Preparation and Evaluation of Itraconazole Nanoparticle Lipid Carrier Gel for Dermatophytosis

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Abstract - Fungal infection is one of the common dermatological diseases. Drug delivery systems for topical use have shown significant advantages in targeting the drug to the action site in the body and also reduces the systemic side effects. Itraconazole was chosen as a model drug with low aqueous solubility. In the present study an attempt was made to prepare itraconazole loaded nanostructured lipid carrier (NLC). Different formulations were prepared by hot homogenization technique using solid lipid and liquid lipid (Compritol 888 & Caprol PGE 860) and surfactants (Poloxamer 188, tween 80). All the Formulations were characterized for drug content, entrapment efficiency, particle size, poly dispersity index, zeta potential &*in vitro* drug release and FTIR was done to study any interaction between excipients. The best formulation shows better drug release and entrapment efficiency 83.2%. the best formulation F3 showed better in-vitro drug release 55.46% at the end of 8th hour. The F3 was made into gel using Carbopol as a gelling agent. SEM study revealed that the NLC gel shows the particle size was found to be 500 nm in size smooth surfaces. NLC gel shows Drug release of NLC gel followed non- Fickian diffusion. NLC gel were stable at 40 ± 2°C and 75 ± 5% RH. Thus, the prepared NLC gel proved to be a potential candidate as a topical nanoparticulate sustained drug delivery system for itraconazole (BCS class II drug).

keywords: Itraconazole, Nanoparticle Lipid Carrier, Lipids.

INTRODUCTION

Fungal infections of the skin are the overall dermatological illness. The predominance of superficial infections of skin, hair and nails are far and wide. Around 40 million individuals have influenced from contagious diseases in advanced and immature countries. Dermatophytosis (Cutaneous Mycoses) have been accounted for worldwide as the most widely recognized cutaneous illnesses among people in clinical practice. Nano Lipid Carriers are good colloidal lipid nano transporter for skin application because of their different attractive consequences for skin, the capacity to secure chemically liable ingredients against chemical decay, film arrangement, controlled occlusion, skin hydration, improvement in bioavailability, physical stability and to alter drug release. [1] Itraconazole (ITR) is a triazole antifungal specialist utilized in the treatment of disease brought about by an assortment of pathogenic fungi. It is a BCS II class medication and its absorption from gastrointestinal tract varied, dose dependent inadequate when controlled through oral route. Also, the conventional oral treatment of ITR is regularly connected with many adverse drug reactions like, stomach discomfort (constipation) migraine and seldom cardiovascular failure. So, the present study was aimed to develop nano lipid carrier gel of itraconazole for dermatophytosis.[2]

MATERIALS

Itraconazole were procured from Nanoceut therapeutics, Pondicherry. Double distilled water was used in all experiments. All other chemical used were from Sigma Aldrich LTD.

METHODOLOGY

SOLUBILITY STUDY:

The solubility of itraconazole was determined using supersaturation method, to 2ml of different solvents like acidic buffer pH 1.2, phosphate buffer of pH 6.8, pH 7.4 and water, pure drug of itraconazole was added until it gets saturated. After that, it was kept undisturbed for 24hours, then it was filtered and further dilution was made with respective solvent and absorbance was measured using UV spectrophotometer. [3]

INFRARED SPECTRAL STUDIES:

To determine the compatibility between the drug and the excipients, FT-IR spectra matching approach was used. 1mg of itraconazole was mixed with 100mg of KBr (which is transparent to IR) and then thoroughly grinds the mixture in motor and made small pellets using this mixture and placed it in a Fourier Transform infrared (FTIR) spectrophotometer. The IR of the pure drug was

compared with that of mixture of drug and excipient and peak matching was done.[4]

SOLUBILITY STUDY PARAMETER:

SELECTION OF SOLID LIPIDS:

To assess the strong lipid and the liquid lipid (oil) appropriate for effective topical application which breaks down the highest concentration of Itraconazole, were added to the lipids and shaken for 2 h at 85 ◦C with 550 rpm utilizing a Thermomixer comfort (Eppendorf, Germany). Moreover, the miscibility of the strong lipid and the fluid lipid just as the solubility of Itraconazole in the combination of the lipids with the best solubility of Itraconazole was assessed under similar conditions.[5]

SELECTION OF LIQUID LIPIDS:

The solubility of ITR in various oils was determined by dissolving an excess amount of it separately in2mL of mixture of selected different oils and dichloro methane (1:1) in stoppered vials. These vials were kept at 37±1.0 _C in an isothermal shaker for 24 h to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 8000 rpm for10 min. The supernatant was filtered through a 0.45-µm-membrane filter and diluted appropriately with methanol. Samples were then analysed by UV–Visible spectrophotometer at ƛmax of 262 nm to estimate the amount of ITR dissolved in particular solvent.[6]

PREPARATION OF NLC:

Itraconazole-NLC was set up with hot emulsification technique followed by probe sonication. To sum things up, itraconazole was dispersed in the combination of liquid and fluid lipids at 85°C. In a different measuring glass, a clear surfactant solution was made. The surfactant blend was preheated to 85°C and added to the melted lipid drug combination gradually with consistent mixing in the magnetic stirrer which forms a hot emulsion. The hot emulsion was additionally exposed to highspeed homogenizer followed by probe sonication. At that point acquired solution was kept in lyophilizer to get the final product [7].

Table 1: Formulation of ITR-NLC dispersion

Formulation code	Itraconazole (g)	Caprol PGE 860 (g)	Compritol ATO 888 (g)	Poloxamer 188 (g)	Tween 80 (g)	Distilled water	Sonication time (minutes)
F1	0.1	0.56	0.5	0.5	0.5	100	30
F2	0.1	1.12	0.5	0.5	0.5	100	30
F3	0.1	1.68	0.5	0.5	0.5	100	30
F4	0.1	0.56	1	0.5	0.5	100	30
F5	0.1	0.56	1.5	0.5	0.5	100	30
F6	0.1	0.56	0.5	0.5	0.5	100	35
F7	0.1	0.56	0.5	0.5	0.5	100	40

PREPARATION OF ITR- NLC GEL:[8]

2 g of Carbopol was taken and moved gradually into 100 ml distilled water taken in a measuring glass. This solution was mixed at 200 rpm for 3 h under magnetic stirring. To this 10 g base gel, determined measure of lyophilized nanostructured lipid transporter of itraconazole was added and blended at 100 rpm for 2 h; trailed by the option of methyl paraben and propyl paraben. Triethanolamine around 1-3 drops was added to get the appropriate gel consistency

EVALUATION OF ITR - NLC DRUG CONTENT ESTIMATION:

Accurately weighed 1 g of the gel transferred to the 100 ml of volumetric flask containing 20 ml of phosphate buffer pH 5.5. The volumetric flask was shaken for 30 min and the volume was made up to 100 ml with phosphate buffer pH 5.5 solution. After suitable dilution, the sample was analyzed using Agilent technologies carry 60 UV- visible spectrophotometer at 262 nm.

DETERMINATION OF EE%:

Entrapment efficiency was determined by determining the amount of free drug spectrophotometrically at 264 nm in the supernatant after centrifugation of the known amount of nanoparticulate dispersion at 10000 rpm using REMI centrifuge for 15minutes.The entrapment efficiency was calculated using the equation.

The EE% was calculated as follows:

$$EE\ \% = \frac{\text{Total drug content (mg)} - \text{unentrapped drug amount (mg)}}{\text{Total drug content (mg)}} \times 100$$

PARTICLE SIZE ANALYSIS:

The particle size of nanostructure lipid carrier (NLC), were measured by using Malvern Zeta sizer Nano ZS-90. Before analysis, nanosuspension was further diluted with HPLC graded water followed by sonication for 30 min. The mean particle size was derived from the particle size distribution data.

ZETA POTENTIAL:

Zeta potential is defined as a measure of the magnitude of the electrostatic or charge repulsion or to allure between particles in liquid suspension. Its measurement will give detailed insight into the causes of dispersion, or flocculation, aggregation and can be applied to enhance the composition of dispersions, emulsions and suspensions. The unit of zeta potential is usually milli volt (mV). Before analysis, nanosuspension was further diluted with HPLC graded water followed by sonication for 30 min. [54]

***IN VITRO* DRUG RELEASE:** [9]

In vitro drug release for itraconazole nanostructured lipid carrier the in vitro drug release profile of itraconazole loaded NLC were studied using vertical diffusion cell. The dialysis membrane was soaked overnight in the pH 5.5 phosphate buffer. Then determined measure of product was kept in the donor compartment above the dialysis membrane. 250 ml of pH 5.5 phosphate buffer was taken in 250 ml beaker. Then the beaker was put over a magnetic stirrer, the temperature and rpm were kept up at 34±0.5°c and 100 rpm al through the study. samples (5 ml) were withdrawn at predetermined intervals of time (0,0.5,1, 2, 3, 4, 5, 6, 7, and 8 h) and supplemented with equal measures of fresh buffer. After appropriate dilution the samples were analyzed for drug concentration by UV spectrophotometer at 262 nm.

PREPARATION AND CHARACTERIZATION OF ITRACONAZOLE NLC GEL [10]

SCANNING ELECTRON MICROSCOPY (SEM):

SEM photographs were taken for the prepared nanoparticles using a scanning electron microscope (Carl Zeisus FESEM model number: Ultra 55 USA.) at different required magnifications at room temperature. The photographs were analyzed for morphological characteristics

pH DETERMINATION:

The pH of the prepared ITR –NLC gel was evaluated using digital pH meter which was calibrated with standard buffer solutions. After the calibration glass electrode was immersed in the gel and pH was noted.

SPREADABILITY:

The spreadability of formulations was determined by using horizontal glass plate method. A standard weight (5 g) was tied to the upper glass plate and about 1 g of itraconazole nanostructure lipid carrier gel was placed between two horizontal glass plates. The whole set was hold in the vertical position. The time required for the plate to slide off from the other plate was noted. The spreadability was calculated from the formula.

Spreadability = M * L/ T

M = Weight tied to upper slide (g)

L = Length of glass slide (cm)

T = Time taken (sec)

VISCOSITY:

The viscosities of gel-based formulations with different concentrations were measured using DVII Brookfield viscometer. The formulations whose viscosity was to be determined was added to the beaker and was allowed to settle down for 30 minutes at the assay temperature (25˚± 1˚c) before the measurement was taken. All measurements were made at room temperature in triplicate using spindle number **94 at 20 rpm.** [11]

EXTRUDABILITY

Extrudability measures the quantity of gel extruded when a constant force is applied to the folded tube. Prepared gel equivalent to 14 g was filled into a collapsible tube and a constant weight of 500 g was placed on to the edge of the tube to extrude the gel. The quantity of gel extruded was measured.

KINETIC ANALYSIS OF DRUG RELEASE

To reveal the kinetics of drug release from the NLC gel, the outcomes got from in vitro release studies examines was fitted to different kinetic models such as first-order, zero order, Higuchi and Krosmeyer Peppas model. The point of reference for choosing the most convenient model was based on a decency-of-fit test.

STABILITY STUDIES

Stability studies were done on most satisfactory formulation according to ICH rules at 40±2°C and 75 ±5% RH. The most good formulation put away in a fixed in aluminium foil. These were put away at room temperature. Following 45days, molecule size, zeta potential, entrapment efficiency, *in vitro* drug release of most good formulation was determined. [12S]

RESULTS AND DISCUSSION

DRUG-POLYMER COMPATIBILITY STUDIES:

The IR Spectra of drug, lipid excipients are shown in Figure 4,11. The spectrum was studied at 4000 cm-1 – 400 cm-1. From the spectra it was clear that there was no interaction between the selected lipids, drug and mixtures. Hence the selected lipid was found to be compatible in entrapping the selected drug with carriers without any mutual interactions.

Figure 1: IR spectra of itraconazole

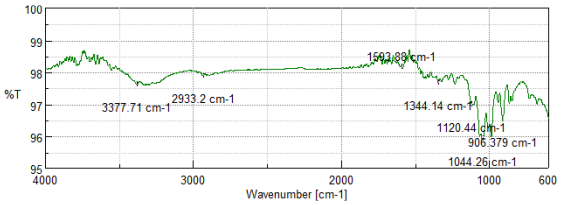
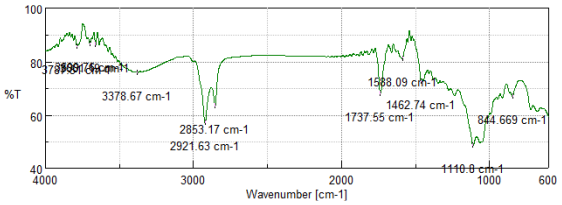


Figure 2: IR spectra of itraconazole, Compritol 888 ATO, caprol PGE 860, poloxamer 188& Carbopol mixture



PREPARATION OF ITRACONAZOLE LOADED NLC

Homogenization followed by ultrasonication is a reliable, simple and reproducible method for preparing itraconazole NLC. The prepared NLC dispersion was found to be uniform and homogenous in appearance.

CHARACTERIZATION OF ITRACONAZOLE LOADED NANOPARTICLE LIPID CARRIER:

DETERMINATION OF PHYSICOCHEMICAL PROPERTIES

The formulated NLC dispersion was shows milky white in appearance, odourless, and fluid in nature. It was stable and did not show sedimentation even after centrifugation at 2000 rpm for 30 min.

DETERMINATION OF ENTRAPMENT EFFICIENCY

The results of entrapment efficiency of formulated NLCs are shown in the Table 2. The entrapment efficiency of the formulations F1-F7. The entrapment efficiency of F3 reached 83.2% which suggests that most of the itraconazole had been better entrapped into the lipid matrix of NLC. From the above results it shows that the entrapment efficiency of the formulations increases with increase of lipid concentration. This was because that when the lipid concentration increases, there would be more lipid to entrap the drug molecules.

PARTICLE SIZE, ZETA POTENTIAL AND POLY DISPERSIBILITY INDEX OF ITR-NLC

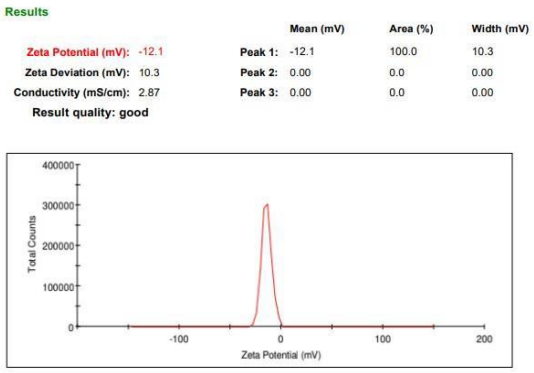
Average particle sizes of itraconazole NLC were found in the range of 185.83 nm to 908.8 nm. It showed that the particles were in nanometre range. The polydispersity index (PDI) was found in the range of 0.27 to 0.8as mentioned in (Table 2). This showed the polydispersity of particle was below 1 which infers the more homogeneity of the particles. The stability of the itraconazole nanostructured lipid carrier was evaluated by measuring the zeta potential of the NLC. The zeta potential of the formulations ranges from -11.6 to -22.6 mV. The zeta potential of best formulation F7 was found to be -12.1 mV which indicates that the formulation was stable. From the observations it was found that the nanostructured lipid carrier has been good homogeneity because poly dispersity index was found to be less than one. The zeta potential is negative due to presence of negative surface charge of the drug.

Table 2: Entrapment Efficiency, Particle Size, Zeta Potential & Polydispersibility of F1-F7

Formulation code	Entrapment Efficiency (%)	Mean diameter	PDI	Zeta potential(mV)
F1	72.1 ± 0.01	478.0	0.315	-13.65
F2	77.6 ± 0.02	381.1	0.056	-14.26
F3	83.2 ± 0.02	185.2	0.018	-22.26
F4	75.36 ± 0.03	908.8	0.854	-11.6
F5	79.25 ±	625.1	0.58	-21.26

	0.04		4	
F6	79.60 ± 0.01	382.4	0.508	-11.26
F7	80.36 ± 0.02	5.229	0.273	-12.1

Figure 4: Particle size of F7



IN- VITRO RELEASE STUDIES OF ITR-NLC DISPERSION

In vitro drug released study was carried out for 8 h for formulation F1-F7. The cumulative percent drug release after 8 h was found. % Decrease in drug release was found as the liquid lipid is increased, this may be due to high viscous and thickness of lipid layer, Table: 3 and Fig: 5.

Table 3: % Cumulative release of F1-F7

Time in hr	cumulative drug release %						
	F1	F2	F3	F4	F5	F6	F7
0.5	10.54	11.68	10.78	16.45	15.92	12.02	16.35
1	25.35	26.49	17.03	21.67	19.18	23.65	24.36
2	28.65	29.47	25.14	25.87	20.94	30.24	29.66
3	35.55	34.65	29.24	30.65	35.55	35.55	32.45
4	39.45	35.68	32.87	35.61	37.65	39.67	45.12
5	43.35	40.65	39.68	46.56	43.76	45.54	49.43
6	48.46	45.67	44.12	56.67	56.25	47.65	51.54
7	53.57	52.56	46.75	60.32	62.76	56.36	54.76
8	65.67	63.78	55.46	67.63	70.69	62.12	61.35

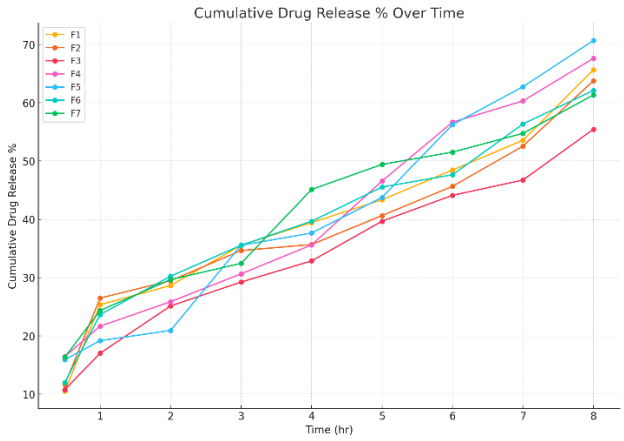


Figure 5: *in vitro* drug release of F1- F7

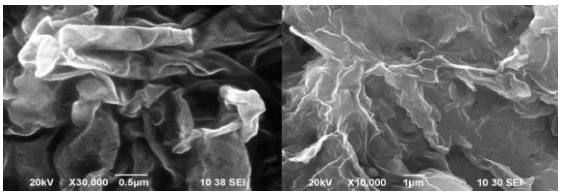
CHARACTERIZATION OF ITRACONAZOLE NLC GEL

The optimized NLC dispersion F3 was prepared as gel. Carbopol 974 was used as a gelling agent for the preparation of ITR-NLC gel. Carbopol based gels were prepared and evaluated for different parameters.

SCANNING ELECTRON MICROSCOPY (SEM) STUDIES

The morphology of itraconazole loaded NLC gel was examined by scanning electron microscope. The SEM photograph was shown in the Figure 6. It revealed that the NLC gel shows the particle size was found to be 500 nm in size smooth surfaces.

Figure 6: SEM image of ITR-NLC gel



EVALUATION OF NANOSTRUCTURED LIPID CARRIER GEL OF ITRACONAZOLE

ITR-NLC gel prepared was subjected to various evaluation test. The mean pH was in the range 6.1, which lies in the normal pH range of the skin and would not produce any skin irritation. The spreadability of the prepared gel F3 was found to be 3.8cm, and the value of spreadability indicates the gel was easily spreadable by small amount of shear. The extrudability is the concentration of gelling agent. The extrudability of the prepared gel was found to be 9.5 g/cm3. Thus, the prepared gel possesses optimum extrudability. The drug content of the formulated gel was estimated

spectrophotometrically at ƛ max 262 nm. The drug content of the NLC gel was found to be 74.08%. the viscosity of the prepared gel was found to be 7361 centipoises given in the table 4.

Table 4: Evaluation Of ITR-NLC Gel

Formulation code	pH mean	SPREADABILITY COEFFICIENT (cm) mean	Viscosity (cps) mean	Extrudability g/cm^3	Drug content (%) mean
F3	6.1	3.8	7361	9.5	74.08

IN VITRO DRUG RELEASE STUDY OF ITR-NLC GEL

Table 5: *In Vitro* Drug Release Of ITR-NLC Gel

Time (hr)	% cumulative drug release mean
1	9.292 ± 0.01
2	12.465 ± 0.02
3	16.321± 0.03
4	26.456 ± 0.01
5	31.265 ± 0.01
6	33.652 ± 0.05
7	42.163 ± 0.02
8	50.236 ± 0.01

IN VITRO DRUG RELEASE KINETICS

The kinetics and mechanism of drug release were studied by release kinetics, the n, k and r2 values are indicated in the Table 6. A formulation shows first-order release which had higher linearity than the zero-order or Higuchi model.

The drug release profile demonstrated a biphasic pattern with initial fast release followed by sustained release. Initially the dissolution and diffusion of the drugs was expected form the surface of the NLC followed by the release of the drug by nano particle erosion. The hydrophobic lipid erosion takes time which is expected to prolong the release of itraconazole. The sustained drug release may attribute to the prolonged action in the skin tissue after topical application.

The exact mechanism of the release kinetics was determined by Higuchi Peppas model. Results indicated that the NLC formulations followed non-Fickian model of release kinetics given in the figure 32,33,34,35.

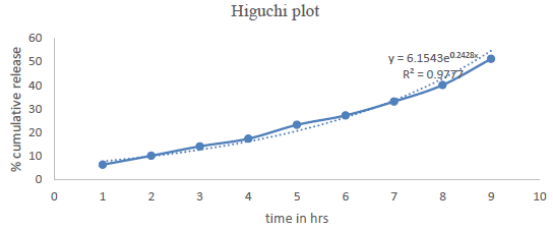


Figure 7: Higuchi model of drug release

Table 6: Kinetic Modelling Data

Formulation	Zero order	First order	Higuchi model	Korsemeyer-peppas	
	R^2	R^2	R^2	n	R^2
F3	0.967	0.8077	0.9777	0.9227	0.974

Stability studies:

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of variety of test period for the drug substance or a shelf life for the drug product and recommended storage conditions. Here the prepared NLC-gel was loaded at accelerated condition at 40 ◦c ± 2◦c, 70% ± 5% RH in a stability chamber. Sample was withdrawn at initial, 30 & 45 days and evaluated for physical appearance, drug content, pH, spreability, extrudability, viscosity and in-vitro diffusion studies. The result showed that the storage conditions had no effect on those parameters. In the stability study, after every 30 days samples were withdrawn and retested for viscosity (cps) and total drug content. The formulation did not show any significant change in both parameters. It indicates that this formulation was able to retain its stability upto 45 days. Stability data had showed in table 7.

Table 7: Stability studies of F3 Formulation

S.no	Parameters	Initial	30th day	45th day
1	Physical appearance	Milky white colour	No change	No change
2	Drug content	74.08 %	73.26 %	73.12 %
3	pH	6.1	6.0	6.0
4	Spreability	3.8	3.7	3.6
5	Viscosity	7361	7352	7319
6	Extrudability	9.5	9.3	9.1
7	% cumulative release	50.236	50.120	49.995 %

CONCLUSION

Nanoparticle lipid carrier (NLC) are very potential formulations for topical delivery of antifungal drugs. In this present study itraconazole loaded nano structured lipid carrier were formulated by using hot emulsification followed by probe sonication. The results showed that it was possible to prepare stable and effective lipid nano structures with mixed lipids like Compritol 888 ATO and Caprol PGE 860 and poloxamer 188 & tween 80 as surfactant. The physicochemical properties and the in vitro release study for all ITR-NLCs were investigated. It was observed that F3(1.68g of Caprol PGE 860 &

0.5g of Compritol 888 ATO) showed particle size 381.1 nm, Polydipersibility index 0.018 & Zeta potential -22.26 Mv with better entrapment efficiency 83.2% and sustained release up to 55% till 8hrs. The optimized F3 was made into gel using Carbopol 0.5% as gelling agent. SEM study revealed that the NLC gel showed the particle size was found to be 500 nm in size smooth surfaces. The exact mechanism of the release kinetics was determined by Higuchi-peppas model. Results indicated that the NLC formulations followed non-fickian model of release kinetics. The obtained results reflect the potential of NLC as a carrier for topical administration of ITR. In conclusion, the developed systems are promising alternative drug carriers for topical pharmaceutics however, further studies are necessary to confirm *in vivo* efficacy.

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