INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN: 2320-2831

IJPAR |Vol.11 | Issue 2 | Apr - Jun -2022
Journal Home page: www.ijpar.com

Research article *Open Access*

Preparation and Characterization of Isoconazole Loaded Nanolipidic Carrier Gel for Antifungal action

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ABSTRACT

Nanoparticulate systems present potential platform for drug therapy that can improve its performance and rise above its limitations. Among the different investigated nano-systems, lipid nanoparticles keep immense promise in the area of drug delivery. As its lipid content represented only by solid lipid, low drug payload is the major challenge for applicability owing to internal rearrangement of crystal lattice and then drug expulsion. In order to increase drug loading, the second generation lipid nanoparticles; nanostructured lipid carriers (NLCs) was developed. Isoconazole loaded NLC based gel evaluated for Visual appearance and pH, spreadability, viscosity and drug content, In-vitro drug permeation and stability for the optimized formulation. The present investigation deals with formulation and Characterization of Isoconazole loaded NLC based gel. The ingredient s like lipid (Glyceryl mono stearate), liquid lipid (olive oil), surfactant (tween 80) and soya lecithin were selected for formulation using ethanol as solvent. All the nine formulation prepared were evaluated for particle size, entrapment efficiency, zeta potential. The mean particle size was observed in between range of 121±8.14nm to 342±8.55nm, the least mean particle obtained for formulation FINLC5. Entrapment efficiency was obtained in between the range of 68±2.88 %to 88±1.74%.The highest entrapment efficiency was found for FINLC5. Zeta potential for all nine formulation obtained in between -21.3 to -32.1 mV. A surface study of the Isoconazole loaded NLC s using SEM displayed aggregation and fusion of the particles, which could be attributed to the mechanical stress of ice crystals. The Best formulation of Isoconazole loaded NLC (FINLC5) was converted into nine different Gel preparation using Cold method. This Method incorporates the Poloxamer and Carbopol 934 as gelling agent. The best gel found to be FINLCG5 having spreadibility 5.95gm/s, viscosity 2314 centipoise (cP) and drug content 98.76%. In-vitro drug permeation for the optimized FINLCG5. Showed the best result by releasing Isoconazole 98.35% in 12hr. There was no significant changes in Physical appearance, viscosity and drug content was observed in selected FINLCG5 gel formulation after various time point and temperature condition which refers it as a stable formulation. From above data it was concluded that the Isoconazole loaded NLC based gel FINLCG5 Gel is the best formulation can be considered for animal study.

Keywords: Isoconazole, Nanolipidic carrier gel, Antifungal action.

INTRODUCTION

Nanotechnology

Nanoparticulate systems present potential platform for drug therapy that can improve its performance and rise above its limitations. Among the different investigated nano-systems, lipid nanoparticles keep immense promise in the area of drug delivery.

As its lipid content represented only by solid lipid, low drug payload is the major challenge for applicability owing to internal rearrangement of crystal lattice and then drug expulsion. In order to increase drug loading, the second generation lipid nanoparticles; nanostructured lipid carriers (NLCs) was developed. NLCs are binary system which contains both solid and liquid lipids which in turn produced less ordered lipidic core.

This imperfection of internal arrangement aids more drug accommodation. So, NLCs overweigh NLC (Fig. 1) as the former can encapsulate higher drug amounts, contains lower water content and improves drug entrapment with minimized leakage during storage. They also since then, researchers paid attention to NLCs and discovered different applications.

Solid lipid nanoparticles (NLCs) and nanostructured lipid carriers (NLCs).

NLCs comprise of lipids that stay high at room and body temperature and surfactants that act as stabilisers. It is not harmful, prohibits the use of organic solvents and can be investigated in order to target pharmaceutical products and controlled releases of drugs. NLCs have shown that pharmaceutical stability is improved and easy to sterilize.

NLCs are able to create crystalline phases and thus reduce the imperfections within the space net leading to drug eviction and low payload. NLCs consisting of a blend of solid and liquid lipid (ol) have been developed to address this obstacle, resulting in structural imperfections that lead to better drug loading [25]. For this reason. Nonetheless, the lipid stability and nanotoxicity challenges for the NLCs are also present. Both NLCs and NLC have many advantages such as improved lipophilic solubility, increased dissolving, improved stay times and higher lymphatic absorption.

Advantages of Nanostructured Lipid Carriers

More drug loading potential, Water is less dispersed, Prevent or decrease drug expulsion while being processed, Targeted Drug Release and Monitoring, Loading of both lipophilic and hydrophilic medicines, Utilization of biologically and biofriendly lipids.

Types of NLC

Depending on the situation of incorporated drug moieties in NLC, following three sorts of morphological models (Figure 1) has been proposed:

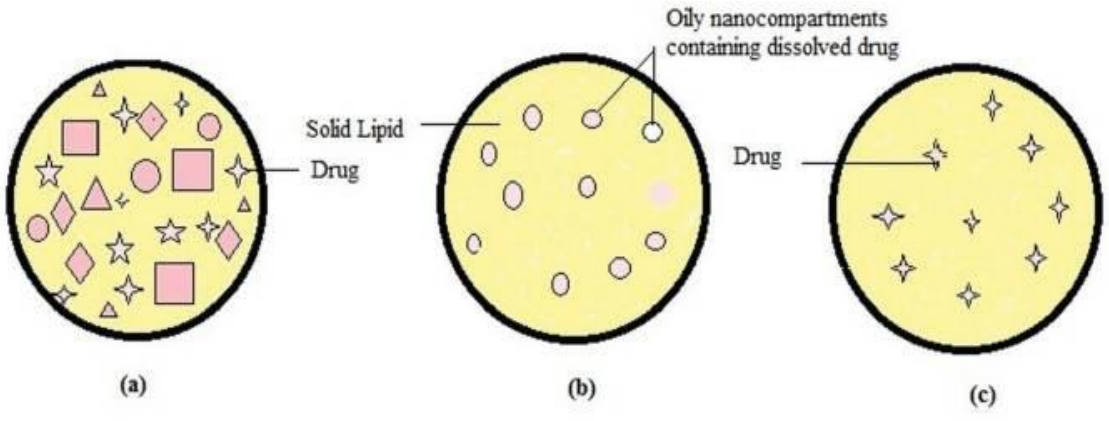

Fig 1: Types of nanostructure lipid carrier

NLC type I (imperfect crystal model)

Uncertain type of glass The NLC consists of a very uneven matrix with many vacuums and areas capable of accommodating more drug molecules in amorphous clusters. These crystal imperfections are obtained by the blending of solid lipids and ample amount of liquid fluids (oils).

The matrix of the NLC is not ready for forming a highly ordered structure due to the various chain lengths of fatty acids, and hence the mixture of mono- di- and triacylglycerols. The mixing of spatially different lipids improves drug usefulness, but this model provides minimum entangling efficiency.

NLC type II (multiple type)

Multiple NLC type is oil/fat/water type. Lipophilic medicines in liquid lipid products are more soluble than solid lipid. This concept results in the use of high liquid lipid content for multiple NLC forms. Oil moieties are easily distributed within the lipid matrix at low concentrations. The addition of oil over and above its solubility leads to a separation of phases that forms small nano compartments of oil within the solid matrix. Oil Form II provides advantages such as high efficiency of drug trapping, managed drug releases and reduced drug leakages.

NLC type III (amorphous model)

Amorphous NLC is formulated to minimize the drug leakage due to phase of crystallization by carefully mixing lipids.. Substantive, but non-crystalline, lipid formations such as hydroximy-loctacaine, hydroxyl stearate, isopropyl myristate or dibutyladicate. During homogenous amorphous conditions, the lipid matrix is available.

MATERIALS AND METHODS

Materials and Equipment

The following materials and equipment's were used for experiment.

Table 1: List of Materials

S.NO	RAW MATERIALS	MANUFACTURER
1.	Isoconazole	MSN laboratories PVT. LTD, Hyderabad
2.	Soybean lecithin	Advantage Oils Pvt. Ltd.
3.	Glycerol monostearate	Spectrum chemical mfg.corp
4.	Poloxamer 188	Research lab Fine Chem .Industries
5.	Carbopal 934	Research lab Fine Chem .Industries
6	Ethanol	Research lab Fine Chem .Industries
7.	Oleic acid	Research lab Fine Chem .Industries
8.	Tween 80	Fisher scientific
9.	Medium chain triglyceride (Olive oil)	Research lab Fine Chem .Industries

Table 2: List of Equipment

S.NO	EQUIPMENT	MAKER
1.	Weighing balance	Shimadzu
2.	FT-IR spectrophotometer	Bruker
3.	UV-Visible spectrophotometer	Shimadzu
4.	Magnetic stirrer	2 MLH
5.	Ph meter	Lab India
6.	Viscometer	Brookfield
7.	Probe sonicator	Sonics
8.	Micro centrifuge	Radical scientific private limited
9.	Stability Chamber	Cintex
10.	Refrigerator	Whirl Pool
11.	Scan electron microscope	Hitachi
12.	Nanopart analyser	Horiba

Formulation of Isoconazole Loaded Nano Lipidic Carrier

Table 3: Formulation Table for Isoconazole loaded NLC

Ingredients	FINLC1	FINLC2	FINLC3	FINLC4	FINLC5	FINLC6	FINLC7	FINLC8	FINLC9
Isoconazole (mg)	10	10	10	10	10	10	10	10	10
GMS(g)	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Olive oil(mg)	800	750	700	650	600	550	500	450	400
Soya lecithin(mgs)	100	150	200	250	300	350	400	450	500
Tween 80(ml)	5	5	5	5	5	5	5	5	5
Ethanol(ml)	20	20	20	20	20	20	20	20	20

Preparation of Isoconazole loaded NLC

NLCs loaded with Isoconazole were prepared by modified emulsification evaporation method at a high temperature and solidification at a low temperature [17]. Approximately 1.4 g of solid lipid GMS (glyceryl mono stearate), liquid lipid (Olive oil) and lecithin were weighed precisely and then co-dissolved into ethanol in water bath at 75°C. The resultant organic solution was added drop wise into aqueous phase containing Tween 80, was added under mechanical agitation with 1000 rpm in water bath maintained at 75 °C for 5 h. The Nano emulsion thus obtained was immediately dispersed into cold distilled water (0–2 °C) with constant stirring at 1000 rpm for 1 h to obtain the drug loaded NLCs dispersion. Loading of NLC with pure drug (10 mg) was performed, respectively, by adding drug to the melted lipid**.**

Evaluation of NLC formulation

Particle size and particle size distribution

- A Horiba Nanoparticle Size Analyzer was used to quantify the particle size, mean particular size (PS-Z average in nm), NLC's Pole Dispersion Index (PI) (SZ-100 Nanopartica series). Samples were prepared with the desired NLC dilution twice deionized with purified water. Samples were prepared.
- The analyzes were carried out using the 0,45µ membrane filter to filter the above solution. A system centered on the medium, i.e. 90° light dispersing for low viscous samples and 170o light scattering for high viscous samples, automatically set the dynamic light dispersing rate.
- The NLC particle size should be from 10 to 1000 nm and the PI < 0.3 which is the uniform size distribution of the single model or uniform mono size dispersion distribution. Triplicate (n=3) is obtained for both measurements.
- Using the Horiba Nanoparticle size analyser, the zeta potential or surface charge potential was determined (SZ-100 nanopartica series). In an electrophoretic cell, an 80 mV electronic field was supplied to the probe with diluted NLC dilution [69]. Triplicate all tests at 25°C have been completed.

Entrapment efficiency

- The entrapment efficiency Percentage(EE%) of the NLC s was determined by indirect method wherein the amount of unentrapped drug in the aqueous surfactant solution i.e., supernatant after centrifugation at 22,000 rpm for 45 min was determined, against the total amount of drug added to the formulation.
- The supernatant was diluted appropriately with 0.1 N HCl and analyzed by using U.V Spectrophotometer. The below mentioned formula was used for the calculation of entrapment efficiency

$$\text{Entrapment efficiency} = \frac{Total\ amount\ of\ drug - amount\ of\ free\ drug}{Total\ weight\ of\ the\ nanoparticles} \times 100$$

Scanning Electron Microscope (SEM) studies

The Scan Electron Microscope observed the surface morphology of the NLCs for the optimized formulation (Hitachi S-3000N). Lyophilized powder samples of NLC 600 Å platinum were bound by a sputter cover and tested by SEM. Coated NLC was then placed and scaned on a sample holder by an electron beam.

The electron beam struck the NLC particles and emitted secondary electrons based on the surface nature which gives the NLC's surface morphology image. The medium size NLC obtained by the SEM is then compared to the NLC size obtained by the Horiba Nanopart analyzer**.**

Preparation of Isoconazole loaded NLC Gel

The NLC dispersions were gelled using different concentrations of poloxamer 407 and carbopol 940 by a cold method. Briefly, 1% of carbopol 940 and poloxamer407 were selected as a gelling agent. Carbopol 940 (1% w/w) was added in small quantities to NLC s formulation with continuous strirring with a mechanical overhead stirrer at 400rpm until a homogenous dispersion was obtained. The resultant gel was evaluated for pH, Viscosity, Spreadability, Invitro drug release

Evaluation of Isoconazole loaded NLC gel

Appearance and Homogeneity

Physical appearance and homogeneity of the repared gels were evaluated by visual perception.

pH

The pH of Isoconazole loaded NLC gel formulation were measured by using a calibrated pH meter.

Viscosity

Brookfield digital viscometer was used to measure the viscosity of prepared gel formulations. The spindle no. 6 was rotated at 10rpm. The reading, near to 100% torque was noted. Samples were measured at 30 ± 1°C.

Spreadability

Two sets of glass slides of standard dimensions were taken. The gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slides. Hundred g weight of gel was placed on the upper slides so that the gel was between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only upper slides to slip off freely by the force of weight tied on it. A 20 g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated for three times and the mean time was taken for calculation.

Spreadability was calculated by using the following formula:

$$S = m \times l/t$$

where,

S= spreadability, m-weight tied to upper slides (20 g),
l- length of the glass slide (7.5 cm),
t- time taken in sec.

Drug content

Gel equivalent to 100mg of Isoconazole was taken and dissolved in 100mL of methanol in a volumetric flask. Gel solution was subjected to shaking for 2h on mechanical shaker to obtain complete solubility of drug. The solution was estimated spectro-photometrically at 272.2nm using methanol as solvent.

In vitro Drug Release Study

- In vitro release studies were performed using the static vertical Franz diffusion cells with a diffusion area of 0.636 cm2 and a receptor compartment of 5 mL. A dialysis cellulose membrane, as artificial membrane, was placed between both compartments, and a receptor solution composed of phosphate buffer at a pH of 6 was used, ensuring sink condition This receptor compartment was stirred at 400 rpm and maintained at 34 ± 0.5 °C by a thermostatic water pump, which circulated water through each chamber jacket, mimicking mucosa conditions.

- Formulations NLC gel were applied in the donor compartment. Subsequently, 5ml of receptor medium was collected according to the intervals and immediately replaced with the same volume of fresh solution. Withdrawn samples were analysed for the drug content using the UV method.

Stability

Stability Studies Selected formulations (FINLCG5) were stored at different storage conditions at elevated temperatures such as 25^0 C±2^0 C/60%±5%RH, and 40 ^{0} C± 2^0C / 75%±5% RH at for 180days.

The samples were withdrawn at intervals of 30 days and checked for physical changes, viscosity and Drug content %.

RESULTS AND DISCUSSION

The study deals with the Preparation and characterization of Isoconazole loaded Nano lipidic carrier (NLC), Best Nano lipidic carrier is converted into Gel formulation and evaluated. Total Nine NLC formulation were prepared by emulsification evaporation method at a high temperature and solidification at a low temperature, Glyceryl mono stearate was used as lipid in this formulation, All nine formulation were characterized for particle size, entrapment efficiency and zeta potential and drug was scanned by FTIR. Isoconazole loaded NLC based gel evaluated for Visual appearance and pH, spreadibility, viscosity and drug content, In-vitro drug permeation and stability for the optimized formulation.

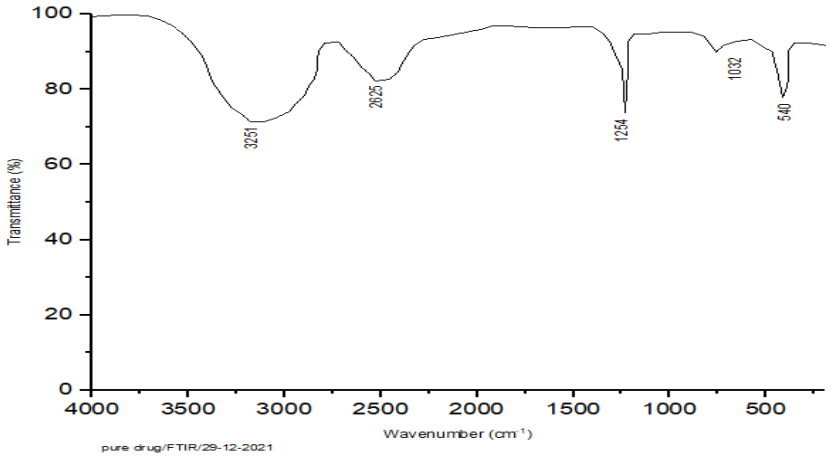

Fig 2: FTIR of Pure Isoconazole

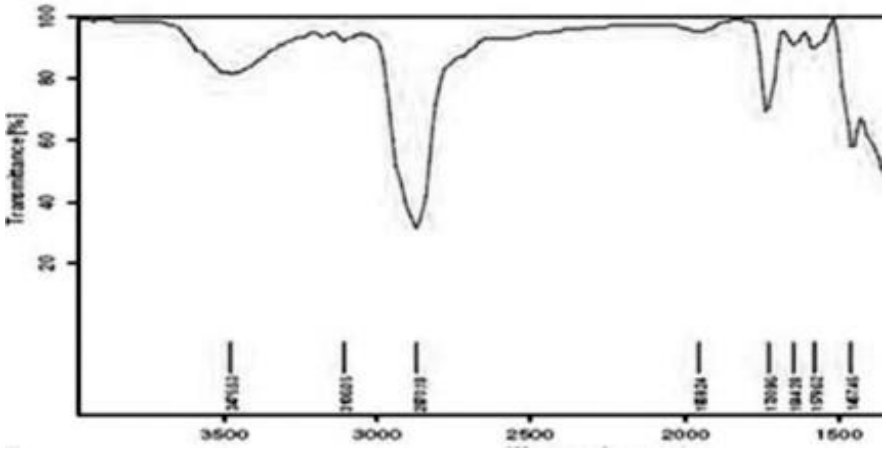

Fig 3: FTIR of Isoconazole with polymers

Table 4: Formulation Table for Isoconazole loaded NLC

Ingredients	FINLC1	FINLC2	FINLC3	FINLC4	FINLC5	FINLC6	FINLC7	FINLC8	FINLC9
Isoconazole(mg)	10	10	10	10	10	10	10	10	10
GMS(g)	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Olive oil (mg)	800	750	700	650	600	550	500	450	400
Soya lecithin(mgs)	100	150	200	250	300	350	400	450	500
Tween 80(ml)	5	5	5	5	5	5	5	5	5
Ethanol(ml)	20	20	20	20	20	20	20	20	20

Table 5: Characterization of Isoconazole loaded NLC (FINLC)

Batch	Particle size(nm)	EE%	Zeta potential
FINLC1	311±10.01	72±1.02	-32.1
FINLC2	288±9.12	76±2.31	-21.3
FINLC3	271±8.74	74±1.14	-26.1
FINLC4	301±9.88	80±1.08	-25.4
FINLC5	121±8.14	88±1.74	-22.5
FINLC6	342±8.55	74±1.31	-29.1
FINLC7	289±7.03	77±3.01	-28.4
FINLC8	271±6.31	68±2.88	-26.4
FINLC9	265±5.24	69±2.14	-24.7

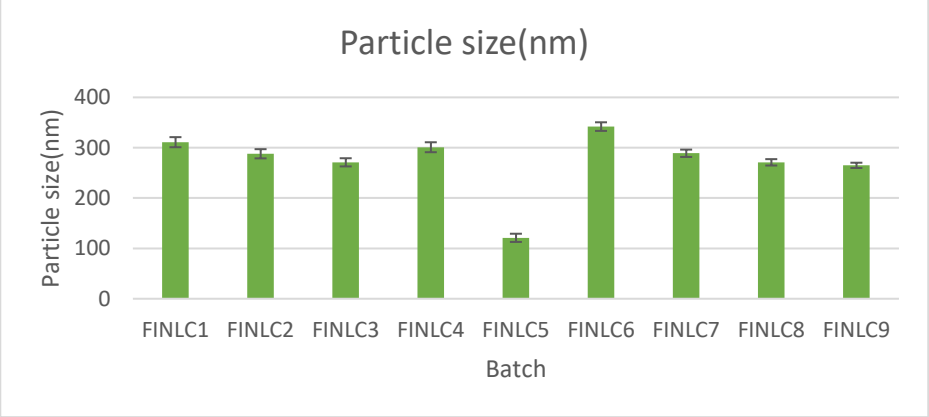

Fig 4: Particle size of Isoconazole loaded NLC (FINLC)

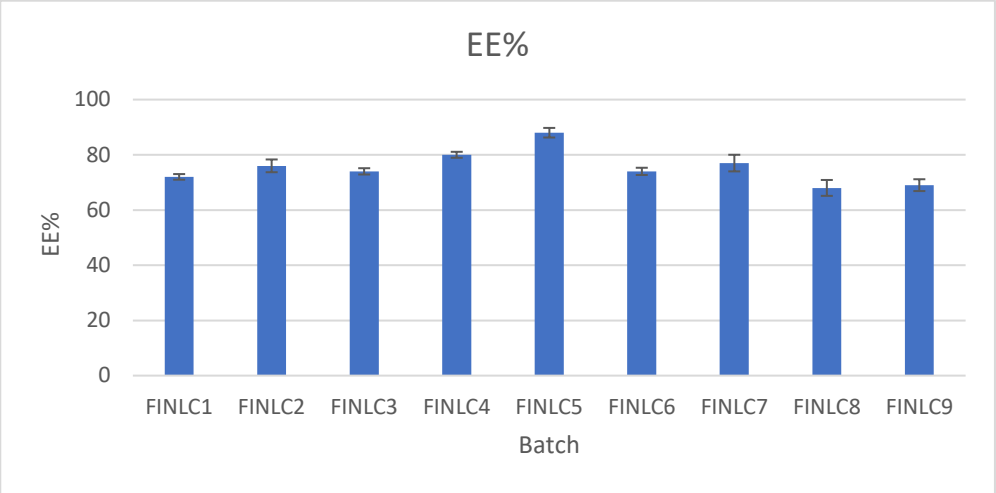

Fig 5: Entrapment Efficiency% of Isoconazole loaded NLC (FINLC)

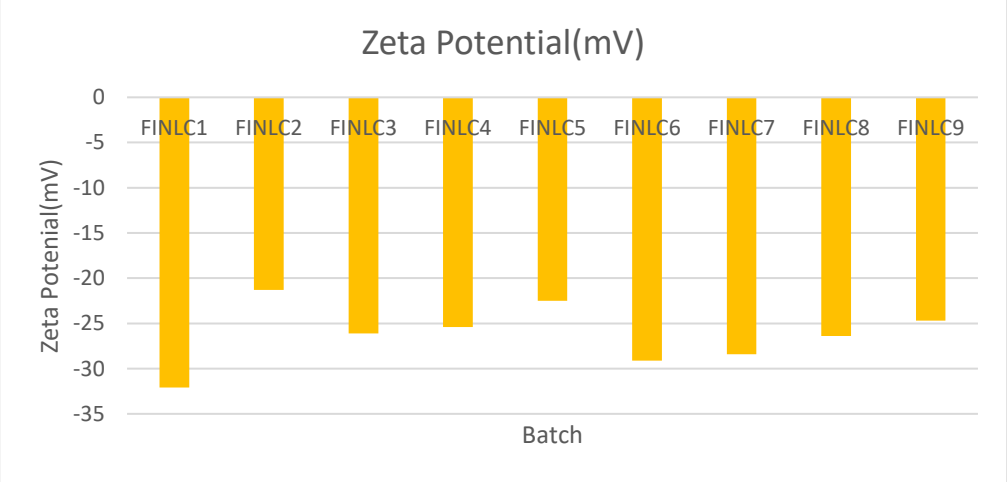

Fig 6: Zeta Potential of Isoconazole loaded NLC (FINLC)

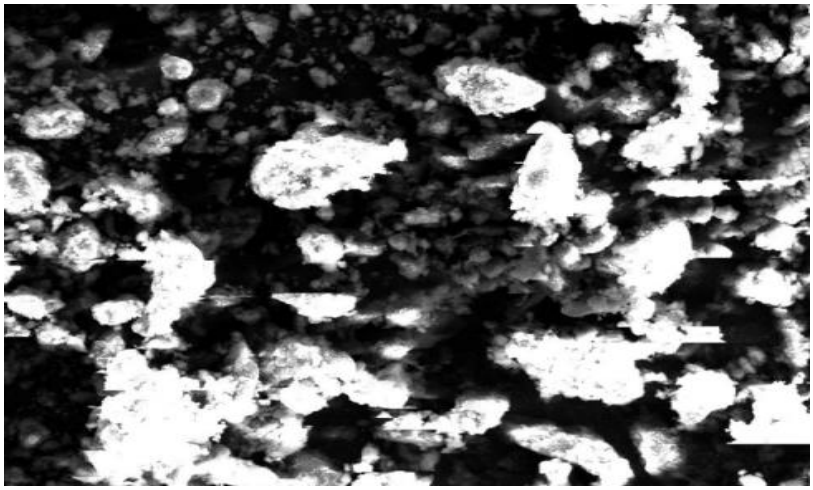

Fig 7: Scanning electron microscope of Isoconazole loaded NLC dispersion

Characterization of Isoconazole loaded NLC

- **Particle size:**The mean particle size was observed in between range of 121±8.14nm to 342±8.55 nm. The least mean particle obtained for formulations FINLC5 due to less content of lipid, while maximum particle size was obtained for FINLC6. Results of mean particle size in table 7.2.1.
- **Entrapment Efficiency%:** The entrapment efficiency in developed formulations produces higher load for all formulations. Entrapment efficiency was obtained in between the range of 68±2.88 to 88±1.74 .The highest entrapment efficiency was found for FINLC5 due to less content of lipid and higher content of surfactant. Results of EE% size in table 7.2.1.
- **Zeta potential:** Zeta potential for all nine formulation obtained in between -21.3 to -32.1 mV.
- **Scanning electron microscope:** A surface study of the Isoconazole loaded NLC dispersions using SEM (Figure 7.1.9) is displayed aggregation and fusion of the particles, which could be attributed to the mechanical stress of ice crystals that are formed during the freeze drying process, on the vesicles wall as previously mentioned.

Evaluation for Isoconazole loaded NLC based gel

- **Visual appearance and pH:** The physical appearance of Isoconazole loaded NLC based gel was found to be yellow in colour, smooth in texture and the pH of gel was found to be 6.4.
- **Spreadability:** All the formulation of NLC based gels are evaluated for spreadability test and the spreadability of different formulation can be observed from given table the spreadability of optimized gel was found to be 5.95gm/s. The observations of spreadability of few formulations.

Table 6: Spreadability

S.NO	Formulation code	Spreadability (gcm/s)
1	**FINLCG1**	4.80
2	**FINLCG2**	4.23
3	**FINLCG3**	5.82
4	**FINLCG4**	5.54
5	**FINLCG5**	5.95
6	**FINLCG6**	5.10
7	**FINLCG7**	4.87
8	**FINLCG8**	4.37
9	**FINLCG9**	3.62

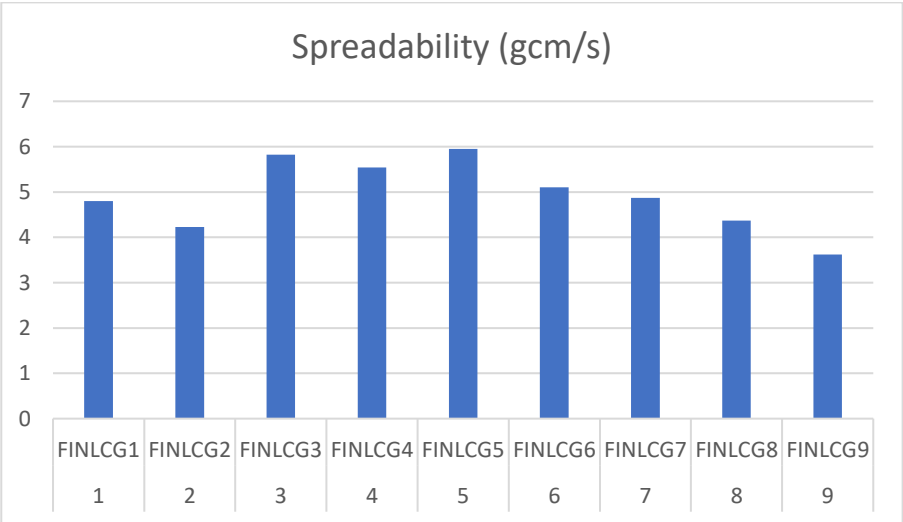

Fig 8: Spreadability of of Isoconazole loaded NLC based gel

Table 7: Viscosity

S.NO	Formulation code	Viscosity cps
1	**FINLCG1**	1057
2	**FINLCG2**	1354
3	**FINLCG3**	1276
4	**FINLCG4**	1801
5	**FINLCG5**	2314
6	**FINLCG6**	1782
7	**FINLCG7**	1329
8	**FINLCG8**	1278
9	**FINLCG9**	1032

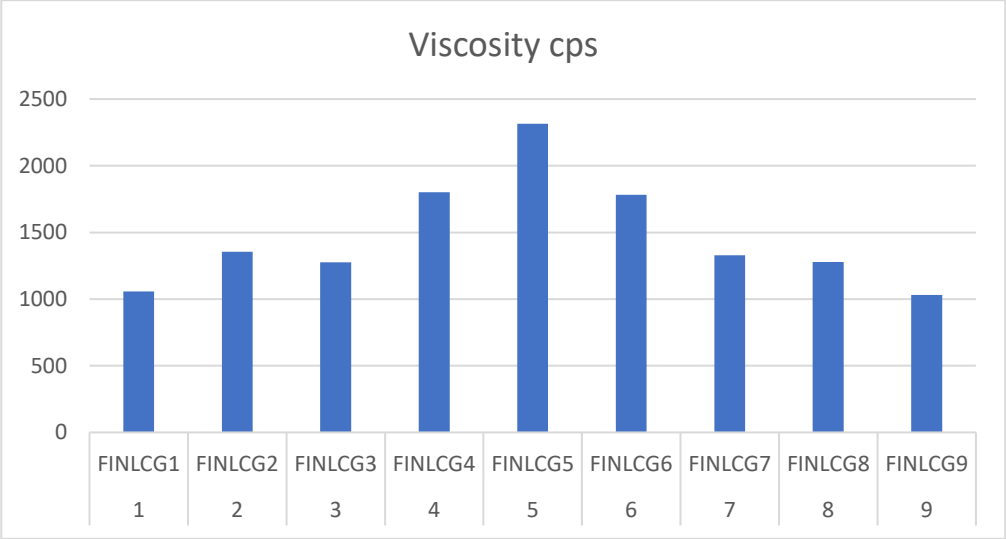

Fig 9: Viscosity of Isoconazole loaded NLC based gel

Viscosity of gel thickened microemulsion is increased with decreased in concentration surfactant cosurfactant mixture in the formulations. Viscosity of different formulations were done and the optimize F5 formulation the viscosity is found as 1834 ±10.02 cps respectively.

Viscosity of the formulation is important since higher the viscosity of the formulation greater will be contact time for absorption.It is determined using the brooke field viscometer. As viscosity increases shear rate decreases. Viscosity of NLC gel increased with decrease in shear rate the thixotropic nature of gel formulation is desirable as it allows case in local application of NLC gel.

All the formulation of NLC based gels are evaluated for Drug content and the drug content of different formulation can be observed from given table 7.3.5 the drug content FINLCG5 gel was found to be 98.76%.

Table 8: Drug content

S.No	Formulation code	Drug content (%)
1	**FINLCG1**	96.23%
2	**FINLCG2**	90.45%
3	**FINLCG3**	87.12%
4	**FINLCG4**	85.89%
5	**FINLCG5**	98.76%
6	**FINLCG6**	90.28%

7	**FINLCG7**	89.27%
8	**FINLCG8**	85.23%
9	**FINLCG9**	87.21%

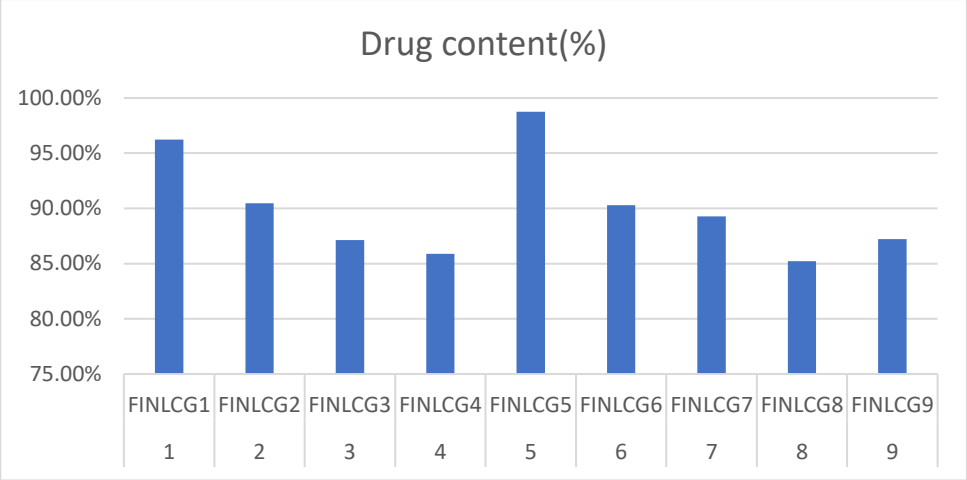

Fig 10: Drug content of Isoconazole loaded NLC based gel

Table 9: Invitro Permeation of Isoconazole loaded NLC based Gel:

Time(hr)	FINLCG1	FINLCG2	FINLCG3	FINLCG4	FINLCG5	FINLCG6	FINLCG7	FINLCG8	FINLCG9
0	0	0	0	0	0	0	0	0	0
0.5	12	34	25	12	20	16	29	34	39
1	21	48	32	44	32	46	37	47	48
2	34	68	59	52	46	50	62	56	58
3	42	81	75	75	61	59	77	78	79
4	53	88	86	88	68	72	84	89	87
6	59	90	91	90	78	83	93	96	89
8	61	91	92	92	88	91	94	92	90
12	74	92	92	92	98.35	92	93	93	90

The In-vitro drug Permeation for all nine formulation was evaluated by the Franz diffusion cell technique In-vitro drug release for the FINLCG1 is 74% in 12hr, FINLCG2 show 90% in 6hr FINLCG5. Showed the best result by releasing Isoconazole 98.35% in 12hr.

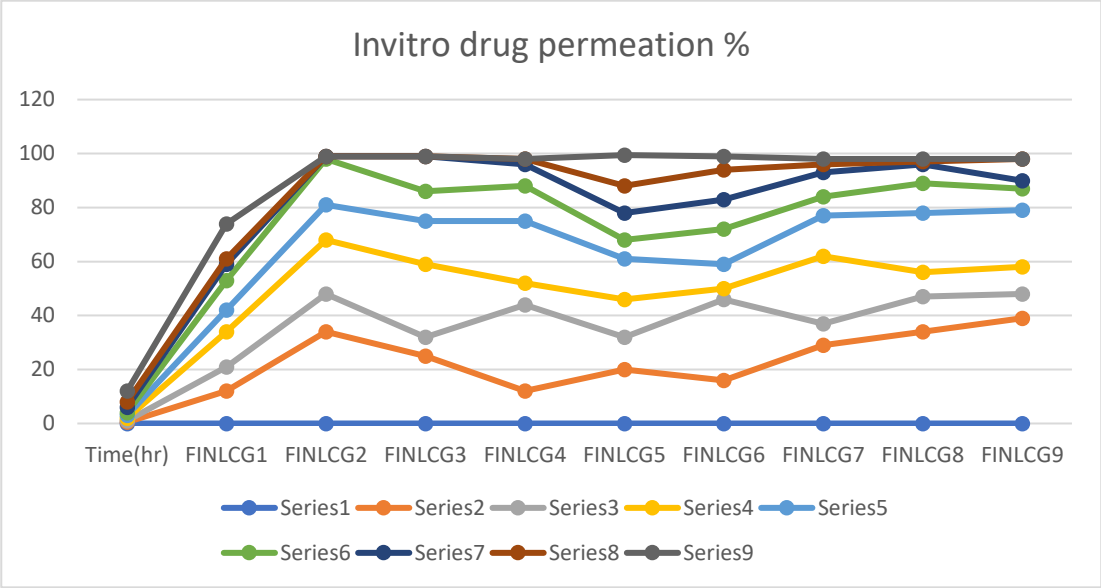

Fig 11: Invitro Permeation study of Isoconazole gel

Stability Study

Table 10: Stability Results for best formula FNLCG5

Days	25^0 C±2^0 C/60%±5%RH			40^0 C± 2^0C /75%±5% RH		
	Physical Appearance	Viscosity cPs	Drug Content %	Appearance	Viscosity cPs	Drug Content %
0	NC	2314	98.76	NC	2314	98.76
30	NC	2314	98.76	NC	2314	98.76
60	NC	2313.99	98.75	NC	2314	98.75
90	NC	2313.98	98.75	NC	2313.98	98.75
180	NC	2313	98.75	NC	2313.97	98.75

NC=No changes

There was no significant changes in Physical appearance, Viscosity and Drug content were observed in selected FNLCG5 Gel formulation after various time point and temperature condition which refers it as a stable formulation.

SUMMARY AND CONCLUSSION

The present investigation deals with formulation and Characterization of Isoconazole loaded NLC based gel. The ingredient s like lipid (Glyceryl mono stearate), liquid lipid (olive oil), surfactant (tween 80) and soya lecithin were selected for formulation using ethanol as solvent. A solution of Isoconazole in methanol was scanned in UV range between 200 to 400 nm (Lab India UV1601spectrophotometer, India). Isoconazole showed maximum absorbance at 272.2 nm in methanol. All the nine formulation prepared were evaluated for particle size, entrapment efficiency, zeta potential. The mean particle size was observed in between range of 121±8.14nm to 342± 8.55nm. The least mean particle obtained for formulation FINLC5. Entrapment efficiency was obtained in between the range of 68±2.88 %to 88±1.74%.The highest entrapment efficiency was found for FINLC5. Zeta potential for all nine formulation obtained in between -21.3 to -32.1 mV. A surface study of the Isoconazole loaded NLC s using SEM displayed aggregation and fusion of the particles, which could be attributed to the mechanical stress of ice crystals. The Best formulation of Isoconazole loaded NLC (FINLC5) was converted into nine different Gel preparation using Cold method. This Method incorporates the Poloxamer and Carbopol 934 as gelling agent. The best gel found to be FINLCG5 having spreadibility 5.95gm/s, viscosity 2314 centipoise (cP) and drug content 98.76%. In-vitro drug permeation for the optimized FINLCG5. Showed the best result by

releasing Isoconazole 98.35% in 12hr. There was no significant changes in Physical appearance, viscosity and drug content was observed in selected FINLCG5 gel formulation after various time point and temperature condition which refers it as a stable formulation. From above data it was concluded that the Isoconazole loaded NLC based gel FINLCG5 Gel is the best formulation can be considered for animal study.

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