PREPARATION AND IN VITRO EVALUATION OF MICONAZOLE NITRATE NANOEMULSION USING TWEEN 20 AS SURFACTANT FOR EFFECTIVE TOPICAL / TRANSDERMAL DELIVERY

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ABSTRACT

The present study has aimed to investigate the effective transdermal delivery of anti-fungal miconazole nitrate (MN) by formulating as nanoemulsion (NE). The NE was prepared by spontaneous emulsification technique and the corresponding gel was prepared using carbopol 934. Clove oil, tween 20 & ethanol were chosen as oil, surfactant & cosurfactant respectively after optimization. The ratio of surfactant and cosurfactant (Smix), oil and Smix were optimized as 1:1. The NE's were coded as F1 to F4 and the corresponding gel as F1(G) to F4(G). The NE's were characterized in vitro for transparency, drug content, compatibility, droplet size, zeta potential, viscosity, pH, transmission electron microscopic (TEM) analysis, drug release, release kinetics and stability studies. *In vitro* skin permeation and antifungal assay were conducted for F4 (G) and compared with a marketed preparation. Transparent NE of narrow size distribution, suitable zeta potential, pH and viscosity were obtained. TEM revealed the formation of discrete nanosized (247.5nm - 511nm) droplets. Maximum drug loading and compatibility of the drug with excipients were obtained. NE F3 had produced a maximum drug release of 94.8%±2.6% upto 8h. The gel F3 (G) has yielded a significant MN permeation of 93.7%±2.1% in 12h across the cellulose acetate membrane compared with marketed gel permeation of 42.9%±1.8%. The release process followed higuchi kinetics and fickian diffusion mechanism (n<0.5). Anti-fungal assay for F3 (G) revealed higher % zone of inhibition against *Aspergillus niger* and *Candida albicans* compared to marketed sample. The NE was stable at room temperature and effectively delivered MN transdermally compared with the marketed gel.

KEYWORDS: Nan-oemulsion (NE), Miconazole nitrate (MN), transdermal delivery, *in vitro* characterization, TEM, antifungal screening.

INTRODUCTION

Nanoemulsion (NE) offers several advantages as drug carrier for transdermal delivery of pharmaceutical agents including controlled droplet size, lower concentration of surfactant and the ability to effectively dissolve lipophilic drugs, enhanced skin permeation and extended release of lipophilic drugs. Moreover, they exert good sensorial and physical properties such as complete dispersion on skin and skin hydration. The drug Miconazole nitrate is fungicidal, used to treat topical and transdermal fungal infections (Samira Sadat Abolmaali, 2011). Miconazole Nitrate is a highly lipophilic and its physicochemical properties suggest its suitability for topical and transdermal delivery. It is being available as a conventional gel in the market and the development of nanoemulsion was aimed with a view to increase its effectiveness through transdermal delivery.

MATERIALS AND METHODS

Miconazole nitrate was procured from Fourts (India) Laboratories pvt.Ltd, India. Tween 20 was obtained from Merck Pvt Ltd, Mumbai. Span 20, Castor oil, Clove oil, Acetone, Ethanol, Ethyl acetate were obtained from S.D Fine Chemicals, Mumbai, Sodium Chloride, Potassium dihydrogen orthophosphate were purchased from Lobachemie, Mumbai, Dimethyl sulfoxide was obtained from Qualigens, Mumbai.

Preparation of miconazole nitrate nanoemulsion: The homogeneous organic phase (S1) composed of clove oil in water–miscible solvent of 5ml acetone and 5ml methanol. The homogeneous aqueous phase (S2) of 30ml composed Tween 20. The organic phase was injected into the aqueous phase under magnetic stirring (1500rpm). The O/W emulsion was formed instantaneously leading to the formation of nanodroplets. The water miscible solvent was removed by evaporation. The prepared nanoemulsion was stored in screw caped vials and kept at room temperature. The transparency observed visually in terms of clarity is shown in Figure 1.

Preparation of miconazole nitrate nanoemulsion gel: The gel was prepared by dispersing 1g of carbopol 934 in sufficient quantity of distilled water. After complete dispersion, the solution was kept in dark for 24h for complete swelling. Then the 5mg MN loaded nanoemulsion was slowly added to the viscous solution of carbopol 934 under moderate magnetic stirring at 550rpm for 10-15min. The pH value was subsequently regulated to 6-9 and then isopropyl alcohol, PEG 400, propylene glycol and triethanolamine were added to obtain homogeneously dispersed gel.

Optimization of miconazole nitrate nanoemulsion: The Nanoemulsion was optimized with respect to oil, ratio of surfactant & co-surfactant, addition time and speed. The oil was chosen based on the maximum solubility of drug. The surfactant of no or less skin irritation, hydrophilic lipophilic balance (HLB) > 10 was selected. Safety is a major

determining factor in choosing a surfactant, as a large amount of surfactants may cause skin irritation, very less concentration of was selected.

Compatibility determination: The possible interaction between Miconazole nitrate and the surfactants of nanoemulsion was analysed using fourier transform infrared spectroscopy by KBr disk technique (3mg sample for 300mg dry KBr) using Perkin Elmer 1600 spectrophotometer, India.

Transparency of nanoemulsion: The transparencies were tested visually for clarity of the preparations against a dark background.

Drug content determination: One ml of nanoemulsion was mixed with 10ml of suitable solvent (methanol), sonicated and filtered Aliquots of different concentrations were prepared by suitable dilution and the absorbance was measured at λ max of 231 nm.(Ankur Jain, 2011).

Droplet size, pH& viscosity determination: The droplet size depends on the rate of emulsification process. The formulation (0.1ml) was dispersed in 50ml of water in a volumetric flask and gently mixed by inverting the flask. Measurement was done using a Zetasizer (Malvern, Germany). Light scattering was monitored at 25^0C at 90^0 angle (Praveen Kumar Gupta,2010). The pH value of the formulations (NE) was measured using a calibrated pH meter (Sartorious, Germany). The viscosity of the nanoemulsion was determined by a viscometer (Brookfield DV-E viscometer,) which was rotated for 10min at 100 maximum rotation per minute with spindle 61 (Shivhare, 2009).

Determination of zeta potential: The formulation (0.1 ml) was diluted 100 times using double distilled water and the zeta potential was measured by the instrument Zetasizer (Malvern, Germany). Emulsifiers not only act as a mechanical barrier but also create surface charges, which can produce repulsive electrical forces among approaching oil droplets and this hinders coalescence. The more negative zeta potential, greater the net charge of droplets and more stable the emulsion is. Zeta potential values lower than -30mv generally indicate a high degree of physical stability (Praveen Kumar Gupta, 2010).

Transmission electron microscopy: Transmission electron microscopic analysis was carried to observe the shape of dispersed oil droplets. A drop of diluted nanoemulsion was applied to a copper grid and was left for 1min. The grid was inverted and a drop of phosphotungstic acid (PTA) was applied to the grid for 10sec. Excess of PTA was removed by absorbing on filter paper and the grid was analyzed using the TECNAI 10 (PHILIPS), Japan operated at 70-80kV at 17500x magnification (Vikas Bali, 2010).

In vitro miconazole nitrate release from Nanoemulsion and gel: The dissolution studies were conducted in a micro dialysis setup. The nanoemulsion (20ml) was suspended in phosphate buffer of pH 7.4. The bulk solution was stirred continuously by a magnetic stirrer at 550rpm and 5ml sample solution was drawn at regular intervals by replacing with equal volume of fresh buffer. The quantity of released drug was analyzed by means of a UV spectrophotometer(UV-1700 CE, Shimadzu corporation, Japan), In case of gel, 1g of gel containing 100g of drug was used for the study (Swarnalatha, 2008).

To study the release kinetics, data obtained from *in vitro* release studies were plotted in various kinetic models such as zero order, first order, higuchi and Hixson crowell to find the mechanism of drug release, kosermeyer peppas model was applied and the n-value was calculated.

***In vitro* Permeation study:** The *in vitro* permeation was performed using a locally fabricated Franz diffusion cell with an effective diffusional area of 3.14cm². The cellulose acetate membrane was clamped between the donor and receiver compartment of the cell. 10ml of nanoemulsion was administered in the donor compartment. The receptor compartment was filled with 24ml of phosphate buffer pH 7.4. The diffusion cell was thermo stated at 37ºC and stirred at 600rpm. Samples of 1ml aliquots were drawn from the receiver compartment and replaced immediately with an equal volume of fresh phosphate buffer pH 7.4. All the samples were analyzed by UV (Swarnalatha, 2008; Prabagar Balakrishnan, 2009).

Antifungal screening by agar well method: Sabouraud agar plates were prepared aseptically to get a thickness of 5-6mm; the plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 37°C before inoculation. The organisms were inoculated in the plates by dipping a sterile swab in the previously standardized inoculums, removing the excess of inoculums by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times, rotating the plates through an angle of 60° after each application. Finally the swab was pressed round the edge of the agar surface. It was allowed to dry at room temperature with the lid closed. Then, wells of about 3mm diameter were punched using sterile core borer into the agar medium and filled with control (solution of MN in methanol), nanoemulsion gel and marketed gel. The plates were kept in refrigerator for one hour to facilitate uniform diffusion of the drug. Then the plates were incubated for 18-24h. Observation were made for zone of inhibition around the well and compared with that of standard 100g of drug was present in all the formulations. All the formulations were tested for antifungal activity against two organism's *Aspergillus niger* and *Candida albicans* was tested (Dongsheng Mou, 2008). The fungal growth was observed and the percentage inhibition was measured using the following formula.

Percentage inhibition = $L_2 / L_1 \times 100$

Where,   L2 = zone of inhibition of test sample

L1 = zone of inhibition of control sample.

Thermodynamic stability studies: The studies were conducted for the optimized NE F3 by centrifugation followed by a, freeze thaw cycle. The stability was studied via clarity and phase separation observation at room temperature for 3months.

Statistical analysis: Statistical analysis was done using ANOVA. The value $P < 0.05$ was kept as significant.

RESULTS& DISCUSSION

Selection of oil, surfactant, cosurfactant & their ratio: Clove oil was selected as oil phase by virtue of its maximum MN solubility (0.043mg ±0.024) compared to eucalyptus and castor oil. Transparent nanoemulsion was formed for the combination of Tween 20 and ethanol at a ratio of 1:1.

The surfactant Tween 20 lower the necessary energy to form the Nanoemulsion consequently improves the drug solubility. The addition of cosurfactant ethanol enhances the NE stability by reducing the droplet size. This reduction in size may be due to the rapid diffusion of cosurfactant by way of evaporation (12). Tween 20 has the HLB value of 16.7. Whereas, the co-surfactant ethanol has 4.2. Hence, the required HLB value i.e. >10 could be arrived in the formation of o/w nanoemulsion. Also tween20 possesses high miscibility with the oil phase (Jignesh, 2011).

Oil and Smix ratio: Seventeen different combinations of oil and Smix (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1) were employed. The ratio of 1:3 (F1), 1:2 (F2), 1:1 (F3) and 2:1 (F4) were chosen due to the formation of transparent NE (Vikas Bali, 2010).

Addition time and RPM: To ascertain the individual effects of addition time and rpm variables on nanoemulsion, a full factorial design for two variables at three levels comprising nine formulations were applied i.e 3^2 factorial design. Different replicates were carried out in different days. Oil and Smix ratio of 1:1 was selected for the preparation of nanoemulsion. The result showed significant influence of these two variables Table 1. Nanoemulsions of suitable size and stability were obtained at an optimum speed of 1500rpm in a time period of 10min. These conditions were kept constant in the preparation of NE.

Compatibility determination: The wave numbers 3429.38cm^{-1}, 3417.89cm^{-1} and 3429.25cm^{-1} indicates molecular vibrations due to hydrogen-bonded O-H Stretching of phenols or alcohols or carboxylic acid. The peaks at 2695.32cm^{-1} and 2913.56cm^{-1} due to aldehydes like ethanol were present in case of MN and tween 20 whereas absent in case of formulation F3 which might be due to evaporation of alcohol during the process. The peaks strongly illustrate no interaction between the MN and tween 20 (Figures 2,3 &,4).

Drug content determination: Drug content was determined at λmax of 231nm which had ranged from 8.35mg ± 0.096 mg to 9.31mg ± 0.069 mg

Droplet size, pH & viscosity determination: The particle size and their distribution of NE formulations are given in Table 2 and in Figure 5 to 8 respectively. The particle size of optimized NE F3 was found to be 247.5nm which is very small in size compared to other formulations. This might be due to the effective ratio of oil and surfactant concentration in addition to the agitation speed and time of NE preparation. A small particle size is very much prerequisite for drug delivery as the oil droplets tend to fuse with the skin thus providing a channel for drug delivery (10). The pH values of all prepared formulation ranged from 5.92 to 6.87 which are considerd acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

Zeta potential: Emulsifiers not only act as a mechanical barrier but also create surface charges called zeta potential, which causes repulsive electrical forces among approaching oil droplets and this hinders coalescence. The more negative zeta potential, greater the net charge of droplets and more stable the emulsion is. Zeta potential values lower than -10mv generally indicate a high degree of physical stability. The formulation F3 possess good physical stability since the value of zeta potential was -13.8.as shown in Figure 9.

Transmission electron microscopy: The TEM of nanoemulsion appeared dark and the surroundings was bright (Figure 10), The droplet size measured using TEM, provides point-to-point resolution. The droplet size is in coordination with the Zetasizer measurement. The picture represents the formation of discrete, spherical & smooth surfaced droplets of NE clearly. The size of these oil globules were ranged from 58.3nm to 117.63nm.

In vitro **miconazole nitrate release from nanoemulsion:** The in vitro release profile of miconazole nitrate nanoemulsion is given in Figure 11. A burst release followed by a steady release has been observed and the drug release process ranked in the order of F2 > F3 > F1> F4. The amount of drug released after 210 min were found to be 90.6%±1.7, 88.9%±3.1, 88.0%±1.2 and 85.3%±3.2 respectively for F2, F3, F1 and F4. Thus significant ($P<0.5$) higher drug release was observed for formulation F2 compared to others. The optimized formulation F3 followed the higuchi release kinetics ($R^2=0.0034$) that is based on diffusion controlled. According to the "n" value 0.158 obtained from korsemeyer peppas model, F3 followed non – fickian case II diffusion mechanism.

The *in vitro* release profile of miconazole nitrate from its emulsified gel formulations is shown in Figure 12. The release process is in accordance with the release process of NE and has followed the order of F3 > F4 > F1 > F2 >Marketed sample, where the percentage of drug released after 8h were 94.8%±1.9%, 88.4%± 2.3%, 86.2%± 2.3%, 79.1%±2% and 30.2% ±2.7% respectively. Significant higher drug release was obtained for the formulation F3(G) which was greater than the marketed sample. This may be due to equal ratio of surfactant and cosurfactant and the small very small droplet size of F3 formulation.

***In vitro* permeation study:** The results of the permeation study are shown Figure 13. Slow release of MN was elicited by NE F3 (93.7%±1.9) at the end of 12h and for the marketed sample only 42.9%±2.4 of MN had permeated. Whereas the miconazole nitrate Gel formulation F3(G) had produced a twofold higher permeation. The enhanced permeation obtained with NE formulation might be due to the increased thermodynamic activity and solubility of MN. The thermodynamic activity of drug is a significant driving force for the release and permeation (Bouchemal, 2004). Nanoemulsions could also be act as drug reservoir where drug is released gradually from the inner phase to the outer phase and thus led to extended release.

Antifungal screening by agar well method: The zone of inhibition that remains free from fungal growth was appeared around the test product on the agar plate. Greatest activity was observed for F3 (G) in which the percentage inhibition reached up to 73% for *Aspergillus niger* and 77% for *Candida albicans*. The results are shown in Figure 14.

Stability studies: The prepared miconazole nitrate nanoemulsion was found to be clear and no phase separation had been observed in a study period of 3 months indicating the physical stability of NE.

Table 1. Process Variables Screening for Addition Time and RPM

Addition Time	RPM	Nanoemulsion Formation
3min	500	NE- NF
	1500	NE- NF
	3000	NE- NF
10min	500	NE- NF
	1500	NE- F
	3000	NE- F
20min	500	NE- NF
	1500	NE- F
	3000	NE- F

NE-NF – Nanoemulsion Not Formed, NE- F – Nanoemulsion Formed

Table.2.Particle Size, pH & Viscosity of Miconazole Nitrate Nanoemulsion

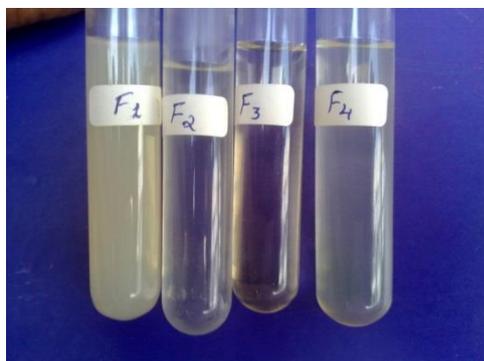| Formulation	Particle Size (nm)	pH	Viscosity
F 1	493.3	6.51	0.8290
F 2	511.0	6.27	0.8650
F 3	247.5	6.83	0.8729
F 4	414.0	5.92	0.8610

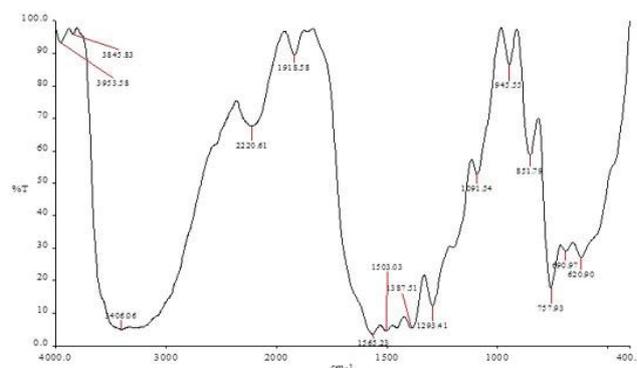**Figure.1.Transparency of Nanoemulsion F1, F2,F3 and F4**

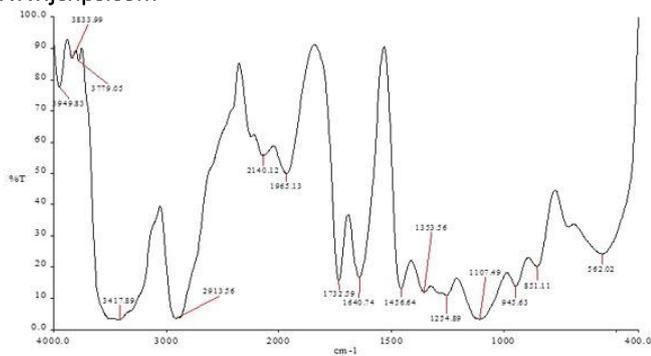**Figure.2.FTIR Spectrum of Miconazole nitrate**

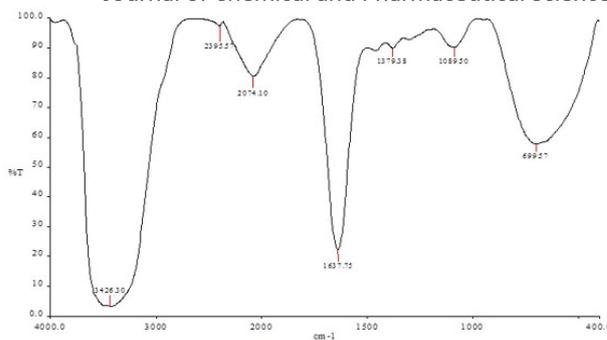**Figure.3.FTIR Spectrum of Tween 20**

Figure.4.FTIR Spectrum of Optimized Nanoemulsion (F3)

Figure 5. Particle Size & distribution of Miconazole Nitrate Nanoemulsion (F$_3$)
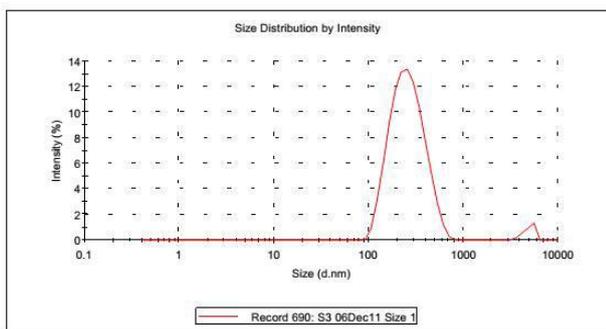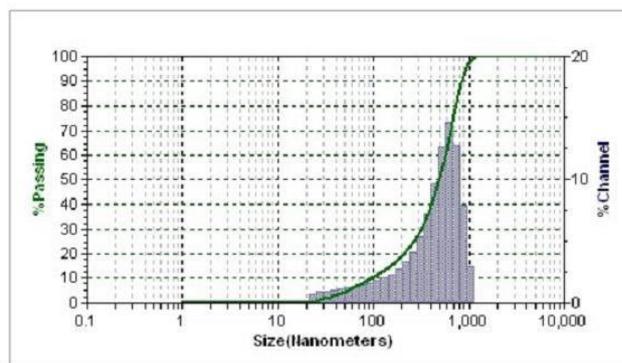

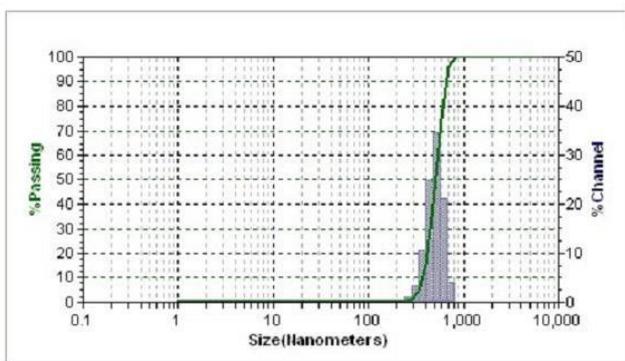**Figure 6. Particle Size & distribution of Miconazole Nitrate Nanoemulsion(F2)**

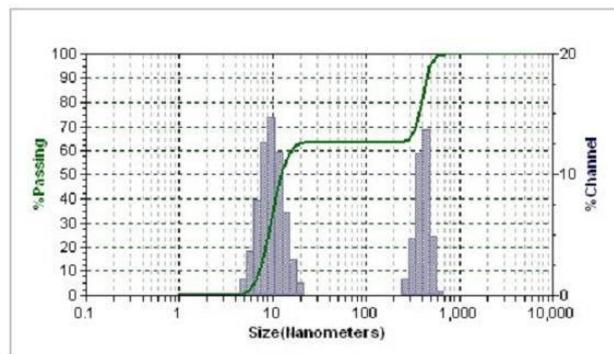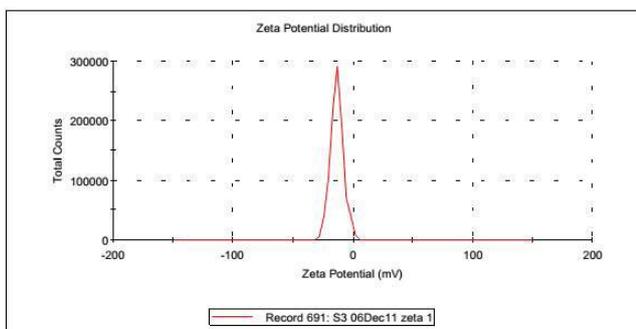**Figure 7. Particle Size & distribution of Miconazole Nitrate Nanoemulsion (F1)**

Figure 8. Particle Size & distribution of Miconazole Nitrate Nanoemulsion (F$_4$)

Figure 9. Zeta Potential of Miconazole Nitrate Nanoemulsion (F$_3$)
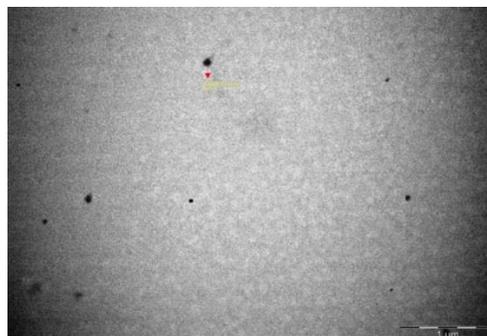

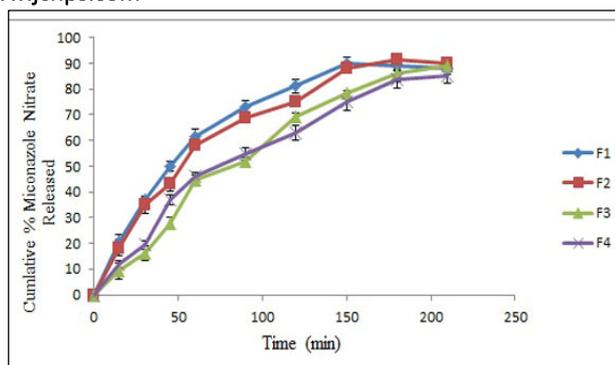**Figure 10. Transmission Electron Microscopic image of Nanoemulsion F3**

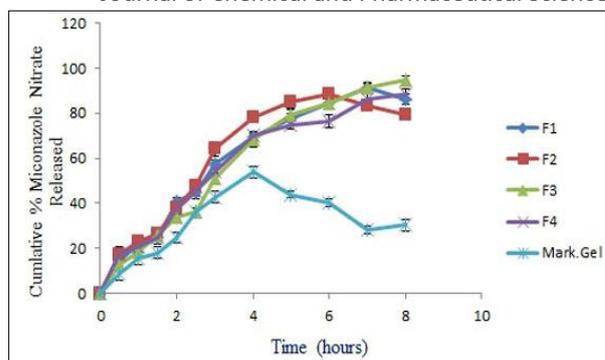**Figure 11.In vitro release of Miconazole nitrate Nanoemulsions**

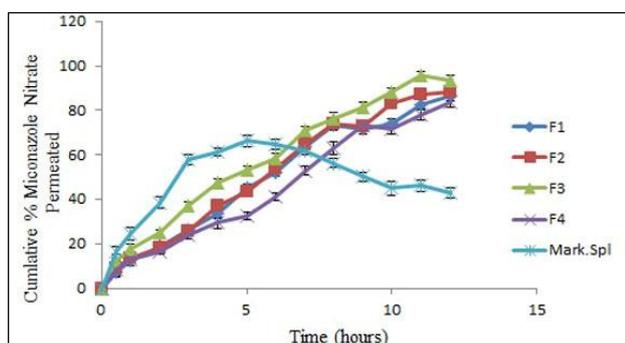**Figure 12. In vitro release of MN Nanoemulsion Gels**

Figure 13. *In vitro* permeation of Miconazole Nitrate Nanoemulsions, Gel and Marketed sample
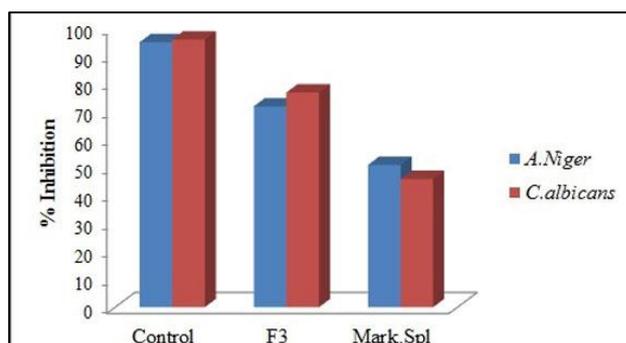

Figure 14. Comparisons of anti-fungal activity of Miconazole Nitrate Nanoemulsion Gel against *A.niger* & *C.albicans*

CONCLUSION

The findings of the work revealed that the process variables of oil, surfactant, their concentration, agitation time & agitation speed influences the formation of NE. There was a significant enhancement of MN release and permeation from the NE gel than the marketed gel preparation used in this study effective anifungal activity was found for the NE gel against *Aspergillus niger* and *Candida albicans*. Overall, it could be concluded that transdermal delivery of antifungal drug MN using clove oil Nanoemulsion is more effective compared to conventional gel.

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