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Formulation and evaluation of microemulsion containing griseofulvin

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***Abstract*---**The aim of the following research is to formulate a microemulsion containing drug Griseofulvin. Griseofulvin is an antifungal agent which inhibits the mitosis. It is recommended orally for treatment of fungal disease. It is used to treat skin infections such as jock itch, athletes foot and ringworm. Also it is used to treat the fungal infections of scalp, toenails and fingernails. Griseofulvin is a BCS II class drug. It is highly lipophilic poorly soluble drug with low oral bioavailability. The present aim was to increase the solubility by microemulsion system for topical delivery. Microemulsions are clear, stable and isotropic liquid. They show advantages such as spontaneous preparation, scale up, ease of preparation, improving

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drug solubility of hydrophobic drugs. It is also used to increase the bioavailability of drug. These also aim at controlling the bioavailability of various drug molecules. The review put forward the development in microemulsion containing system. They improve solubility, chemical stability and oral bioavailability of poorly water soluble drugs. There is formulation of four different batches. The batches differ in quantity of surfactant and co- surfactants.

***Keywords*---**griseofulvin, microemulsion, particle size, zeta potential, scanning electron microscopy.

Introduction

The formulation and development of novel drug delivery system with the nature of enhancing the effectiveness of the existing of drug is an ongoing process in pharmaceutical research. The microemulsion concept was introduced in 1940s by Hoar and Schulman who generated a clear single phase solution by triturating milky emulsion with hexanol[1]. Microemulsion are clear, transparent, thermodynamically stable dispersions of oil and water, stabilized by an interfacial film of surfactant frequently in combination with a co-surfactant[2]. Alternative names for this system are often used such as swollen micelle, transparent emulsion, solubilized oil and micellar solution. Microemulsions are bicontinuous system that are essentially composed of bulk phases of water and oil separated by a surfactant/cosurfactant rich interfacial region[3]. These systems have advantages over conventional emulsions in that they are thermodynamically stable liquid systems and are spontaneously formed.

Griseofulvin is an effective antifungal drug for several species of fungi, such as Microscopium, Epidermophyton and Trichophyton[4].Griseofulvin is practically insoluble in water and is a biopharmaceutics classification system Class II drug, which means that it has a low solubility and a high permability[5]. Due to its low water solubility, Griseofulvin has a dissolution rate, which lead to low drug bioavailability. Low Griseofulvin absorption can be increased by formulating it as a microemulsion, which lead to selection of Griseofulvin microemulsion gel preparation as the topic for this study[6]. Griseofulvin may cause some systemic side effects if given orally over the long term. Side effects that may arise include proteinuria, nephrosis, leukopenia, hepatitis, clotting disorders, liver enzyme elevation, hyperbilirubinemia, and bleeding in the digestive tract. To avoid these adverse effects, a Griseofulvin microemulsion gel preparation for topical use was formulated to overcome these problem.

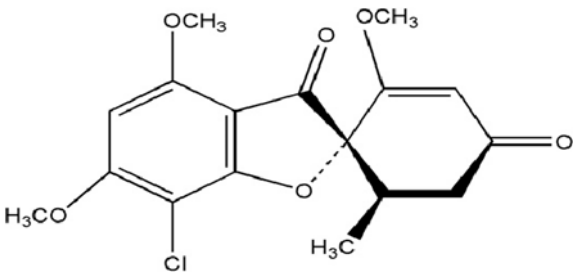

Fig 1. Structure of Griseofulvin

Materials and Methods

Materials

Griseofulvin was purchased from the Solanki suppliers (Pune, India). All the Chemicals used as oleic acid, propylene glycol, Tween 20 were of analytical grade.

Methods

Identification of pure drug

Identification of pure drug was carried by Fourier Transform Infra-red spectrophotometry (Shimadzu 800s) 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 ratio 1:1). The samples were hermetically properly sealed in aluminium pans and heated at a constant rate of 10℃ /min over a temperature range of 40-300℃.

Determination of Melting point

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

Formulation of Microemulsion

Weighed required quantity of drug (Griseofulvin). Griseofulvin drug was dissolved in oil (oleic acid) and this phase is sonicated for 5 min. The phase of surfactant and co- surfactant was prepared. Both the phases are mixed together by using magnetic stirrer. The addition of dropwise water was done to obtain 100 ml microemulsion. 100 ml microemulsion was prepared.

Table 1
Preparation of Optimized Microemulsion

Ingredients	Batch 1	Batch 2	Batch 3	Batch 4
Griseofulvin (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 water (ml)	32	39	46	53

Evaluation of Microemulsion
pH

The pH of Microemulsion was determined using digital pH mete (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. As pH of skin is usually 5.1 to 5.6, the pH of microemulsion of topical delivery should be always considered within this range.

Viscocity

The 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 and100 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 high 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

The conductivity measurement helps in determining whether the formulation is water continuous or oil continuous type. The solubilization of selected oily mixture was measured qualitatively by measuring the electrical conductivity. The conductivity of formulated samples was measured using conductivity meter (Model Systonics -Conductivity meter 304).

Drug content

Drug content of microemulsion was determined by dissolving accurately weighed 1 ml 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. The drug content is determined using an equation that includes a liner regression analysis of the calibration curve.

Zeta potential

Zeta potential is used to determine the stability of the prepared microemulsion. The charge on the surface of the particles is characterized by HORIBA SZ-100 by measuring the Zeta potential of the microemulsions. The sample is injected into a disposable cell and measurement of the particle electrophoretic moiety results in the calculation of Zeta potential.

Particle size determination

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).

Conductivity

The Conductivity of prepared microemulsion was measured by digital conductivity meter. Voltage is applied between two electrodes in a probe immersed in a sample. The drop in voltage caused by resistance of sample is used to calculate the conductivity per centimetre

Scanning Electron Microscopy

SEM provides detailed image of the structure which is not possible by TEM. It is also used in the particle counting and size determination. The average magnification of scanning electron microscopy is 20X to 30,000X.

Results and Discussion

UV Spectroscopy

Calibration is defined as the process of assessment and refinement of the accuracy and precision method. It is the general method for determining the concentration of substance in an unknown sample by comparing to the unknown to set of standard samples of unknown concentration.

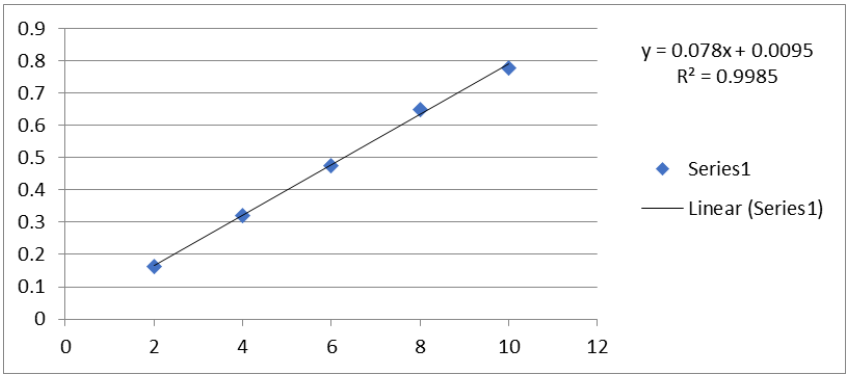

Fig 2. Calibration curve of Griseofulvin

IR Spectroscopy

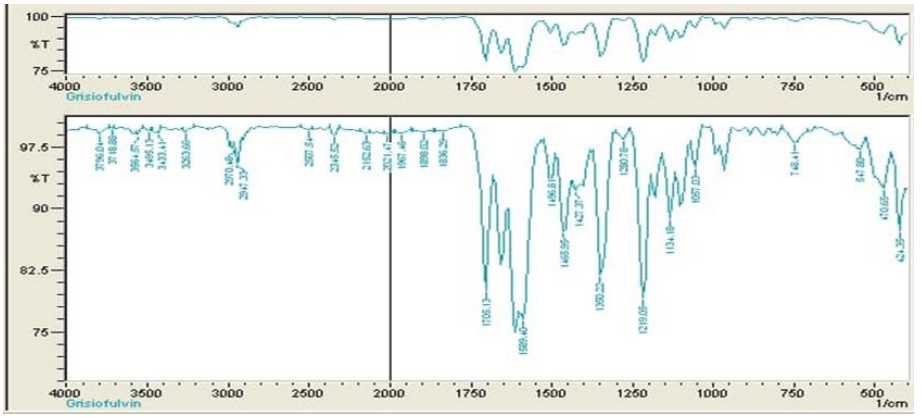

Fig 3. Interpretation of IR of Griseofulvin

Table 2
Interpretation of IR of Griseofulvin

S. No	Reference peak wavenumber	Observed peak wavenumber	Functional groups
1	1500-1600	1589	C=C
2	600-1500	1465	C-H
3	3200-3400	3263	O-H
4	1680-1760	1705	C=O

Melting Point

The melting point of Griseofulvin was found to be 220℃.

pH, Drug Content

The pH of microemulsion of topical delivery should be always considered within this range. pH of different microemulsions was checked and it was shown in table. Drug content of all microemulsions was done and result was shown in table:

Table 3
Determination of pH, drug content

Batches	pH	Drug content (%)
1	5.8	94.89
2	6.5	92.78
3	6.2	98.89
4	5.6	90.70

The higher the amount of drug more it will show concentrated formulation. These formulations range from concentrated aqueous.

Viscosity

Viscosity was determined and results was shown in table

Table 4
Viscosities of microemulsion

Viscocity (RPM)	Batch 1 (cP)	Batch 2 (cP)	Batch 3 (cP)	Batch 4 (cP)
10	000	00.0	0.03	1.
20	000	00.0	0.03	1.
50	000	00.0	0.03	1.
100	000	00.0	0.01	1.2

Determination of Particle size and Zeta potential

Table 5
Determination of Particle size and zeta potential

Batches	Particle size	Zeta Potential (mV)
1	5237.3	-36.4
2	785.9	-46.2
3	377.3	-46.5
4	2156.2	-44.5

The particle size of microemulsion is determines the rate and extent of drug release absorption. The small of particle size is required for lead to more rapid absorption as well enhanced the bioavailability of the formulation. Particle size of optimized microemulsion was found to be 377.3 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 negative zeta potential indicates the droplets of microemulsion having no charge i.e. the system is stable. Zeta potential was found to be -46.5mV.

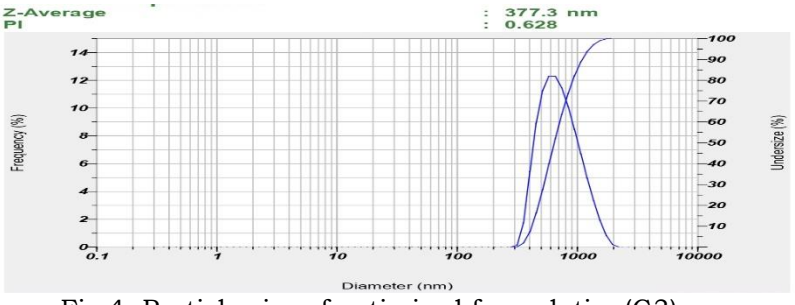

Fig 4. Particle size of optimized formulation(G3)

Zeta Potential (Mean) : -46.5 mV
Electrophoretic Mobility Mean : -0.000360 cm²/Vs

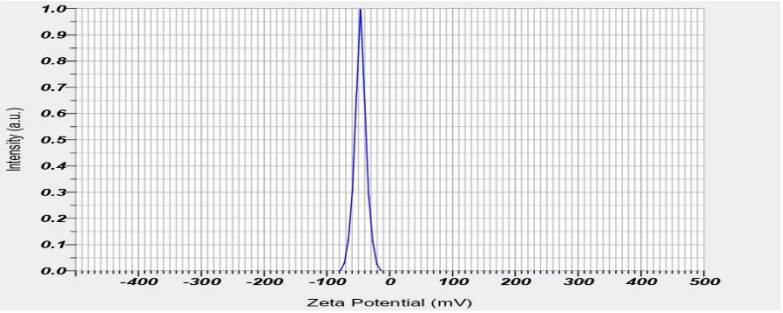

Fig 5. Zeta Potential of optimized formulation(G3)

Electrical Conductivity

Table 6
Determination of electrical conductivity

Batches	200 ms	20 ms	2 ms	200 µs	20 µs
1	000	00.0	0.03	026	1.
2	000	00.0	0.03	031	1.
3	000	00.0	0.03	028	1.
4	000	00.0	0.01	012	1.

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

Scanning Electron microscopy

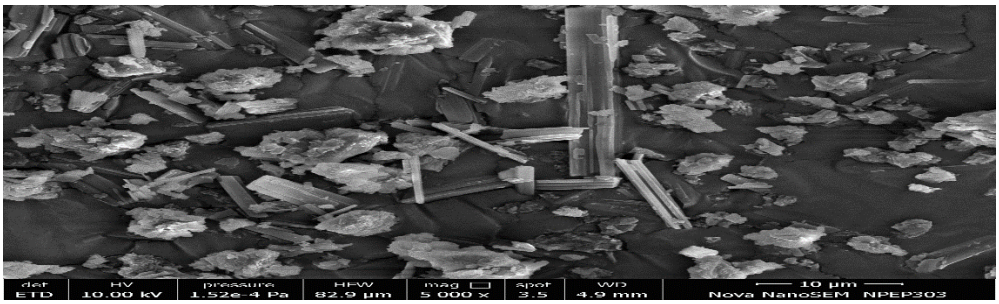

Fig 6. SEM of optimized formulation

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 current research work is focused on the preparation of safe, efficient and more compatible microemulsion which will enhance utility of these novel vehicles. The microemulsion protects labile drug, control drug release and reduce patient variability. The microemulsion can be used to optimize drug targeting without a concomitant increase in systemic absorption. The role of microemulsion is used to overcome problems of poor aqueous solubility of highly lipophilic drug compound. It provides high, consistent and reproducible bioavailability. The microemulsion preparation shows transparent yellow colour. The zeta potential determines the stability of formulation. To avoid the adverse side effect, a Griseofulvin microemulsion for topical use was formulated.

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