topical drug delivery, Econazole nitrate, Viscosity, *In-vitro* drug release etc.

Article Info: Received 09 June 2019; Review Completed 19 July 2019; Accepted 20 July 2019; Available online 15 August 2019



Cite this article as:

Shaikh NM, Vijayendra Swamy SM, Nagoba SN, Kulkarni KB, Formulation and Evaluation of Nanoemulsion for Topical Application, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):370-375 <http://dx.doi.org/10.22270/jddt.v9i4-s.3301>

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INTRODUCTION:

Nanoemulsion are defined as isotropic, thermodynamically stable transparent or translucent systems of oil and water which stabilize by surfactant with a droplet size usually in the range of 5 to 200 nm. Nanoemulsion having various advantages over the macroemulsion are as follows i.e., Nanoemulsions have a much higher surface area and free energy than macroemulsions that make them an effective transport system. This system does not show the problems of inherent creaming, flocculation, coalescence and sedimentation, which are commonly associated with macroemulsions. Nanoemulsions can be developed by spontaneous emulsification method to enhance the solubility and bioavailability of poorly water soluble drugs. These are non-toxic non-irritant hence can be easily applied to skin and mucous membranes. The use of nanoemulsion as delivery systems can improve the efficacy of a drug, allowing the total dose to be reduced and thus minimizing side effects.

Nanoemulsion used as drug carrier in topical treatment of diseases especially in skin disease. They are able to incorporate a variety of hydrophobic and hydrophilic drugs to improve the accumulation of drug at the site of action and

to decrease side effects. Nanoemulsion can provide sustained and controlled release of entrapped drug.

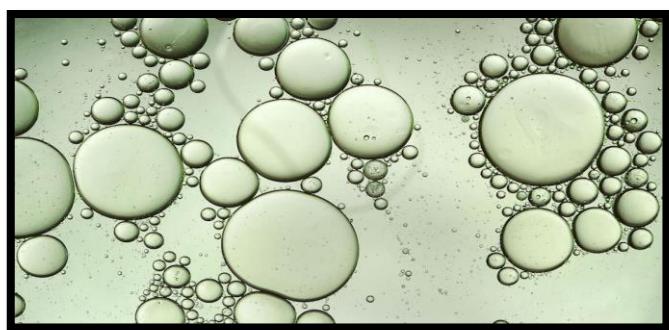


Figure 1: Nanoemulsion

In this formulation antifungal drug *Econazole nitrate* used to formulate nanoemulsion. Various conventional topical doses forms of *Econazole nitrate* are available such as cream and lotion however side effects are associated with *Econazole nitrate* therapy such as irritation, pain and redness; to overcome these problems *Econazole nitrate* nanoemulsion is prepared. *Econazole nitrate* belongs to BCS class II i.e. poorly soluble and highly permeable drug. Due to poor solubility, it

is incompletely absorbed after oral dosing and bioavailability varies among individuals. *Econazole nitrate* drug is imidazole ring containing broad-spectrum antifungal agent, which interacts with 14-demethylase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol. *Econazole* inhibits the synthesis of ergosterol which is an essential component of the fungal cell membrane; this will increase the cellular permeability causing leakage of cellular contents, resulting in fungal cell death. This drug achieves excellent skin levels with minimal dosing. It is effective topically for the managing of cutaneous candidiasis and tinea infections of the skin. Tinea infection is characterized by changes in skin pigment due to colonization of the stratum corneum by a lipophilic fungus of the normal flora of the skin, known as *Malassezia furfur*. *M. furfur* is eradicated by the presence of *Econazole nitrate* in the outer skin layers. These preparations are applied on to the skin surface for providing local or systemic effect.^{1,2}

Objectives:

- The main objective of the study is to develop topical nanoemulsion.
- To choose the appropriate excipients based on physicochemical properties of the drug.
- To formulate and evaluate the nanoemulsion.
- To increase the solubility of drug by nanonization.
- To increase therapeutic effect at targeted site.
- To reduce dose frequency and side effects.

MATERIALS AND METHODS:

Econazole nitrate is received as gift sample from Aarti pharmaceutical Pvt. Ltd. Mumbai, oleic acid, PEG 200, Tween 20 are purchased from S.D. Lobachem, Mumbai, India, All chemicals and solvents are of analytical grade.

Methods^{3,4}

Determination of melting point

Melting point of drug is determined by using capillary method. Drug is filled into capillary tube upto the height of 3 mm by sealing its one end. The capillary is introduced into the digital melting point apparatus and the point at which the drug starts melting note that point until the entire sample get melted.

Solubility study:

For the purpose of solubility, beyond saturation additional amount of drug is added in the solvent (either aqueous or non-aqueous) at room temperature and kept for 24 hrs with rare shaking. The supernatant was taken and evaluated by using Shimadzu UV 1800 double beam spectrophotometer.

Identification of drug by FTIR

The pure drug is mixed with IR grade solvent in a proper ratio and applying pressure on IR plate. The sample of drug is then scan over the range of 4000-400 cm⁻¹ in Perkin Elmer FTIR spectrometer. FTIR spectrum of *Econazole nitrate* shows the presence of the peaks which complies with the reference spectra.

Identification of drug by UV- spectroscopy

100 mg of drug (*Econazole nitrate*) is accurately weighed on digital balance and is taken into 100 ml volumetric flask. Sufficient quantity of methanol is added to dissolve the drug. The volume is made up to 100 ml using methanol to prepare stock solution of 100 µg/ml.

Preparation of standard calibration curve of *Econazole nitrate* in methanol

From the stock solution 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 ml of solution is pipette out into 10 ml volumetric flasks and volume is made up to 10 ml to form concentrations of 2, 4, 6, 8, 10, 12 µg/ml with methanol. The absorbance was measured with the help of UV spectrophotometer at 271 nm by taking methanol as reference solution. All study done in triplicate (n=3) with the same instrument.

Compatibility study

The compatibility studies are done on Perkin Elmer FTIR spectrophotometer. Compatibility studies are used for detection of any possible chemical interaction between the drug and excipients used i.e., oil, surfactant and co-surfactant. A physical mixture of drug, oil, surfactant and co-surfactant are prepared and mix with suitable quantity of potassium bromide. About 100 mg of this mixture are compressed to form a transparent pellet using a hydraulic press at 15 tons pressure. This is then subjected to IR radiations ranges from 4000 to 400 cm⁻¹ in a Perkin Elmer FTIR spectrophotometer. The IR spectrum of the physical mixture is compare with those of pure drug, oil, surfactant and cosurfactant and matching is done to detect any appearance or disappearance of peaks.⁵

Pseudo-ternary phase diagram study

Constructing pseudo-ternary phase diagrams is time consuming, particularly when the aim is to accurately define the phase boundary. Care is taken to ensure that observations are not made on metastable systems, although the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous. The relationship between the phase behavior of a mixture and its composition can be captured with the aid of a phase diagram. Oleic acid (as oil), Tween 20 (surfactant), and PEG 200 (co-surfactant) are selected to study the phase diagrams in detail. Pseudo ternary phase diagrams are constructed separately for each surfactants mixture ratio to identify the o/w nanoemulsion regions. The pseudo-ternary phase diagram was developed by using the aqueous titration method. Surfactant (Tween 20) and co-surfactant (PEG 200) are mixed (surfactants mixture) in different volume ratios (1:1, 1:2, 2:1). These surfactants mixture ratios are chosen to reflect the increasing concentration of the co-surfactant with respect to surfactant for the detailed study of the phase diagrams in the nanoemulsion formulation. Oleic acid optimize as an oil phase based on the solubility study. For each phase diagram, oil (oleic acid) and specific surfactants mixture ratios were mixed thoroughly in different volume ratios from 1:9 to 9:1. Thirteen different combinations of oil and surfactants mixture (1:1, 1:2) are made for the study to define the boundaries of the phases precisely formed in the phase diagrams. Aqueous phase is slowly titrated for each combination of oil and surfactants mixture separately. 5 ml of aqueous phase is added at each interval upto 50ml under magnetic stirring and visually observed for phase clarity and flow ability. Calculations for the ratios of oil and surfactants mixture are also done simultaneously. The physical state is plotted on a pseudo-three-component phase diagram with one axis representing the aqueous phase, the second representing the oil phase, and third representing a mixture of surfactant and co-surfactant (surfactants mixture) at a fixed volume ratio.^{5,6}

Preparation method of Nanoemulsion:

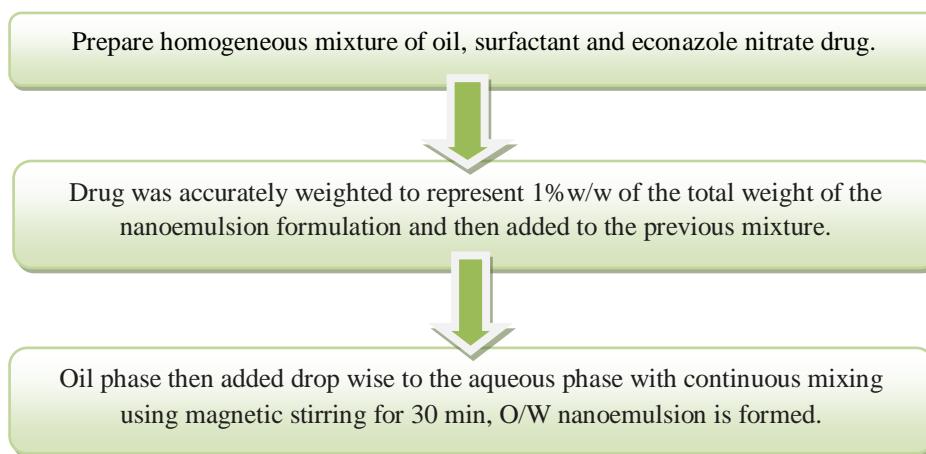


Figure 2: Spontaneous emulsification method

Table 1: Formulation of nanoemulsion

Sr. No.	Formulation	surfactants mixture (ratio)	Oil/surfactants mixture (ratio)	%w/w of components in Nanoemulsion formulation			Drug % w/w
				Oil	S.mix	Water	
1	F1	1:1	1:9	5.00	45.00	50	1
2	F2	1:1	1:8	5.00	40.00	55	1
3	F3	1:1	1:7	5.00	35.00	60	1
4	F4	1:2	1:9	5.00	45.00	50	1
5	F5	1:2	1:8	5.00	40.00	55	1
6	F6	1:2	1:7	5.00	35.00	60	1

EVALUATION OF NANOEMULSION^{7,8}:

Thermodynamic stability

The selected formulation is subjected to different thermodynamic stability tests.

Heating cooling cycle

The temperature of refrigerators between 4° and 45° of six cycles with storage at each temperature of not less than 48 hr is studied. Those formulations, which are stable at these temperatures, are subjected to centrifugation.

Centrifugation

The prepared formulations that are passed for centrifugation are centrifuged at 5000 rpm for 30 min by using centrifuge. The formulations that did not show any phase separated were taken to further tests.

Measurement of pH: pH of various nanoemulsions formulations are determining by using digital pH meter. 1 gm of nanoemulsion is dissolved in 100 ml of distilled water and pH was measured. The measurement of formulation is done in triplicate to avoid error.

Percentage drug content: 1 ml of nanoemulsion is mixed with 10 ml of suitable solvent. Aliquots of different concentration are prepared and by using suitable dilutions

after filtering the stock solution; absorbance is measured by UV spectroscopy. Drug content is calculated by using the equation obtains from linear regression analysis of calibration curve.

Determination of % transparency and drug precipitation

Formulations of different ratio are selected on the basis of ternary phase diagram. Transparency study is made to find out the maximum % transparency and drug precipitation between oil, surfactants mixture (surfactant and co-surfactant) and water containing 1% drug. (Nanoemulsion system is a clear transparent system when diluted with distilled water).

Viscosity determination

Viscosity of nanoemulsion is determined by using Brookfield viscometer. 20 ml of nanoemulsion is filled in a 25 ml beaker and the viscosity is measured using spindle number 6 at 10 rpm.

In vitro Diffusion studies:

The diffusion studies of the prepared nanoemulsions are performed by using Franz diffusion cell with the aid of cellophane membrane. Nanoemulsion sample (5ml) is taken in cellophane membrane and the diffusion studies are

carried out at 37 ± 1 °C using 250 ml of (25%) methanolic phosphate buffer (pH 7.4) as the dissolution medium. 5 ml of each sample was withdrawn periodically at 1, 2, 3, 4, 5, 6, 7 and 8 hrs and each sample is replaced with equal volume of fresh dissolution medium in order to maintain sink condition. Samples are analyzed by UV- spectrophotometer at 271 nm for drug content.

RESULT AND DISCUSSION:

The present study was attempted to prepare nanoemulsion of *Econazolenitrate* for effective treatment of fungal disease. Total six nanoemulsion formulations are prepared and evaluated for various parameters:

Organoleptic properties

Organoleptic properties of *Econazole nitrate* are found to be as per USP NF monograph.

Table 2: Organoleptic properties

Sr. No.	Test	Specification	Observation
1.	Colour	White	White
2.	Odour	Odorless	Odorless
3.	Nature	Amorphous	Amorphous

Spreadability: The Spreadability is good and sticks well on skin.

Melting point analysis

The melting point of *Econazole nitrate* was observed to be 160 °C which complies with melting range of standard 161-165 °C.

Solubility: Solubility of *Econazole nitrate* was found to be in different solvents are given below-

Table 3: Solubility study

Sr. No.	Solvent system	Specification as per USP	Result
1	Ethanol	Freely soluble	Freely soluble
2	Oleic acid	Soluble	Soluble
3	Tween 20	Soluble	Soluble
4	PEG 200	Soluble	Soluble
5	Methanol	Freely Soluble	Freely Soluble
6	Water	Slightly soluble	Slightly soluble
7	chloroform	Insoluble	Insoluble
8	Ether	Insoluble	Insoluble

Identification of drug through UV spectroscopy

Standard calibration curve of *Econazole nitrate* as pure drug in methanol

Table 4: Standard calibration curve of Econazole nitrate

Concentration (μg/ml)	Absorbance
2	0.204
4	0.440
6	0.675
8	0.898
10	1.110
12	1.310

UV shows that *Econazole nitrate* gives maximum absorption at 271 nm and figured in linear standard calibration curve shown in fig no 3.

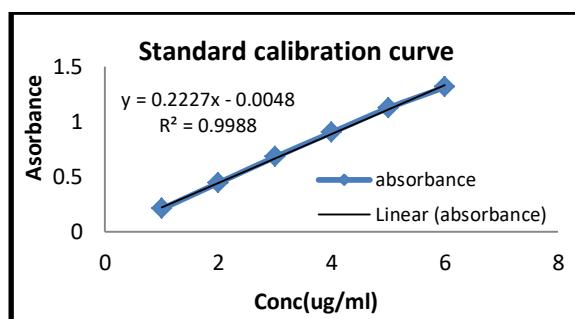


Figure 3: Standard calibration curve of Econazolenitrate in methanol

Determination of drug-excipients compatibility study

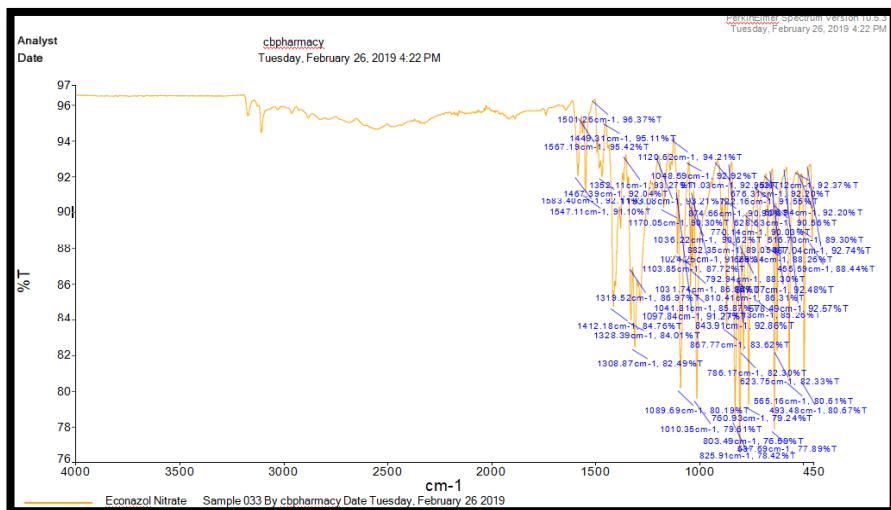


Figure 4: FTIR of Econazolenitrate

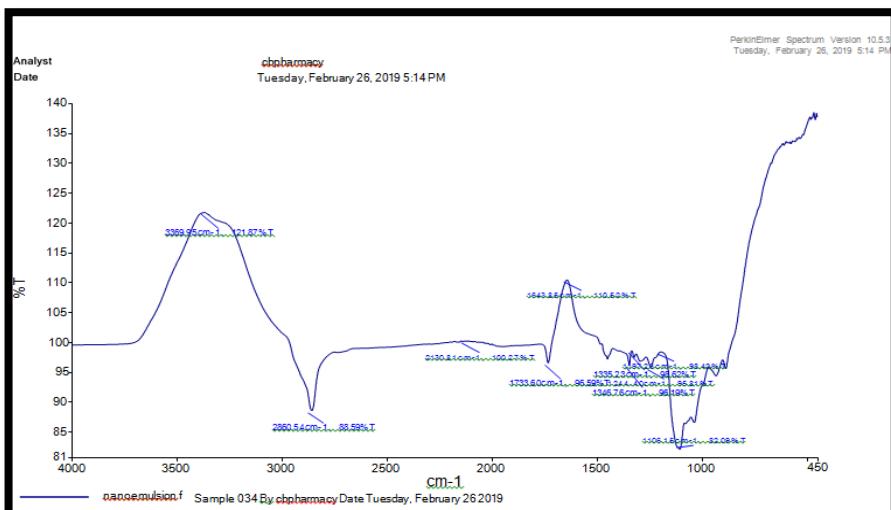


Figure 5: FTIR of Nanoemulsion formulation

From FTIR it can be said that there was no incompatibility between drug and excipient as shown in fig. no. 4 & 5.

Thermodynamic stability

Table 5: Thermodynamic stability study

Formulation code	Heating Cooling Cycle	Centrifugation
F1	Stable	No phase separation
F2	Stable	No phase separation
F3	Stable	No phase separation
F4	Stable	No phase separation
F5	Stable	No phase separation
F6	Stable	No phase separation

pH, Viscosity and % Drug content:

Table 6: Characterization of Nanoemulsion

Formula code	pH	Viscosity (cp)	Drug content (%)
F1	5.5	5229	95.92
F2	5.8	4332	96.32
F3	5.2	4320	95.45
F4	5.3	4850	94.66
F5	6.5	5920	98.78
F6	5.2	4530	94.65

pH of prepared nanoemulsion formulations was found in the range of 5.5-6.5 and tabulated in table 6.

The percentage drug content of prepared nanoemulsion formulation was found to be 94 to 98 % and tabulated in table 6.

The mean average viscosity was found to be 4000 to 6000 cp, F5 batch shows highest viscosity and tabulated in table 6.

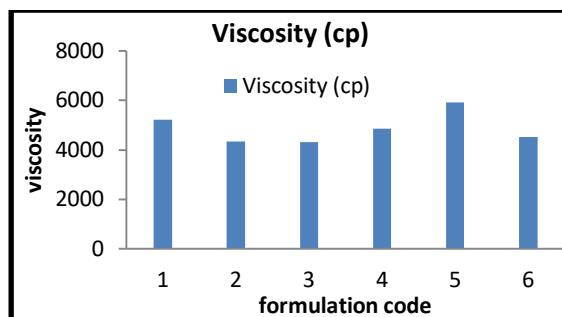


Figure 6: Viscosity of nanoemulsion formulation

In-vitro drug release:

Table 7: *In-vitro* drug release (in %)

Time in hrs	F1	F2	F3	F4	F5	F6
0.5	6.86	5.95	4.5	3.68	8.22	5.79
1	12.77	11.86	10.95	9.59	14.13	7.97
1.5	22.75	15.95	17.29	20.45	25.49	16.09
2	35.04	33.22	30.04	28.68	38.22	27.16
2.5	46.4	44.13	43.22	40.04	52.76	40.36
3	57.31	55.04	53.22	51.58	59.58	50.6
3.5	68.3	65.53	60.12	67.20	69.10	64.23
4	71.2	70.2	71.5	71.21	75.25	71.15
4.5	76.2	75.25	76.35	76.35	85.25	76.45
5	81.45	81.78	81.23	82.10	90.24	81.65
5.5	90.23	90.52	90.23	90.78	94.70	90
6	93.25	92.23	90.7	96.12	98.12	95.25

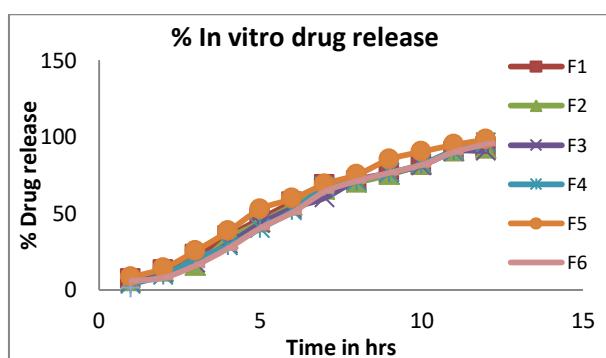


Figure 7: % In vitro drug release

Result of *in-vitro* drug release from different formulations are tabulated in table 7 and graphically shown in fig.no.7. The prepared formulation batch F5 shows the better release profile as compared to other preparation F1, F2, F3, F4 and F6.

CONCLUSION:

The present work concluded that *Econazole Nitrate* containing nanoemulsion formulation for solubility enhancement was successfully prepared by the spontaneous emulsification method. Nanoemulsion is more acceptable for the topical drug administration. Oleic acid, span 80 (surfactant) and ethanol (co-surfactant) was successfully used as a suitable carrier system for incorporating econazole for topical drug delivery. Various formulations were prepared as per the composition and drug loaded in nanoemulsion. The prepared formulation of F5 shows the

better release profile than others. From above result concluded that nanoemulsion drug delivery system can be effective for topical application in the treatment of fungal diseases and further study on an animal being need to perform before its commercial use.

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