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Formulation and evaluation of econazole nitrate microemulsion

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***Abstract*---**Econazole Nitrate microemulsion is presently considered as the novel drug delivery system. It shows prolonged action. It is considered as the imidazole antifungal agent which shows broad spectrum activity. Antifungal agent contains the lipophilic groups which are administered in the microemulsions as they are able to penetrate the system easily. It belongs to BCS Class II drug. i.e., low soluble and highly permeable. The drug efficacy of topical formulation is limited to instability due to poor solubility and low permeability. These are the basic reasons which increase the scope for designing of microemulsion. The topical microemulsion containing Econazole Nitrate prepared using oleic acid as the oil phase, tween 20 as

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surfactant and propylene glycol as co- surfactant. The optimized formulation B1 was evaluated for various tests such as Particle size, Zeta potential, pH, Viscosity, Scanning electron microscopy and Electrical conductivity.

***Keywords*---**econazole nitrate, microemulsion, antifungal.

Introduction

The concept of microemulsion was introduced by Hoar and Schulman in the 1940s, it was a clear single-phase solution by triturating a milky emulsion with hexanol[1]. Microemulsion are stable, isotropic and clear mixture of oil, water, surfactant and co- surfactant. These are the thermodynamically stable mixtures[2]. Topical drug delivery is defined as the application of pharmaceutical dosage form to skin for direct treatment of cutaneous disorder or cutaneous manifestations of general diseases[3,4]. The primary advantage of topical application is the avoidance of first pass metabolism. The skin is properly structured membrane and mainly consists of three layers. Stratum corneum is considered as the outermost layer and is considered as the layer for permeation of drugs. It is mainly composed of dead and keratinized cells[5].

Microemulsion are defined as the good delivery system for delivery of hydrophobic drugs[6]. The main antifungal drugs like azole and imidazole are lipophilic in nature and it is very difficult to incorporate in aqueous gel. To solve that microemulsion came into picture. Lipophilic drugs can be easily incorporated in droplets of o/w microemulsion[7]. Econazole nitrate is an antifungal drug containing imidazole ring which interacts with 14 demethylase a cytochrome P-40 enzyme which converts to lanosterol to ergosterol. Econazole inhibits the ergosterol synthesis which is the essential component of fungal cell membrane, this increases the cellular permeability causing leakage of cellular components, resulting in fungal cell death[8.9]. Due to low solubility of Econazole Nitrate is incompletely absorbed after oral administration[10]. It is also topically applicable in the treatment of cutaneous candiasis and tinea infections of skin[11]. The objective of present research was to formulate a stable microemulsion with good consistency, bioavailability, prolonged action and penetration of Econazole Nitrate.

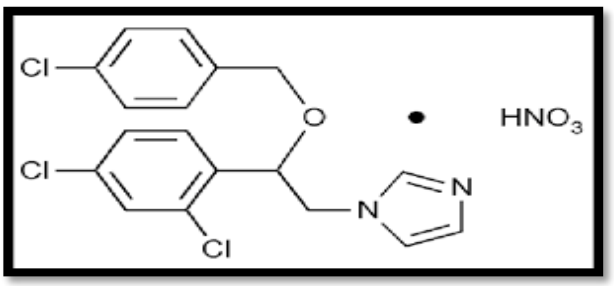

Fig 1. Structure of Econazole Nitrate

Materials and Methods

Materials

Econazole nitrate are procured from Solanki suppliers (Pune, India). Oleic acid and Tween 20 were purchased from Solanki suppliers (Pune, India). All the chemicals and solvent used were of analytical grade.

Methods

Identification of pure drug

Identification of pure drug was carried was by Fourier transform Infra-red Spectrophotometry (Shimadzu 8400S) scanned in the range of 200-400 nm.

Identification of drug by FTIR

FTIR (Shimadzu 8400S) spectrophotometer were used in the range of 400-4000 cm^{-1} using potassium bromide discs (Mixing ratio1:1) The samples were hermetically sealed in aluminium pans and heated at a constant rate of 10°C/ min over a temperature range of 40 to 300°C.

Determination of melting point

Melting point of drug was determined by Thiele's tube method. The small amount of drug in one end closed capillary attached to graduated thermometer and constant heat was supplied to the assembly suspended in the paraffin bath. The temperature at which the drug melts is noted.

Preparation of Microemulsion

Weighed required quantity of drug (Econazole nitrate). Econazole drug was dissolved in oil (oleic acid). The Econazole nitrate was then dissolved with the mixture of surfactant and co-surfactant (Tween 20 and propylene glycol). Finally, the appropriate amount of water is added to the solution mixture drop by drop to get microemulsion.

Table 1
Preparation of Optimized microemulsion

Ingredients	Batch 1	Batch 2	Batch 3	Batch 4
Econazole nitrate (gm)	1	1	1	1
Oleic acid (ml)	7	7	7	7
Tween 20 (ml)	30	25	20	15
Propylene glycol (ml)	30	28	26	24
Distilled (ml)	32	39	49	53

Evaluation of Microemulsion

- **pH**[12]**:**
  The pH of Microemulsion was determined using digital pH meter (Model EQ-610). Before measuring the pH of optimized microemulsion, the pH meter was calibrated with phosphate buffer 4 and 7. Then microemulsion was taken in glass beaker and electrode of pH meter was dipped into it for a minute and pH was noted.
- **Viscosity**[13,14]:
  Viscosity of microemulsion was determined by using Brookfield viscometer (Model LV) using spindle no 62. The apparent viscosity was measured at 10, 20, 30, 50 and 100 rpm. The Brookfield viscometer consist of cup which is stationery and spindle which is rotating. Different rotating size spindles are used and immersed in liquid. For liquids with low viscosity, large sized spindles are used (large diameter and surface area) are used and for higher viscosity small spindles (small diameter and surface area) are used. Rotate the spindle in microemulsion till we get a constant dial reading in the display of viscometer. This procedure is repeated for three times to get a reproducible result.
- **Electrical conductivity**[15]**:**
  The conductivity measurement helps in determining whether the formulation is water continuous or oil continuous type. The solubilization of selected oily mixture was measured quantitatively by measuring the electrical conductivity. The conductivity of formulated samples was measured using conductivity meter (Model Systonics- Conductivity meter 304).
- **Drug content**[16,17]**:**
  Drug content of microemulsion was determine by dissolving accurately weighed 1ml of microemulsion in 10 ml of methanol. After suitable dilution absorbance was recorded by using UV- visible spectrophotometer (UV – 1800 Shimadzu, Japan) at 270 nm.
- **Zeta potential**[18]**:**
  HORIBA Scientific SZ -100 measures the Zeta potential of microemulsion to determine the charge on the surface of particles. The material is introduced into the disposable cell, and the Zeta potential is calculated by measuring particle electrophoretic moiety.
- **Particle size determination**[19]**:**
  Samples were diluted using distilled water followed by measurement of particle size and Zeta potential in the triplicates and average values. Particle size of microemulsions was determined using HORIBA sz-100 (z type) and the average values ±SD were recorded.
- **Scanning Electron Microscopy**[20]**:**
  Scanning electron microscopy provides high resolution imaging that may be used to evaluate diverse materials for surface cracks, defects contaminants and corrosion. When a focused stream of secondary electron interacts with atoms in sample, multiple singles produced that include information about the surface topography using Nova NanoSEM NPEP. All the images are canned at 10000 x with a 5 m dimension scale 303.

Result and Discussion

Fourier Transform Infrared Spectroscopy (FT-IR)

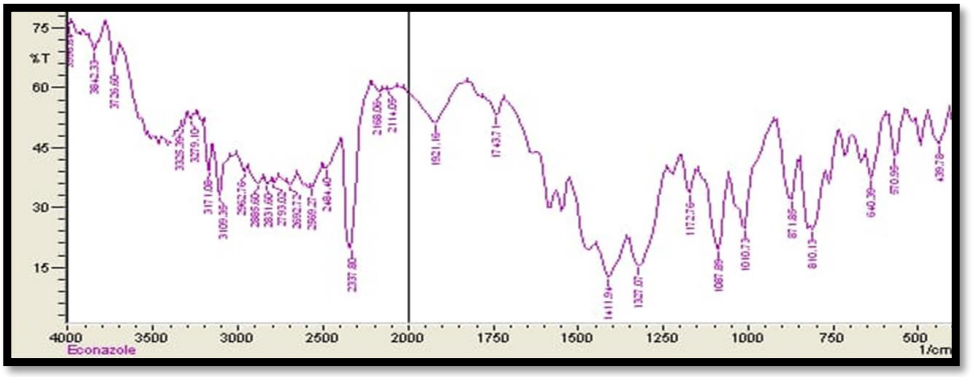

Fig 2. Fourier Transform Infrared Spectroscopy (FT-IR)

Identification and confirmation of pure drug (Econazole Nitrate) was carried out by observing obtained spectra. It showed characteristics peak at 3325.39 (-C-N stretching) imidazole ring; 3109.35 (-C-H stretching); 1411.94 (-C-C stretching);640 (-C-Cl stretching). These peaks value was in accordance with previously reported spectra of Econazole Nitrate.

UV spectroscopy

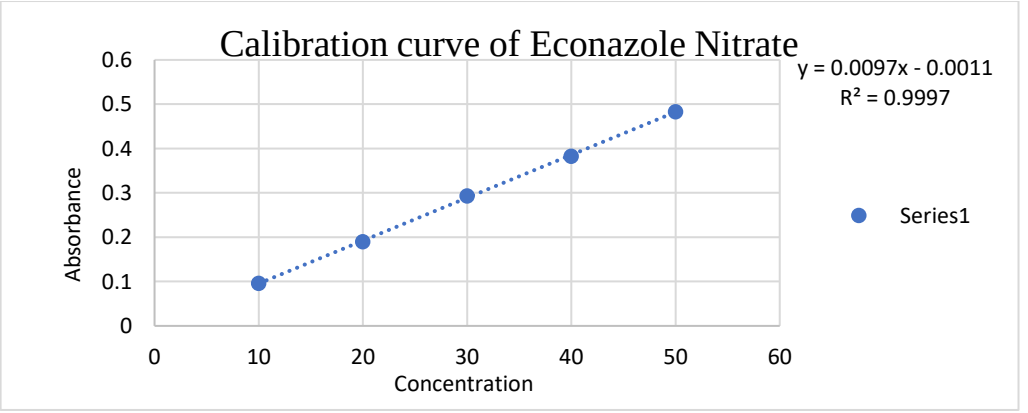

Fig 3. Calibration curve of Econazole Nitrate

The standard calibration curve of Econazole Nitrate was obtained by plotting absorbance against concentration. The calibration curve obeys Beers- Lamberts law and it shows good linearity. Econazole Nitrate in methanol showed maximum absorbance at 270 nm. The concentration range of 0- 50 µg/ml at 270 nm showed coefficient of correlation value of 0.9997.

Melting Point

The melting point of Econazole Nitrate was found to be 162℃.

pH, Drug content (%)

The pH value of optimized Econazole Nitrate microemulsion formulation (B1) was suitable for topical application because the pH of skin in the range 5.5 to 7.0. pH of different microemulsion was checked and it was shown in table. Drug content of all microemulsions was done and result was shown in table:

Table 2
Characterisation of microemulsion

Batches	pH	Drug Content (%)
1	6.2	98.45
2	5.8	96.05
3	5.6	93.67
4	6.1	94.34

Viscosity

Viscosity was determined and result was shown in table

Table 3
Viscosities of microemulsion

Viscosity (RPM)	Batch 1 (cP)	Batch 2 (cP)	Batch 3 (cP)	Batch 4 (cP)
10	126	138	255	348
20	147	157.5	199	238.5
30	153	163	175	192
50	158	166	150	149.4
100	160.5	169	121.5	109

The rheological properties of the microemulsion are evaluated by Brookfield viscometer. These viscosities determination confirm whether the system is o/w or w/o microemulsion.

Determination of Particle size and Zeta potential

Table 4
Determination of Particle size and Zeta potential

Batches	Particle size (nm)	Zeta Potential (mV)
1	35.2	17.5
2	89.6	17.3
3	544.8	21.7
4	1732.6	19.0

The droplet size of microemulsion determines the rate and extent of drug release absorption. The small of particle size of microemulsion lead to more rapid absorption as well enhanced the bioavailability of the formulation. Particle size of

optimized microemulsion (B1) was found to be 35.2 nm; such globules were considered to be suitable for topical administration. The zeta potential governs the stability of microemulsion, it measures the value for stability sample. The high value of zeta potential indicates electrostatic repulsion between droplets. Zeta potential of B1 was found to be 17.5mV.

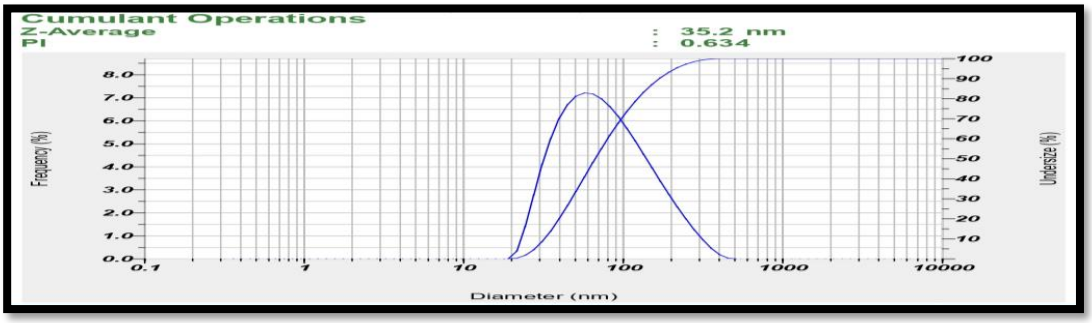

Fig 4. Particle size of optimized formulation (B1)

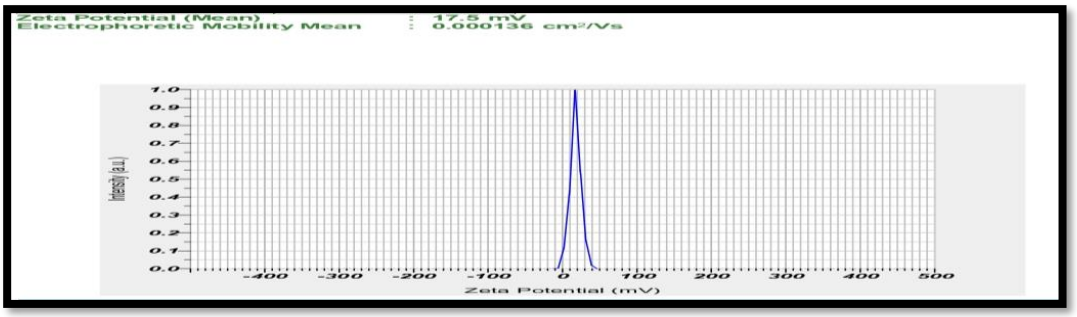

Fig 5. Zeta potential of optimized formulation (B1)

Electrical Conductivity

Table 5
Determination of electrical conductivity

Batches	200 ms	20 ms	2 ms	200 µs	20 µs
1	000	00.2	0.19	190	1.
2	000	00.2	0.22	1	1.
3	000	00.2	0.22	1	1.
4	000	00.1	0.18	177	1.

Electrical conductivity is utilized to identify nature of o/w or w/o microemulsion. It is measured using electro conductometer, used to identify whether there is an oil or water as continuous phase. It also identifies the phase inversion phenomenon.

Scanning Electron Microscopy

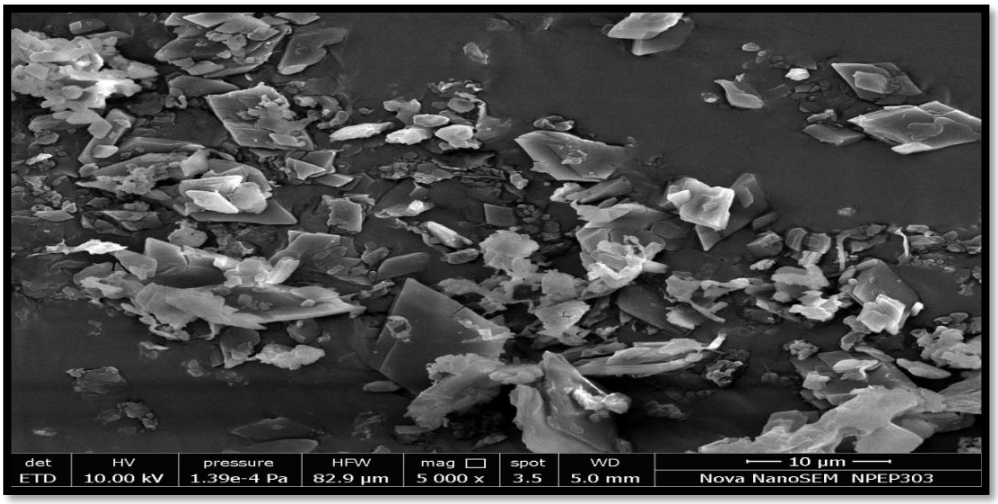

Fig 5. SEM of optimized formulation

The optimized batch of microemulsion was subjected to SEM analysis for morphology and surface topography. The SEM analysis of the microemulsion shows hexagonal and bicontinuous structure.

Conclusion

The topical route is most popular and successful for controlled and targeted drug delivery. The aim of this work is to develop Econazole Nitrate microemulsion for prolonged release based on topical delivery and for also treating skin fungal infection in short duration of time. The microemulsion containing Econazole Nitrate for solubility enhancement was prepared by phase titration method. The microemulsion is most accepted for topical drug delivery as it shows prolonged shelf life. The various formulation was prepared as per the different composition. The prepared formulation of B1 was optimized for results such as particle size, zeta potential and scanning electron microscopy. The stability behaviour can be determined using zeta potential. The formula is prepared with complete and extend release of poorly soluble antifungal drug can be prepared with expected higher penetration through the skin due to small particle size.

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