Preparation and Evaluation of Nanoemulsions as Carrier for Poorly Water Soluble Drug: Ketoconazole

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ABSTRACT

The objective of the present study was to develop and characterize an optimal stable nanoemulsion formulation of Ketoconazole with an aim to increase its bioavailability. The components for the formulation of nanoemulsion were olive and clove oil selected as the oil phase, surfactants namely Tween 40and 80 and the co-surfactants, Ethanol were selected. Different concentrations of oil and surfactant, which formed Nano emulsions were selected based on the thermodynamic stability and dispensability test. Optimized formulation was selected for in vitro study on the basis of higher drug release, optimum globule size, minimum lower viscosity, and overall lower surfactant concentration and co-surfactant. The diffusion of drug from nanoemulsion was compared with marketed formulation and we obtained better result from it. Thus nanoemulsion could be used effectively to improve the bioavailability of poorly water soluble drugs to improve their bioavailability. The nanoemulsion were optimized optical transparency, viscosity measurement, phase separation determination of pH, measurement of globule size, zeta potential, drug content, in vitro diffusion study, stability study.

Keywords: Ketoconazole, FTIR studies, oils, surfactants, Co-surfactants, In vitro drug release studies

I. INTRODUCTION

Nano emulsions, also known as submicron emulsions, ultrafine emulsions and miniemulsions, are submicron sized colloidal particulate systems considered as thermodynamically and kinetically stable isotropic dispersions, which consist of two immiscible liquids like water and oil, stabilized by an interfacial film consisting of a suitable surfactant and co-surfactant to form a single phase.[1] Nanoemulsions are positioned to play a crucial role in boosting medication delivery and enhancing patient outcomes as this field of study continues to advance.[2] Ketoconazole is a broad spectrum imidazole antifungal agent marketed as creams and tablets. It interacts with 14-demethylase, a cytochrome P-450 enzyme and inhibits ergosterol synthesis and increased fungal cellular permeability and is used against a wide variety of fungi and yeasts. It is readily but incompletely absorbed after oral dosing and is highly variable. The ketoconazole nanoemulsion was prepared.[3] The optically clear and low-viscous formulation with enhanced solubility and minimum droplet size diameter would pose a definite promise in improving the significance of poorly soluble drug. So, the objective of the present research work was to formulate nanoemulsion of ketoconazole for improving the solubility and bioavailability of drug.[4,5]

II. MATERIALS

Ketoconazolewas obtained from Hetero lab, HYD. Oils, Surfactants, Co surfactants were procured fromSynpharma Research Labs, Hyderabad, and other chemicals, and the reagents used were of analytical grade.

Formulation of nanoemulsion

Nanoemulsion formulation was performed using spontaneous emulsification method. Ketoconazole is added to the oil phase which has added butylated hydroxyanisole to homogeneous, add a smix solution which is a mixture of surfactant and cosurfactant, then stirred with the magnetic stirrer until homogeneous. Aquades is added by means of titration, stirred continuously until nanoemulsion is formed which is marked by the formation of a translucent solution.[6,7]

Table-1: Composition of Nano emulsion

F. no	Drug (mg)	Oil (mg)	S mix ratio	S mix (mg/ml)		Water (ml)
				Surfactant	Co-Surfactant	
F1	250	10	4:1	32	8	50
F2	250	20	4:1	25	5	50

F3	250	18.18	4:1	21.82	5.45	54.55
F4	250	14.04	4:1	16.84	4.21	64.91
F5	250	11.94	4:1	14.33	3.58	70.15
F6	250	14.81	4:1	23.71	5.92	55.56
F7	250	11.63	4:1	18.61	4.65	65.12
F8	250	11.11	4:1	19.45	19.44	50

CHARACTERIZATION

Viscosity: The viscosity was measured to determine rheological properties of formulations. Brookfield Rheometer viscometer at 30˚C with a CPE 61 spindle at 30 rpm was used to serve this purpose. Results were taken in triplicate and the average was taken in to consideration. [8]

pH: Another important parameter of Nano emulsion is pH. The excipients used in the formulation decide the pH of the final preparation and hence the route of administration. The change in the pH may affect the zeta potential of the formulation which in turn can affect the stability of preparation. The pH of the formulations was measured using digital pH meter. Results were taken in triplicate and the average was taken in to consideration. [9]

Drug content: The drug content of Drug nanoemulsion formulation was measured using UV visible spectroscopic method. The 2 µg/ml of aliquot was prepared using nanoemulsion formulation using diluting solvent. The samples were measured as 258 nm using UV VIS spectroscopic method. Results were taken in triplicate and the average was taken in to consideration. [10]

Centrifugation: This parameter was characterized to check the physical stability. The nanoemulsion system was centrifuged at 5000 rpm for 10 minutes to determine whether the system shows signs of creaming or phase separation. The system was observed visually for appearance. [11]

Conductivity: Electrical conductivity of formulated samples was measured using a digital conduct meter at ambient temperature. Results were taken in triplicate and the average was taken in to consideration. [12]

Dilution test: If the continuous phase is added in nanoemulsion, it will not crack or separate into phases. Maximum amount of water and oil were added to o/w and w/o formulations respectively and then inspected visually for clarity and phase separation. Here 50 and 100 times aqueous dilution of the formulation were visually checked for phase separation and clarity. Results were taken in triplicate and the average was taken in to consideration.[13]

Globule size and Zeta potential analysis: Nanoemulsion formulation was diluted 50 times and 100 tomes with distilled water. The resultant samples were prepared by gentle agitation for 5 min using a magnetic stirrer. In addition, globule size distribution (PSD) and zeta potential of the final nanoemulsion were determined using dynamic light scattering technique by Malvern zetasizer (NANO ZS). [14]

SEM Analysis

Morphological evaluation of Ketoconazole Nano emulsion was conducted by scanning electron microscopy. The samples were placed over a copper grid coated with carbon film and air-dried, and then were stained with 0.1% phosphotungstic acid. Finally, the samples were air dried and then observed with an H-7650 Scanning electron microscope (Hitachi Ltd, Tokyo, Japan).[15]

Drug encapsulation efficiency

Precisely weighed 100mg of Ketoconazole Nano emulsion were suspended in 100ml of phosphate buffer (pH 7.4) and kept in sonication for 2hrs. Then the samples were centrifuged at 1000rpm for 20mins to remove the supernatant layer, if any. The samples were filtered. From this filtered solution 1 ml of sample was withdrawn and diluted to 100 ml with phosphate buffer (pH 7.4). Then it was analysed spectrophotometrically at 258 nm.[16]

Diffusion study

In vitro release of Ketoconazole Nanoemulsion was conducted by a Franz diffusion cell apparatus. The dialysis membrane having a pore size of 2.4 mm with 10 ml of pH 7.4 phosphate buffer at 37°C. Briefly in a 10 ml beaker 10 ml of pH 7.4 phosphate buffer was taken. A 2 ml of formulation was taken into a dialysis bag and dipped into the buffer solution. The flask was kept on a magnetic stirrer. Stirring was maintained at 300 rpm and the temperature of the buffer was maintained at 37°C. Sampling was done by withdrawing 1 ml of aliquots from a beaker. Immediately 1 ml of new buffer was added to keep the sink condition. Samples were analyzed after sufficiently diluting with buffer by using a UV/Spectrophotometer at a

wavelength of 258 nm. Each test was conducted thrice and average value taken for the calculation.[17,18]

Stability studies

Storage stability was studied by storing the lyophilized Nanoemulsion samples at 4°C and room temperature for 3 months. In addition, Ketoconazole stability in the Nano emulsion was examined by determining the amount of parent drug remaining after specific storage periods.[19]

III. RESULTS AND DISCUSSION

Drug - excipient compatibility studies

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and excipients were studied. The peaks obtained in the spectra of each formulation correlates with the peaks of drug spectrum. This indicates that the drug was compatible with the formulation components.

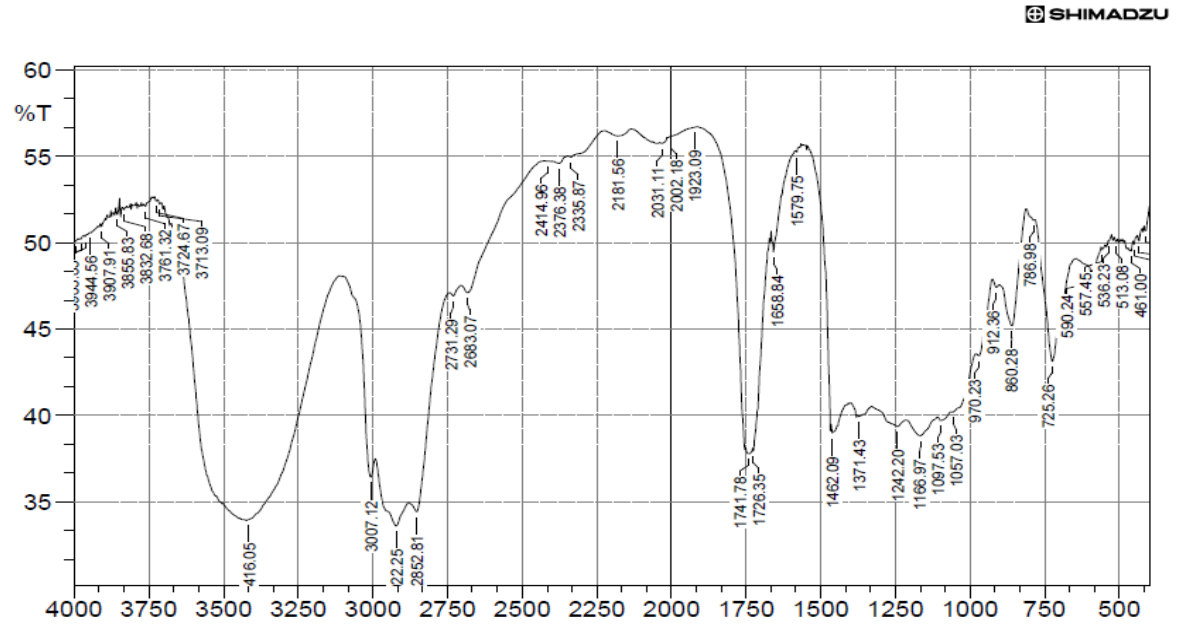

Fig-1: FTIR Studies of ketoconazole

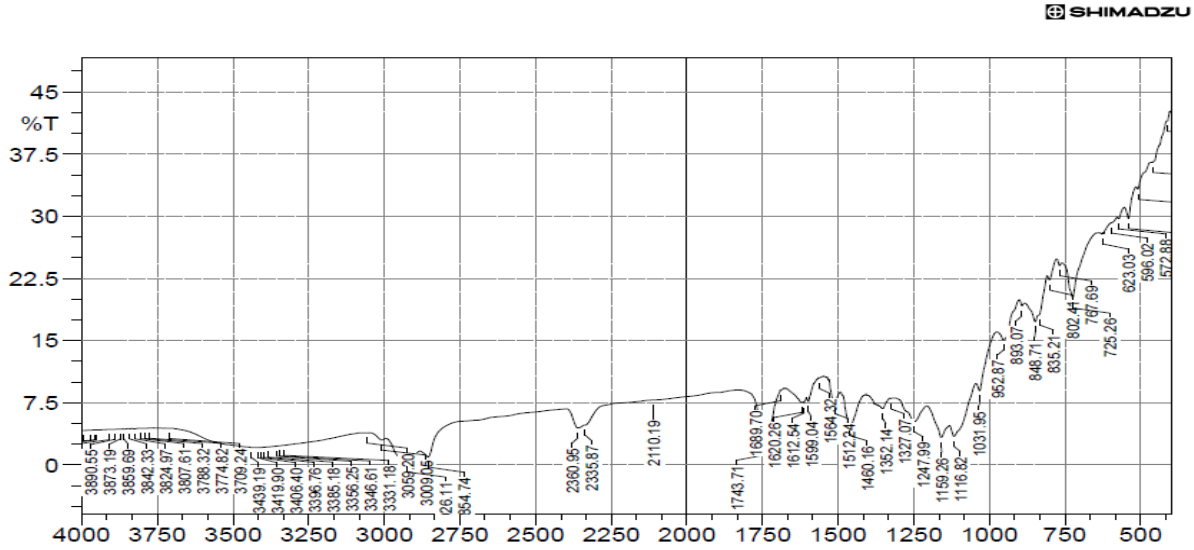

Fig-2: FTIR Studies of optimized formulation

EVALUATION PARAMETERS:

The entrapment efficiency for the prepared formulation was in the range of 72.50±1.27 to 82.30±1.30 %.

The entrapment efficiency increased progressively with increasing the concentration of polymers which

could be attributed to the formation of larger nanoemulsion that entrapped more amount of drug. Nanoemulsions were found to be the finest among all with entrapment efficiency of 82.30±1.30%.

TABLE-2: DRUG ENTRAPMENT EFFICIENCY OF ALL FORMULATION

F.no	Drug entrapment efficiency (%)	pH	Viscosity(mm^2/sec)
F1	76.93±1.56	5.50	0.936±0.56
F2	72.50±1.27	5.86	0.892±0.39
F3	79.60±1.36	5.89	0.925±0.45
F4	80.12±1.40	5.72	0.869±0.47
F5	79.89±±1.25	5.39	0.945±0.50
F6	82.30±1.30	5.48	0.898±0.48
F7	83.56±1.42	5.60	0.856±0.35
F8	81.95±1.53	5.23	0.863±0.41

Viscosity

Increase in viscosity can affect the penetration of formulation through skin. Viscosity of formulation is affected by the sonication amplitude. Lower the viscosity better is the permeation. According to our optimum formulation result viscosity i.e. 0.856±0.35 mm/sec is in good range, so we can assure that our formulation have good permeation due to ideal viscosity range.

pH

pH of formulation has important role for compatibility of formulation with skin. Our formulation has pH value of 5.60 that is compatible to pH of our skin. Skin pH range (4.7 - 7.5).

Clarity test for NE

Nano emulsions have to be clear in appearance. Formulations we prepared were having difficulty in giving clarity to formulation. Eight formulations with different surfactant to oil concentration yielding turbidity except F7 showed little clarity than turbidity so increased the time of sonication from 10 to 24 minutes and yet not clear. Prepared four more formulations in order to obtain clear results but we changed sonication from continuous to periodic sonication. Periodic sonication yields better result in clarity. It shows that sonication was effect on transparency of nanoemulsion.

Determination of globule size and morphology

Sample was coated with gold and allowed the SEM to capture the images at a temperature of - 120^0c and voltage of 5kV.

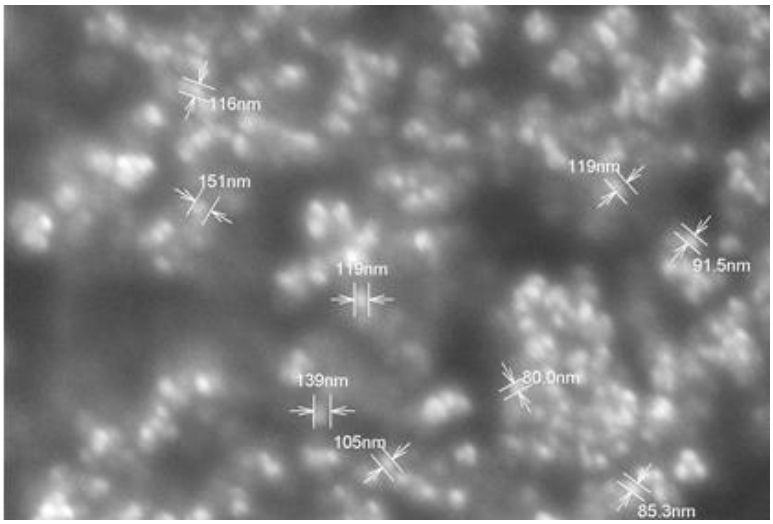

Fig-3: SEM analysis of Optimized Nano emulsion

The SEM photomicrographs of the nanoparticles are shown in Figures, the morphology of the prepared different types of nanoemulsion was found to be almost spherical in shape and have rough surface. The mean particle size of the different formulations of the prepared nanoemulsion was between 50 to 110 nm, it was observed that the particle size increase with increasing in the concentration of polymer and surfactant ratio as shown in the formulations that contain the highest ratio of polymers.

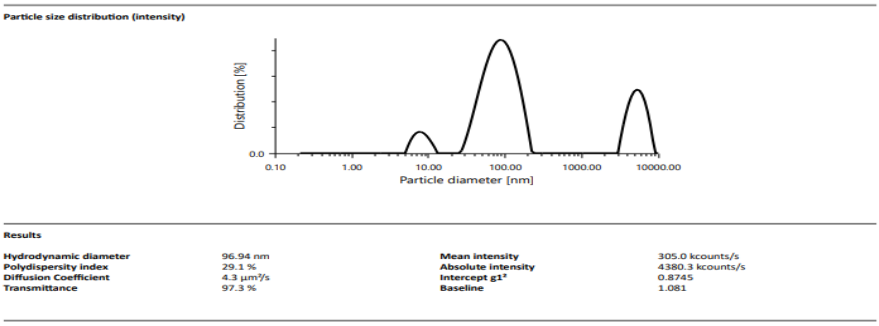

Fig-4: Particle size analysis of Optimized Nano emulsion

Zeta potential

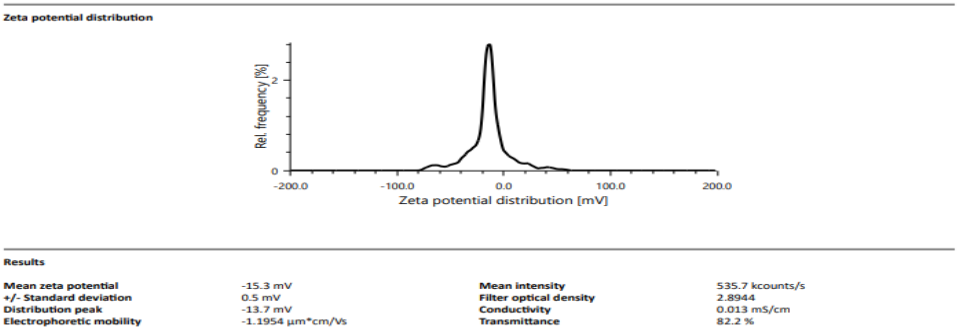

Fig-5: Zeta potential of Ketoconazole nanoemulsion

Table-3: Evaluation Studies of Ketoconazole Nano emulsion particle size and Zeta potential

F. No	Particle size (nm)	ZETA POTENTIAL
F1	89.68±1.25	-20.1±0.46
F2	92.37±1.63	-13.3±0.53
F3	90.47±1.48	-12.6±0.67
F4	88.93±1.22	-13.9±0.59
F5	87.35±1.63	-14.5±0.67
F6	90.12±1.70	-16.8±0.75
F7	96.94±1.69	-15.3±0.74
F8	93.42±1.34	-18.1±0.68

In vitro drug release studies:

Among the four types of nanoemulsion highest amount of release percentage i.e., 97.85±1.68 % was found for Ketoconazole Nano emulsion.

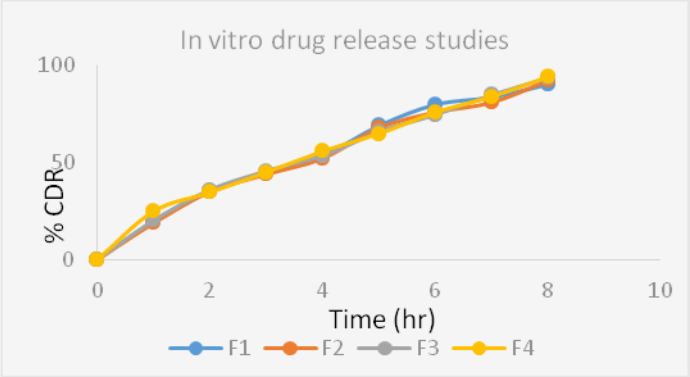

Fig-6: Drugrelease studies of (F1-F4) formulations

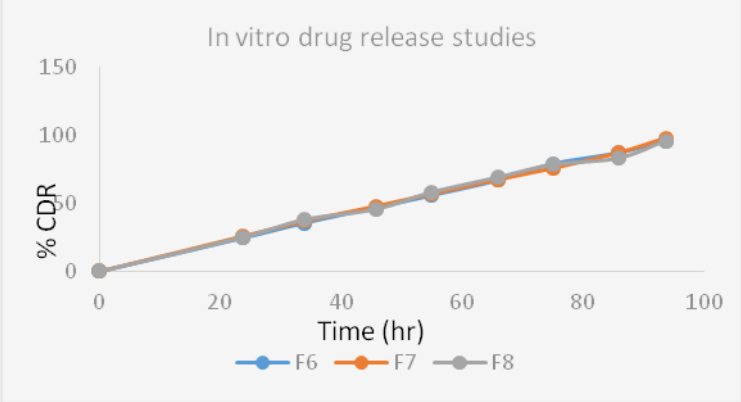

Fig-7: Drug release studies of (F5-F8) formulations

Table-4: Results of Ketoconazole Nano emulsion of all formulations

% Drug Release studies								
Time (hrs)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**

0	0	0	0	0	0	0	0	0
1	19.96±1.35	18.86±1.28	20.20±2.33	24.98±1.88	23.65±1.30	24.58±1.28	25.85±1.96	25.10±2.58
2	35.80±1.47	34.97±1.47	35.96±2.10	34.67±2.56	33.89±1.22	35.64±2.11	36.88±2.37	37.94±2.35
3	44.76±2.10	43.98±1.75	45.75±2.14	44.96±1.30	45.71±1.34	46.89±2.57	47.94±2.67	45.68±1.89
4	53.59±2.13	52.16±1.89	53.61±1.30	55.82±2.69	54.86±2.13	55.75±2.48	56.89±1.47	57.89±1.78
5	68.97±1.49	67.81±1.47	65.89±2.38	64.89±2.47	65.91±2.66	66.97±2.68	67.48±1.75	68.94±2.55
6	79.81±1.50	75.84±3.10	74.71±1.30	75.82±1.49	74.97±2.35	78.90±2.66	75.80±2.33	78.12±1.85
7	83.58±1.28	80.93±2.34	84.86±2.28	83.94±1.32	85.82±2.22	86.94±1.99	87.14±1.75	72.85±1.58
8	90.22±2.56	92.18±1.76	93.58±2.58	94.17±2.38	93.58±1.58	95.86±1.47	97.85±1.68	95.66±2.33

Drug release kinetics of Optimized formulation (F7)

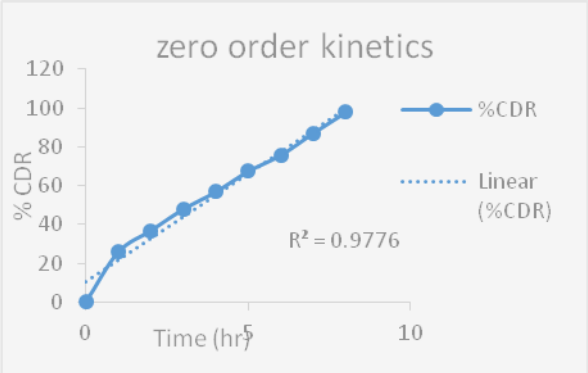

Fig-8: Zero order kinetics

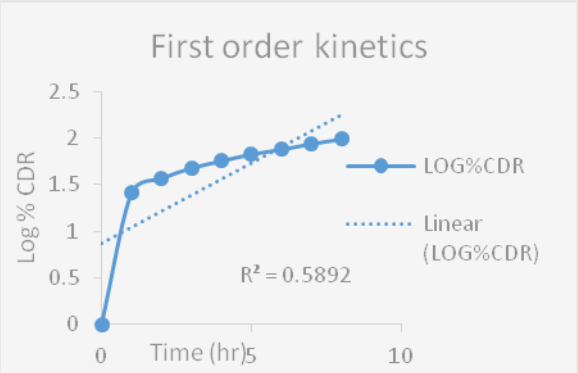

Fig-9: First order kinetics

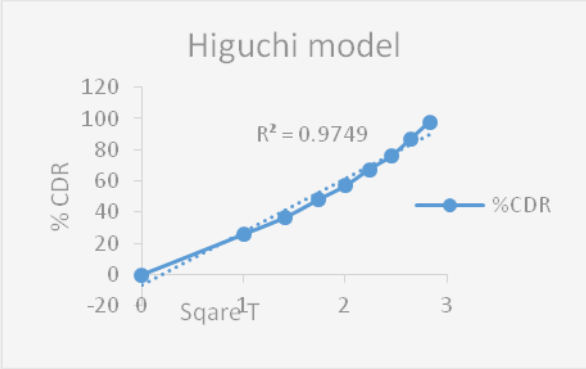

Fig-10: Higuchi model

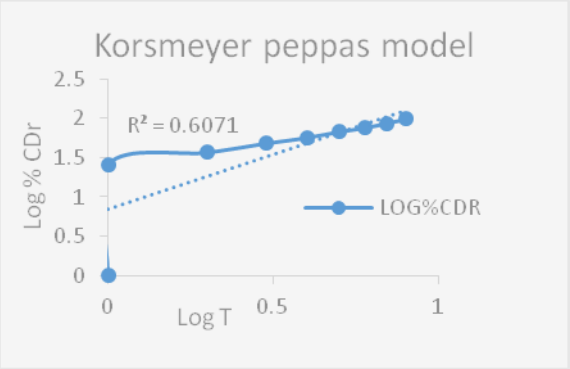

Fig-11: Korsmeyer peppas

Stability studies of Optimized formulation (F7)

Optimized formulation F7 was selected for accelerated stability studies as per ICH guidelines. The patches were observed for color, appearance and flexibility for a period of three months. The folding endurance, weight, drug content, % cumulative drug release of the formulation was found to be decreasing. This decrease may be attributed to the harsh environment (40^0C) maintained during the studies.

Table-5: Stability studies of optimized formulations

% Drug release studies					
Formulation Code	**Initial**	**1st Month**	**2nd Month**	**3rd Month**	**Limits as per Specifications**
F-7	97.85±1.68%	96.89±1.48%	95.83±1.54%	94.82±1.37%	Not less than 85 %
F-7	97.85±1.68%	96.82±1.71%	95.75±1.50%	94.25±1.48%	Not less than 85 %
F-7	97.85±1.68%	96.50±1.37%	95.28±1.68%	94.58±1.42%	Not less than 85 %

CONCLUSION

To conclude this study, cinnamon oil was tested for antifungal activity and findings shows better activity of olive oil over fungal strains than clove oil. Therefore, we proceed our work with olive oil essential oil as dual ingredient having active effect against fungi as well as oily phase excipient for our nanoemulsion formulation. For preparing an optimum Nano emulsion formulation we incorporated olive oil into emulsion system using water as an aqueous phase and surfactant to overcome interfacial tension and maintain stability of colloidal system. Formulations with different ratios were prepared with olive oil as an oil phase and API; the surfactants used were tween 40 and tween 80. Purified water was used as an aqueous phase throughout the study. The most compatible NE formulation was with Tween 40 with periodic sonication gave an optimum result among other formulations. This optimum formulation was then tested for its characterization such as droplet size, PDI and zeta potential for stability of our nanoemulsion system and was accepted depending on findings and result. The final formulation as olive oil based Nano emulsion for the treatment of fungal infections, but according to the good results obtained from the whole study, it can be ensure the effectiveness of NE formulation. From above result concluded that olive oil based nanoemulsion drug delivery system can be effective for topical application in the treatment of fungal diseases and further study on animal being need to perform before its commercial use.

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Conflicts of Interest:

The author declares that there are no conflicts of interest regarding the publication of this article. Additionally, this article does not include any studies involving human subjects. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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