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Tolnaftate loaded sln gel for improved transdermal drug delivery: formulation and evaluation

V.Viswanath[1]*, B. Narasimha Rao[1], K. Gnana prakash[1], D. Sai Padmini[2], B. Gowthami[1]

[1]Department of pharmaceutics & [2]Department of pharmaceutical chemistry, P. Rami Reddy Memorial College of Pharmacy, Utukur, Kadapa, India

***Corresponding author e-mail:** viswanath.prrm@gmail.com

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ABSTRACT

Solid lipid nanoparticles (SLN) were novel colloidal drug carrier systems offering controlled release profiles for many pharmaceutically active agents. The objective of present research work was to develop Tolnaftate-solid lipid nanoparticles using solid lipid as drug carrier. As the drug was inactive orally, it was delivered by topical route. Hot homogenization along with ultrasonication were used for such purpose. Then they were converted to gel. The drug compatibility was checked by using FTIR studies. Seven different formulations were prepared by variable concentrations of lipids and its effect on drug loading was studied. Produced Tolnaftate- SLN gels were evaluated for the parameters like Homogenity, Spreadability, Viscosity, pH, Drug content, Entrapment Efficiency, *invitro* drug release & *invitro* drug permeation studies. Antifungal activity of the optimized formulation (F7) was also studied. Tolnaftate -SLNs have good potential in treating topical fungal infections.

KEYWORDS: Tolnaftate, Span 80, Solid lipid, Carbopol 934.

INTRODUCTION

Solid lipid nanoparticles are introduced in 1991and they serves as an alternative carrier system to other conventional colloidal carriers such as emulsions, liposomes, polymeric nanoparticles etc. [1] SLNS are gaining attraction as useful novel drug colloidal carriers for most of the topical preparations. However they are consumed by various routes such as oral, rectal, nasal, respiratory, occular and by parenteral route. They are submicron colloidal carriers ranging from 50-1000nm[1] which are composed of physiological lipid. Because of their unique properties such as small size, large surface area, high drug loading, interaction of the phases at the interface these represent potent drug delivery system.[2-3]

In this type of dosage form, lipids in the solid form overcome the disadvantages associated with the liquid state of oil droplets (lipid). There was an increasing interest to use lipid based systems, reasons for this are i) lipids increases the bioavailability, ii) it is easy to evaluate lipoid excipients iii) decreases the plasma profile variability, iv) they offer the enhanced ability to solve the key issues of technology transfer and manufacture scale up. Since liquid lipids are replaced by solid lipids it offers many advantages such as low toxicity, [4] good biocompatibility, better delivery of the drugs to targeted organs [5] and it also shows better physical stability.

SLN's are most effective colloidal carriers having lipid based systems introduced earlier in the 19th century. This is the most familiar approach by which oral bioavailability of poorly water soluble drugs is enhanced. They avoids the disadvantages associated with other dosage forms like polymeric nanoparticles, liposomes[6] etc. When compared with the liposomes, solid lipid nanoparticles has no drug leakage,[7] show better stability against the hydrolysis of the drug, prolonged release of the drug from the formulation, comparatively stable against particle growth during

storage. These novel drug delivery systems prevent chemical degradation of active components and changes the release mechanism of drugs incorporated. [8, 9]

Advantages of solid lipid nanoparticles:

- Solid lipid nanoparticles showed better controlled drug release. [10]
- The drug content was high.
- Biocompatibility of the lipids used was good.
- Stability [11] of the dosage forms was increased.
- Manufacture scale up was easy.
- When compared to bio-polymeric nanoparticles, these are easy to formulate and evaluate.
- Chemically reacting compounds can be protected by preparing them in to solid lipid nanoparticles. [12]

Chemically, Tolnaftate was synthetic thiocarbamate with a naphthalene, 3-methyl phenyl rings in its structure. Squalene epoxidase enzyme [13] was used for biosynthesis of Ergosterol which was an important component of fungal cell wall, was inhibited by it. It was insoluble in water, partially soluble in ethanol and freely soluble in acetone and methylene chloride. Because of its poor solubility in water and good solubility in lipids, it was formulated as solid lipid nanoparticles. The formulations were further incorporated in gels to obtain controlled release of drug on skin. Carbopol 934 was suited best for preparation of gel because of its better suitability to incorporate TNF-SLNs. The structure of Tolnaftate was as follows in fig.no.1.

MATERIALS AND METHODS

Tolnaftate, Carbopol 934, methanol, ethanol were brought from Yarrow chem. Products, Mumbai. Stearic acid, Propylene glycol, Dimetyl sulphoxide, Tween 80, Triethanolamine were obtained from Finar Chemicals Pvt. Limited, Ahmedabad. Span 80 was brought from Labo chemi, Mumbai. Methyl & Propyl parabens were brought from S.D.Fine chemicals, Mumbai, India., Accord labs, Secunderabad respectively. Instruments like Magnetic stirrer(Remi equipments Pvt. Ltd), Probe sonicator (Orchid scientifics; PS-250), FTIR (Brucker- Germany), UV-Spectrophotometer (Systronics) were used in the study.

FORMULATION OF SOLID LIPID NANOPARTICLES:

Solid lipid nanoparticles were formulated by using the combination of both Hot Homogenisation and Ultrasonication methods. Various concentrations of lipid (such as 0.25, 0.5, 0.75, 1, 1.25, 1.75, 2% of Stearic acid) was melted separately for each formulation. Weighed quantity of drug (Tolnaftate) and Span 80 (Surfactant) was dissolved in organic solvent like ethanol and this solution was added to the melted lipid and heated to a temperature above the melting point of lipid. On the other hand, aqueous solution of stabilizer (tween80) was heated to the same temperature. The aqueous phase was added to organic phase slowly by continuous stirring and it was kept it in probe sonicator for 10 minutes. Then the dispersion was kept under stirring for 15minutes with the help of a magnetic stirrer in order to get solid lipid nanoparticles dispersion free from particle aggregates. The formula for preparing solid lipid nanoparticles is given in table.no.1.

PREPARATION OF SLN GEL:

100 mg of carbopol was dissolved in 10 ml of water and stirred well to remove air entrapment and lumps. To this ethanolic solutions of methyl and propyl parabens, propylene glycol, Dimethylsulphoxide, Triethanolamine were added and stirred well. From this prepared gel, one gram of gel was taken, weight equivalent to 10mg of Tolnaftate loaded solid lipid nanoparticles dispersion was added and mixed well without any lumps.The formula for preparing the solid lipid nanoparticulate gel was given in the table no.2.

EVALUATION OF TOLNAFTATE LOADED SLN GEL:

Drug excipient compatibility studies: The compatibility between the pure drug and all other pharmaceutical ingredients such as stearic acid, span 80, tween80, all other excipients were studied by using Fourier Transform InfraRed Spectroscopy by using Pressed pellet Technique [14] by using Potassium bromide discs. Then the discs were scanned at wave number of 4000-400$cm^{-1.}$

pH: Weighed quantity (1gm) of gel was dissolved in 100 ml of water and pH of the solution was determined using digital pH meter. [15] The results were tabulated.

Homogeneity: Each formulated gel was placed in suitable containers and it was tested for its homogeneity by visual inspection. Appearance of gel was observed along with the checking of any aggregates.

Spreadability: Weighed quantity (1gram) of each formula was pressed between two slides and allowed

to stand for 5 minutes upto which no more spreading was expected. Diameters of the spreaded circles were measured in cm and were taken as comparative values for spreadability. The experiment was repeated for three times and an average value was determined.

Viscosity: Brookfield viscometer was used to measure the viscosity of the gel prepared. The spindle was rotated at 6 r/min and obtained values were recorded. [16]

***Entrapment Efficiency*:** One gram of TNF-SLN gel was dissolved in 10ml of ethanol and centrifuged for about 10minutes.The supernatant was diluted [17] and suitable concentrations were prepared with ethanol to produce a concentration within Beer's range and the absorbance was measured at 257nm using UV-Visible spectrophotometer. Entrapment Efficiency of each formulation was calculated by using the following formula.

$$\text{Entrapment efficiency} = \frac{\text{Amount of entrapped drug}}{\text{Total amount of drug}} \times 100$$

In-vitro Permeation studies: The rat epidermis was obtained from *wistar* rats and mounted on the keshary chein diffusion cell in such a way that epidermis was in contact with receptor medium. The receptor compartment was filled with mixture of ethanol and phosphate buffer of pH 7.4 in the ratio of 2:8. Stratumcorneum was facing the donor compartment which has the gel formulation on it. The hydrodynamics in the receptor compartment were maintained by stirring with a magnetic bead .one ml of sample was withdrawn regular intervals of time and replaced with fresh medium to maintain sink conditions. Samples taken out were suitably diluted and absorbance was measured by using UV-Visible spectrophotometer. [18, 19]

***In-vitro Diffusion studies*:** Cellulose membrane prepared from egg shell was used for the study. One gram of gel having wt equivalent of TNF-SLN dispersion was spreaded on the cellulose membrane which was previously soaked with mixture of ethanol and phosphate buffer of pH 7.4 in the ratio of 2:8. The drug loaded membrane was tied over the edge of glass tube of 2cm in diameter with the help of a rubber to prevent leakage. Then this tube was in a beaker containing 100ml of dissolution medium. Magnetic stirrer was used to agitate the dissolution medium. Samples were taken out at regular intervals of time and replaced by the fresh dissolution medium. Samples collected were diluted to produce a concentration within Beers's range and the absorbance was measured at 257nm in UV-Visible spectrophotometer. [20]

Drug content determination: One gram of TNF-SLN gel was dissolved in 100 ml of mixture of ethanol and phosphate buffer of pH 7.4 in ratio of 2:8 .The volumetric flask containing gel solution was shaken in order to completely solubilise the drug. The resulting solution was filtered and suitable dilutions were made and produced a concentration within Beers's range. Absorbance of this solution was measured at a wavelength of 257nm using same solvent mixture as blank. [21]

Drug content= {Absorbance/slope} × Dilution factor

Antifungal study: Initially, Sabouraud dextrose agar media was prepared. The media was sterilized in an autoclave. Then the media was cooled to 40-50^{0}c and poured in sterile petri plates. The media was then inoculated with sub culture of fungal strains (*Candida albicans*) by pour plate method and mixed. The media in the plates were allowed to solidify in a sterile area for 5min.By the help of sterile borer, wells were prepared in the media and these wells were filled with placebo gel, optimized formulation, ethanolic drug solution, gel with 10µg concentration. These plates were incubated for 24-48hrs and Zone of inhibition was compared. [22]

RESULTS AND DISCUSSION:

Drug Compatibility Studies by FTIR Technique: FTIR studies by pressed pellet technique show no major change in the peak indicating that there was no incompatibility between the pure drug and other pharmaceutical ingredients. The peaks obtained for each functional group were listed in table.no.3.The FTIR graphs of pure drug and drug with all other excipients were given below from fig no.2-6.

EVALUATION OF TNF-SLN GELS:

Characterization of physical parameters: The results obtained after assessment of biopharmaceutical parameters were tabulated as follows. From the table, it was clear that as the lipid concentration increases parameters such as Viscosity, pH, Spreadability was also increased. After physically examination of all the formulations, they were found to be homogenous. As the concentration lipid was enhanced, the drug loading in the lipid matrix was also increased with increasing drug content in the formulations.The values of drug content, viscosity, pH, diameter of spreading areas were given in table.no.4.

Entrapment efficiency: Tolnaftate was loaded in SLN at a high level because of its lipophilic nature. Maximum entrapment efficiency of 90.3-93.9% was

observed in preparations containing TNF-SLN gels. It was found that enhanced concentration of stearic acid, increases the entrapment efficiency of Formulations.F7 was considered as optmised, because of its high entrapment efficiency. The values obtained for entrapment efficiency were enlisted in table no.5.

In-vitro diffusion studies**:** *In-vitro* drug release studies were performed over a period of 24 hrs maintaining sink conditions. Percentage cumulative drug release were obtained as 97.4, 95, 94.1, 91.9, 89.8, 86, 84.4% respectively. The drug release was retained with the increase in concentration of lipid. Because the drug embedded in solid lipid matrix was high. Hence controlled release of drugs was attained using TNF-SLN gels. The percentage drug release of different formulations was compared by using a graph in fig no.7.

Invitro permeation studies: By using rat skin, *invitro* permeation studies were performed for optimized formulation i.e., seventh formulation for a period of 24 hrs. After 24 hrs of study, 76 percentage of drug was found to be permeated through the rat skin.

Antifungal study:
The zone of inhibition was maximum for the optimized formulation(F7) when compared with the zone of inhibition obtained for the placebo gel saturated ethanolic drug solution and Tolnaftate loaded sln gel with a concentration of 10µg/ml. The corresponding zone of inhibitions was shown in fig no. 8.

CONCLUSION

In the present study, we made an attempt to formulate Tolnaftae loaded solid lipid nanoparticles with variable concentrations of Stearic acid and incorporated them in a topical gel prepared by using carbopol934 as a gelling agent. Large surface area and film forming properties of slns aid in effective permeation of drug from the formulations. Synthesised formulations were characerised for parameters such as pH, spreadability, viscosity, Entrapment efficiency, drug content, in vitro diffusion studies, *in-vitro* permeation studies, antifungal activity. Among the developed formulations, F7 was considered as an optimized one as it showed better entrapment efficiency, drug content, antifungal activity when compared with the other formulations .Sustained drug release was observed with F7 because of presence of high concentrations of lipid .Thus it was concluded that SLN Gels serve as a promising carrier for the delivery of antifungal drug, Tolnaftate for the treatment of fungal infections of skin.

ACKNOLEDGEMENTS

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Table no 1: Composition of solid lipid nanoparticles loaded with Tolnaftate

Ingredients	F1	F2	F3	F4	F5	F6	F7
Stearic acid	0.5%	0.75%	1%	1.25%	1.5%	1.75%	2%
Tolnaftate	200mg	200mg	200mg	200mg	200mg	200mg	200mg
Span 80	2%	2%	2%	2%	2%	2%	2%
Ethanol	5ml	5ml	5ml	5ml	5ml	5ml	5ml
Tween 80	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%
Water	15ml	15ml	15ml	15ml	15ml	15ml	15ml

Table.no.2: Composition of sln gel

Ingredients	F1	F2	F3	F4	F5	F6	F7
Carbopol 934	100mg	100mg	100mg	100mg	100mg	100mg	100mg
Propylene glycol	10%	10%	10%	10%	10%	10%	10%
Dimethyl sulphoxide	10%	10%	10%	10%	10%	10%	10%
Methyl paraben	0.018%	0.018%	0.018%	0.018%	0.018%	0.018%	0.018%
Propyl paraben	0.002%	0.002%	0.002%	0.002%	0.002%	0.002%	0.002%
Triethanolamine	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Water	10ml	10ml	10ml	10ml	10ml	10ml	10ml
SLN Dispersion equivalent to	10mg.	10mg.	10mg.	10mg.	10mg.	10mg.	10mg.

Table.no:3 Functional groups of infrared spectroscopy

Material to be tested	Groups Assigned				
	C-H Stretch	C-N Stretch	C-N Stretch	C=S Stretch	C=C Stretch
Tolnaftate (pure drug)	2927.6	1482.4	1297.8	1964-2000	1626.5
Tolnaftate+Stearic acid	2853.4	1436.1	1296.3	1937.8-2019.1	1601.8
Tolnaftate+Span80	2864.4	1465	1273.8	1964.2-2067.3	1601.9
Tolnaftate+Tween 80	2923.1	1481.2	1249.8	1961.5-2067.3	1643.6
Tolnaftate+All Excipients	2923.7	1457.9	1294.5	1736.3-1964.2	1649.7

Table.no:4 pH, Homogenity, Spreadability, Viscosity and Drug content of all formulations

Formulation Code	Drug Content%	Homogeneity	Spreadability (cm)	Viscosity (dynes/cm)	pH
F1	82	++	6.23	0.94×10^{-3}	6.04
F2	83.8	+++	4.76	1.54×10^{-3}	6.12
F3	84	++	5.1	1.63×10^{-3}	6.24
F4	84	++	4.36	1.67×10^{-3}	6.56
F5	85.3	+++	5.25	1.73×10^{-3}	6.32
F6	83.8	++	5.56	1.79×10^{-3}	6.89
F7	86	+++	4.71	1.80×10^{-3}	6.10

Table.no.5: Entrapment efficiency of F1 - F7

Formulation code	Entrapment (%) Efficiency
F1	90.35
F2	91.15
F3	92.21
F4	92.26
F5	93.70
F6	93.38
F7	93.96

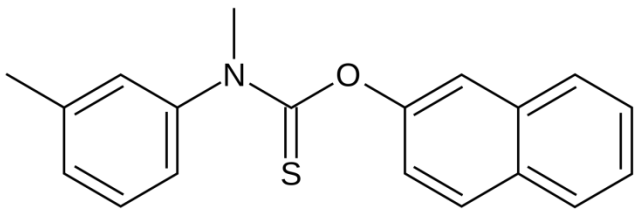

Fig.no: 1 Structure of Tolnaftate

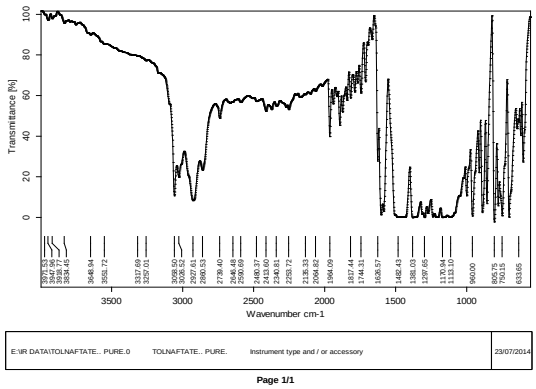

Fig.no.2: FTIR graph of Tolnaftate

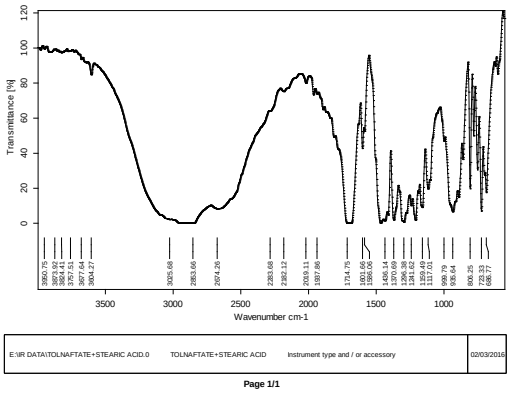

Fig.no.3: FTIR graph of TNF+Stearic acid

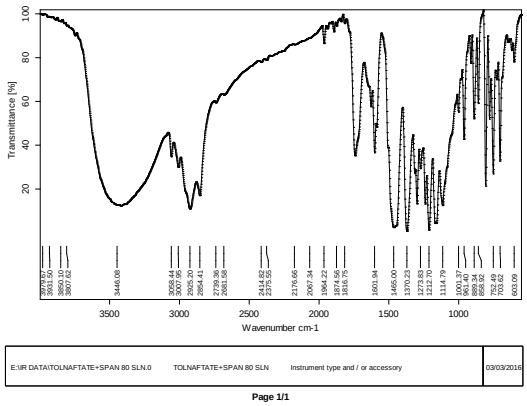

Fig no.4: FTIR graph of TNF+Span 80

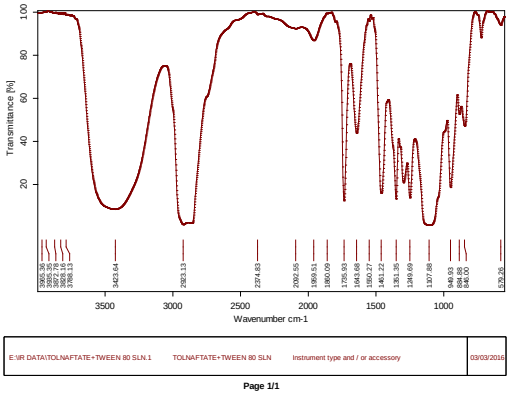

Fig.no.5: FTIR graph of TNF+Tween 80

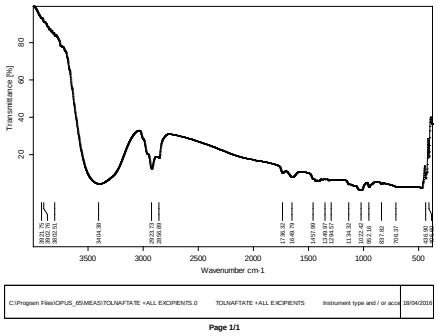

Fig.no.6: FTIR graph of TNF+Excipients

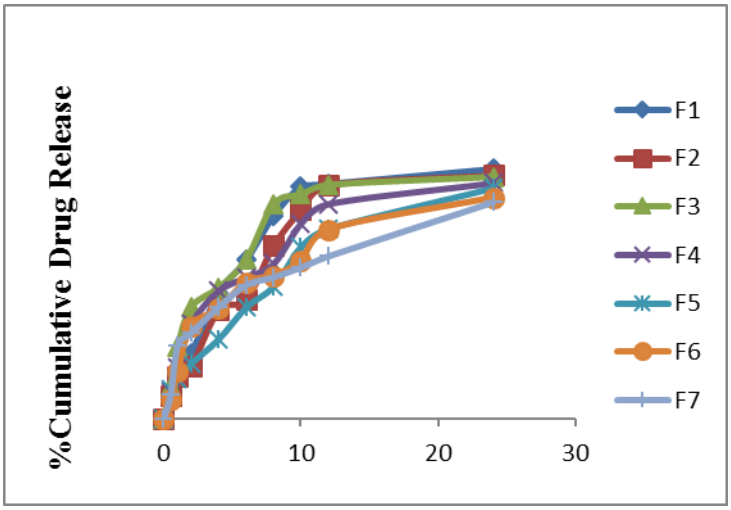

Fig.no.7: Graph showing percentage drug release for F1 -F7

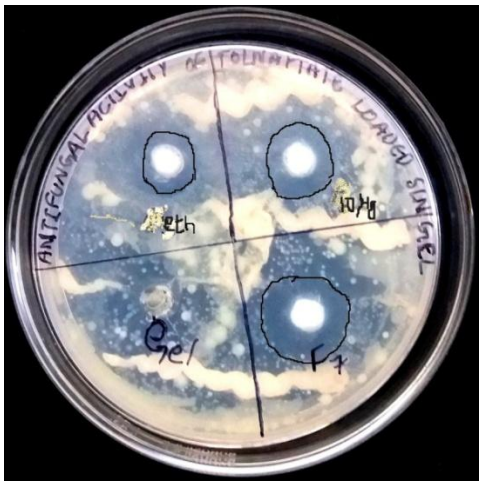

Fig.no.8: Image indicating Zone of inhibition

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